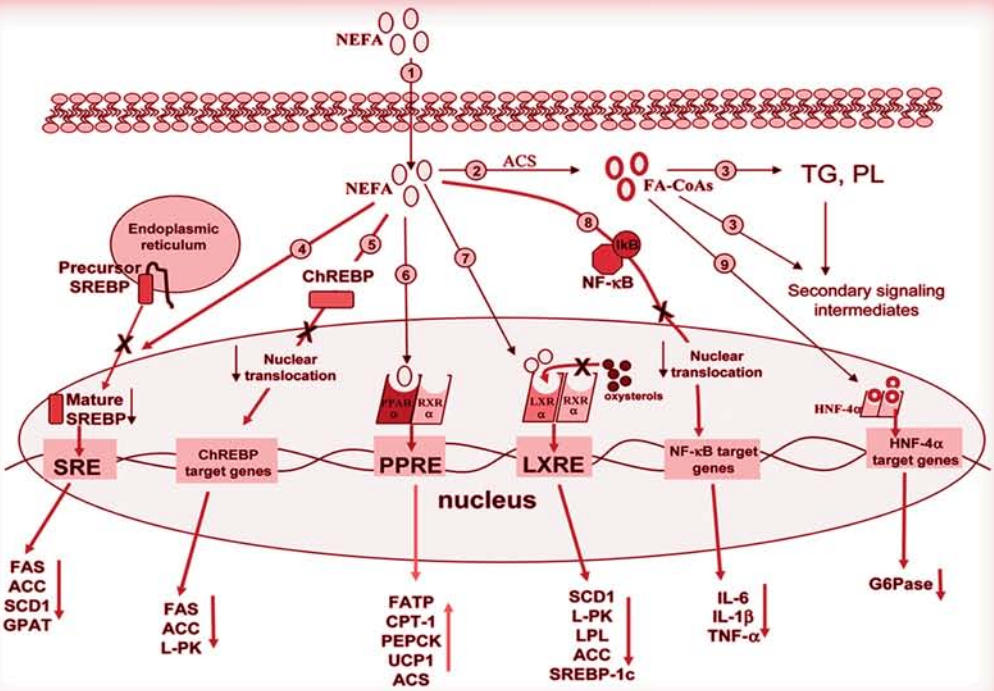
FATTY ACIDS IN FOODS *and their* HEALTH IMPLICATIONS

THIRD EDITION



edited by

CHING KUANG CHOW

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FOOD SCIENCE AND TECHNOLOGY

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Preface

The first edition of this book volume was published in 1992, and the second edition in 1999 by Marcel Dekker, Inc., New York. Since then, volumes of new information on the fatty acids in various foods and food products, as well as their biological health effects have become available. In addition to quantity, the type of fatty acids consumed plays an important role in the etiology of a variety of degenerative diseases, including cardiovascular disease, cancer, immunity and inflammatory disease, renal disease, diabetes, neuromuscular disorders, liver disease, visual dysfunction, psychiatric disorders, and aging. Understanding the mechanisms by which fatty acids exert their biological effects is important in unraveling the pathogenesis of these disorders, and may help providing effective preventive measures.

Both animal- and plant-derived food products contain fat. Food fat provides taste, consistency, and helps us feel full. Fat is a major source of energy for the body, and aids in the absorption of lipid soluble substances including vitamins A, D, E, and K. Dietary fat is essential for normal growth, development, and maintenance, and serves a number of important functions. Increasing evidence indicates that fatty acids and their derived substances may mediate critical cellular events, including activation and expression of genes, and regulation of cellular signaling.

New reports or findings dealing with the health effects of various fatty acids have always commanded a strong public interest. In recent years, omega-3 and *trans* fatty acids have received more attention than others. On September 8, 2004, the Food and Drug Administration announced the availability of a qualified health claim for reduced risk of coronary heart disease on conventional foods that contain omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid. While these fatty acids are not essential to the diet, scientific evidence indicates that these fatty acids may be beneficial in reducing coronary heart disease. On the other hand, recent scientific reports, expert panels, and studies concluded that consumption of *trans* fatty acids contributes to increased low-density lipoprotein cholesterol levels, which increase the risk of coronary heart disease. The Food and Drug Administration's final rule on *trans* fatty acids (or *trans* fat) requires manufacturers to list the amount of *trans* fat per serving on a separate line under saturated fat on the Nutrition Facts panel. As of January 1, 2006, food manufacturers must list the content of *trans* fat on the nutrition label. The health effects of α-linolenic acids and conjugated linoleic acid have also received considerable recent attention.

In recent decades, the prevalence of obesity or overweight has increased steadily in the United States and elsewhere. Currently over 40% of the adult population in the United States are considered as overweight or obese. Obesity is an important risk factor contributing to the development of the three leading causes of death—cardiovascular disease, cancer, and diabetes—and other disorders in the United States. As fat has much higher energy density than that of protein and carbohydrate, dietary fat is often blamed as the source of excess energy, although it is difficult to differentiate the effects of dietary fat and other energy nutrients independent of total energy intake. Evaluating trends in energy nutrient intake is useful in understanding the role of individual energy nutrients in the development of obesity and obesity-related illness over time. Also, investigation of the role of fatty acids in satiating effect and energy homeostasis is important in understanding food intake and energy balance issues.

Partly owing to the high-energy density, concerns over health problems associated with obesity and overweight have led to the development of several fat substitutes. The rapid advance in molecular biology and biotechnology has allowed for selective alteration of fatty acid composition in oil crops. It is now possible to commercially produce oil crops that contain a desirable proportion of specific fatty acids. Owing to distinct biological and health effects of various fatty acids, manipulation of the lipid composition in oil crops is likely to impact our well-being and economy enormously.

In addition to updating original chapters on the basis of available recent information, the following new chapters are added to cover the subject areas that were not covered or not adequately

covered in the second edition: Fatty Acids in Fermented Food Products (Chapter 13), Effect of Heating and Frying on Oil and Food Fatty Acids (Chapter 20), Consumption of Fatty Acids (Chapter 21), Significance of Dietary γ-Linolenate in Biological Systems: Alternation of Inflammation and Proliferative Process (Chapter 32), Biological Effects of α-Linolenic Acid (Chapter 33), Biological Effects of Conjugated Linoleic Acid (Chapter 34), The Role of Omega-3 Polyunsaturated Fatty Acids in Food Intake and Energy Homeostasis (Chapter 35), and Fatty Acid and Cognition, Behavior and Brain Development, and Mood Disease (Chapter 39). Also, the following chapters were completely rewritten: Fatty Acids in Meat and Meat Products (Chapter 5), Fatty Acids in Milk Fat (Chapter 6), The Effects of Dietary Fatty Acids in Fatty Acid Metabolism (Chapter 23), Dietary Fatty Acids and Eicosanoids (Chapter 28), Fatty Acids and Aging (Chapter 40), and Essential Fatty Acids and Visual Dysfunction (Chapter 43). At the same time, several chapters that appeared in the second edition were not included in the third edition.

This updated and expanded book volume presents the current status of fatty acids in common foods and food products. It also aims to provide readers with state-of-the-art information on the widely diversified health implications of fatty acids. However, as the precise role of fatty acids in the etiology of various degenerative disorders is yet to be delineated, it is not the intention of this book volume to present a unified view on the health implications of fatty acids or to provide guidelines for fatty acid consumption.

I would like to express my sincere appreciation to all the authors of this book volume for their cooperation and excellent contributions. Without their participation and efforts this project would not be a reality. Also, I would like to thank Susan Lee and Amber Donley of Taylor & Francis for their assistance and support during the course of the project. Finally I wish to thank my wife Shukwei for her understanding and patience over the past many years.

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1 Fatty Acid Classification and Nomenclature

Kelly Lobb and Ching Kuang Chow

CONTENTS

I. INTRODUCTION

Fats or lipids consist of numerous chemical compounds, including monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, and fatty acids. Fatty acids constitute the main component of phospholipids, triglycerides, diglycerides, monoglycerides, and sterol esters. Fatty acids consist of elements, such as carbon, hydrogen, and oxygen, that are arranged as a linear carbon chain skeleton of variable length with a carboxyl group at one end. Fatty acids can be saturated (no double bond), monounsaturated (one double bond), or polyunsaturated (two or more double bonds), and are essential for energetic, metabolic, and structural activities.

Food scientists, nutritionists, biochemists, chemists, and biomedical scientists alike recognize the need for a coherent nomenclature for fatty acids. There are a number of nomenclature systems for fatty acids, and some researchers continue to name fatty acids traditionally on the basis of the names of the botanical or zoological species from which they are isolated. Such naming system provides no clue as to the structure of fatty acids. The International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB) attempted to deal with this problem by setting up two nomenclature committees, the IUB-IUPAC Joint Commission of Biochemical Nomenclature (JCBN) and the Nomenclature Committee of IUB (NC-IUB). IUPAC states definitive rules of nomenclature for organic chemistry (1960) and lipids (1978), Markely (1960) presents a historical review of chemical nomenclature, and Fletcher et al. (1974) discuss the origin and evolution of organic nomenclature. Other excellent reference sources for fatty acid classifications and nomenclatures include Fahy et al. (2005), Fasman (1989), Gunstone (1996, 1999), Gunstone et al. (1992), Gunstone and Herslof (1992), Hopkins (1972), and Robinson (1982).

II. NOMENCLATURE

According to the strictest rules of nomenclature, a chemical name must identify and describe its chemical structure unambiguously. This is done by using systematic nomenclature, and there is no

possibility of mistaken identity when this system is used. The systematic method names fatty acids solely on the basis of the number of carbon atoms and the number and position of unsaturated bonds relative to the carboxyl group(s). Substituted groups and their positions are identified. Optical activity and geometric configuration at double bonds are also designated. Systematic nomenclature will be described generally for the saturated fatty acids and then for other types of fatty acids as necessary thereafter.

It is also important to note that there are modifications to this naming system that bring novel naming systems into existence. Trivial (common) nomenclature, which includes circumstantially assigned names, for example, source names, is widely used. The English system of trivial name arose from the practice of adding the suffix "-ic" to a root indicative of the natural source or some property of the acid; for example, acetic (ethanoic) acid from the Latin word *acetum*, meaning vinegar; stearic (octadecanoic) acid from the Greek word *stear*, meaning tallow; palmitic (hexadecanoic) acid from palm oil; and oleic (octadecenoic) acid from the Latin word *oleum*, meaning oil.

Semisystematic nomenclature is often found in verbal communication and as an abbreviation in written communication. This system tries to preserve some of the structural features of systematic nomenclature, yet does so using trivial names, illustrating features that seem important for a particular purpose at the time. For example, the systematic name 2-hydroxy-*cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid might become abbreviated to 2-hydroxylinolenic acid.

Also widely used is the structural system in which fatty acids are identified solely by carbon number and number of unsaturated double bonds. Morris (1961) has proposed that chain length be designated as C10, C20, and so forth, and the unsaturated double bonds be serialized. That is, either methylene-interrupted or conjugated, only the number of the carbon where the series begins will be indicated. For example, linolenic acid, or *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid, becomes C18:3Δ9c.

The Greek letters omega (ω) and delta (Δ) are sometimes used with special significance in naming fatty acids. Omega is often used to indicate how far a double bond is from the terminal methyl carbon irrespective of the chain length. Delta, followed by a numeral or numerals, is used to designate the presence and position of one or more double or triple bonds in the hydrocarbon chain counting from the carboxyl carbon. Thus, ordinary oleic acid is also named Δ9-octadecenoic acid.

Another system of nomenclature in use for unsaturated fatty acids is the "ω" or "n" classification, and the "n" system is analogous to the "ω" naming system. This system is often used by biochemists to designate sites of enzyme reactivity or specificity. The terms "ω" and "n" refer to the position of the first double bond in the carbon backbone of the fatty acid, counting from the end opposite to the carboxy group or closest to the methyl end of the molecule. Thus, oleic acid, which has its double bond nine carbons from the methyl end, is an ω-9 (or n-9) fatty acid, and linoleic acid is an ω-6 (or n-6) fatty acid because its second double bond is six carbons from the methyl end of the molecule (or between carbons 12 and 13 from the carboxyl end). Eicosapentaenoic acid, found in many fish oils, and alpha-linolenic acid, found in certain vegetable oils, are both ω-3 (or n-3) fatty acids, which have the first double bond that exists as the third carbon–carbon bond from the terminal methyl end (ω) of the carbon chain. Also, both ω- and n-naming methods give a clear indication of the stereoisomeric species concerned; for example, *cis* C20:4n-6 and *cis* C20:4ω-6. The first number indicates the number of carbon units, the second number refers to the number of double bonds, and the n-6 or ωω-6 designation refers to the position of the last double bond. Both the "ω" and "n" nomenclature methods used to designate the position of the last terminal double bond are interchangeably used, however, the "n-3" designation is the proper IUPAC abbreviation. See Davidson and Contrill (1985) for a comparison of the "n" and "ω" naming systems.

The nomenclature of some common fatty acids using the four common naming systems is shown in Table 1.1. Of which, three systems employ the chain length and the number and position of any double bond. The first two columns show systems based on complete names and the last two columns show systems for denoting fatty acids with abbreviations.

Although the IUPAC system is unambiguous, some authors still select a nomenclature system according to their audience. Also, criteria for a suitable abbreviated terminology have been proposed but have yet to be universally adapted. Questions of which abbreviated terms are most appropriate

TABLE 1.1
Nomenclature of Some Common Fatty Acids

Names		Abbreviation	
Trivial	**IUPAC**	**Carboxyl-Reference**	**n- or ω-Reference**
Palmitic acid	Hexadecanoic acid	16:0	16:0
Stearic acid	Octadecanoic acid	18:0	18:0
Oleic acid	9-Octadecenoic acid	18:1Δ9	18:1n-9 or 18:1–9
Linoleic acid	9,12-Octadecadienoic acid	18:2Δ9,12	18:2n-6 or 18:2–6
Linolenic acid	9,12,15-Octadecatrienoic acid	18:3Δ9,12,15	18:3n-3 or 18:3–3

for representing the long and complicated chemical names will continue until an accepted system of nomenclature for individual fatty acids is agreed upon.

III. SATURATED FATTY ACIDS

The naturally occurring fatty acids can be grouped on the basis of the presence of double or triple bonds into two broad classes termed *saturated* and *unsaturated*. Most of the saturated fatty acids occurring in nature have unbranched structures with an even number of carbon atoms. They are referred to as normal alkanoic acids and may bear the prefix "*n*-," such as in *n*-hexanoic or *n*-octadecanoic. They have the general formula R–COOH, in which the R group is a straight-chain hydrocarbon of the form $CH_3(CH_2)_x$ or C_nH_{2n+1}. These acids range from short-chain-length volatile liquids to waxy solids having chain lengths of ten or more carbon atoms. Fatty acids from 2 to 30 carbons (or longer) do occur, but the most common and important acids contain between 12 and 22 carbons and are found in many different plant and animal fats. Under the systematic rules of nomenclature, the aliphatic acids are regarded as derivatives of hydrocarbons of the same number of carbon atoms ($–CH_3$ is replaced by –COOH). The final "e" of the corresponding hydrocarbon (alkane) is replaced by the suffix "-oic"; for example, alkane becomes alkanoic. The unsaturated fatty acids are named in a similar manner, with alkene becoming alkenoic and alkyne becoming alkynoic. The presently accepted names for the hydrocarbons are given in Table 1.2. The tables in this and the following sections are representative only, not complete. Except for the first four members of the series (meth-, eth-, prop-, but-), which have trivial names, the prefix of the name cites the number of carbon atoms.

Saturated fatty acids are also functionally divided into short- and long-chain acids and are most widely known by their trivial names. Table 1.3 lists some of the most important saturated fatty acids. Also included is a system of abbreviated nomenclature that designates chain length and degree of unsaturation; for example, 18:0 designates an 18-carbon saturated fatty acid, whereas 18:2 indicates two double bonds. The location of unsaturations as well as conformation of double bonds can also be designated; 18:2Δ9c, 12c designates *cis* double bonds at the 9 and 12 carbons from the carboxyl group. Similar designations will be described for hydroxy, keto, and so on.

The short-chain saturated acids (4:0–10:0) are known to occur in milk fats and in a few seed fats. Bovine milk contains butanoic acid as well as smaller amounts of 6:0, 8:0, 10:0, and 12:0 acids. Milk from the sheep and goat also contain these, but decanoic is present in larger amounts. Lauric acid (12:0) and myristic acid (14:0) are major components of seed fats of the Lauraceae and Myristiceae families, which accounts for their trivial names. Palmitic acid is the most prominent saturated fatty acid occurring in fish oils, in the milk and storage fat of many mammals, and in vegetable fats. Stearic acid (18:0) is a minor component in most vegetable fats, and its trivial name derives from the fact that it is a major component in the tallow of ruminants.

The long-chain saturated acids (19:0 and greater) are major components in only a few uncommon seed oils. Although many types of fatty-acid-containing oils are present in natural sources, only

TABLE 1.2
Nomenclature of the Aliphatic Hydrocarbons

Number of Carbons	Alkane C_nH_{2n+2}	Alkene C_nH_{2n}	Alkyne C_nH_{2n-2}
1	Methane		
2	Ethane	Ethene	Ethyne[a]
3	Propane	Propene	Propyne[b]
4	Butane	Butene	Butyne
5	Pentane	Pentene[c]	Pentyne
6	Hexane	Hexene	Hexyne
7	Heptane	Heptene	Heptyne
8	Octane	Octene	Octyne
9	Nonane	Nonene	Nonyne
10	Decane	Decene	Decyne
11	Hendecane[d]	Hendecene	Hendecyne
12	Dodecane	Dodecene	Dodecyne
13	Tridecane	Tridecene	Tridecyne
14	Tetradecane	Tetradecene	Tetradecyne
15	Pentadecane	Pentadecene	Pentadecyne
16	Hexadecane[e]	Hexadecene	Hexadecyne
17	Heptadecane	Heptadecene	Heptadecyne
18	Octadecane	Octadecene	Octadecyne
19	Nonadecane	Nonadecene	Nonadecyne
20	Eicosane	Eicosene	Eicosyne
21	Heneicosane	Heneicosene	Heneicosyne
22	Docosane	Docosene	Docosyne
23	Tricosane	Tricosene	Tricosyne
24	Tetracosane	Tetracosene	Tetracosyne
25	Pentacosane	Pentacosene	Pentacosyne
26	Hexacosane	Hexacosene	Hexacosyne
27	Heptacosane	Heptacosene	Heptacosyne
28	Octacosane	Octacosene	Octacosyne
29	Nonacosane	Nonacosene	Nonacosyne
30	Triacontane	Triacontene	Triacontyne
31	Hentriacontane	Hentriacontene	Hentriacontyne
32	Dotriacontane	Dotriacontene	Dotriacontyne
33	Tritriacontane	Tritriacontene	Tritriacontyne
34	Tetratriacontane	Tetratriacontene	Tetratriacontyne
35	Pentatriacontane	Pentatriacontene	Pentatriacontyne
36	Hexatriacontane	Hexatriacontene	Hexatriacontyne
37	Heptatriacontane	Heptatriacontene	Heptatriacontyne
38	Octatriacontane	Octatriacontene	Octatriacontyne
39	Nonatriacontane	Nonatriacontene	Nonatriacontyne
40	Tetracontane	Tetracontene	Tetracontyne
41	Hentetracontane	Hentetracontene	Hentetracontyne
42	Dotetracontane	Dotetracontene	Dotetracontyne
43	Tritetracontane	Tritetracontene	Tritetracontyne
44	Tetratetracontane	Tetratetracontene	Tetratetracontyne
45	Pentatetracontane	Pentatetracontene	Pentatetracontyne
46	Hexatetracontane	Hexatetracontene	Hexatetracontyne
47	Heptatetracontane	Heptatetracontene	Heptatetracontyne
48	Octatetracontane	Octatetracontene	Octatetracontyne
49	Nonatetracontane	Nonatetracontene	Nonatetracontyne
50	Pentacontane	Pentacontene	Pentacontyne
60	Hexacontane	Hexacontene	Hexacontyne
70	Heptacontane	Heptacontene	Heptacontyne

[a] Formerly ethine, propine, and so forth; [b] Also called allylene; [c] Also called amylene; [d] Formerly undecane; [e] Also called cetane.

TABLE 1.3
Important Saturated Fatty Acids: Molecular Formulas and Trivial Names

Chemical Name	Formula	Abbreviation	Trivial Name
Methanoic	CHOOH	1:0	Formic
Ethanoic	CH_3–COOH	2:0	Acetic
Propanoic	$CH_3(CH_2)$ –COOH	3:0	Propionic
Butanoic	$CH_3(CH_2)_2$–COOH	4:0	Butyric
Pentanoic	$CH_3(CH_2)_3$–COOH	5:0	Valeric
Hexanoic	$CH_3(CH_2)_4$–COOH	6:0	Caproic
Heptanoic	$CH_3(CH_2)_5$–COOH	7:0	Enanthic
Octanoic	$CH_3(CH_2)_6$–COOH	8:0	Caprylic
Nonanoic	$CH_3(CH_2)_7$–COOH	9:0	Pelargonic
Decanoic	$CH_3(CH_2)_8$–COOH	10:0	Capric
Undecanoic	$CH_3(CH_2)_9$–COOH	11:0	
Dodecanoic	$CH_3(CH_2)_{10}$–COOH	12:0	Lauric
Tridecanoic	$CH_3(CH_2)_{11}$–COOH	13:0	
Tetradecanoic	$CH_3(CH_2)_{12}$–COOH	14:0	Myristic
Pentadecanoic	$CH_3(CH_2)_{13}$–COOH	15:0	
Hexadecanoic	$CH_3(CH_2)_{14}$–COOH	16:0	Palmitic
Heptadecanoic	$CH_3(CH_2)_{15}$–COOH	17:0	Margaric or daturic
Octadecanoic	$CH_3(CH_2)_{16}$–COOH	18:0	Stearic
Nonadecanoic	$CH_3(CH_2)_{17}$–COOH	19:0	
Eicosanoic	$CH_3(CH_2)_{18}$–COOH	20:0	Arachidic
Docosanoic	$CH_3(CH_2)_{20}$–COOH	22:0	Behenic
Tetracosanoic	$CH_3(CH_2)_{22}$–COOH	24:0	Lignoceric
Hexacosanoic	$CH_3(CH_2)_{24}$–COOH	26:0	Cerotic
Octacosanoic	$CH_3(CH_2)_{26}$–COOH	28:0	Montanic
Triacontanoic	$CH_3(CH_2)_{28}$–COOH	30:0	Melissic
Dotricosanoic	$CH_3(CH_2)_{30}$–COOH	32:0	Lacceroic or lacceric
Tritricosanoic	$CH_3(CH_2)_{31}$–COOH	33:0	Psyllic or ceromelissic
Tetratricosanoic	$CH_3(CH_2)_{32}$–COOH	34:0	Geddic or gheddic
Pentatricosanoic	$CH_3(CH_2)_{33}$–COOH	35:0	Ceroplastic

about two dozens are the sources for most of the fatty acids. The fatty acid composition of a variety of fats and oils in foods can be found from the chapters of this volume and other sources (Brignoli et al., 1976; Pryde, 1979; Smith, 1979).

Although the majority of natural saturated fatty acids have a straight chain, there are many that have a branched chain. The branched-chain saturated fatty acids have systematic names consisting of two parts; the terminal portion is the name of the longest straight chain present in the compound, that is, the parent chain, and preceding this are the names of side chains. Arabic numbers indicate the locations of the branching. Such prefixes, called *locants*, are listed in numerical sequence from the carboxyl carbon and are separated from each other by commas and from the remainder of the name by a hyphen. The entire name is written as one word; for example, 2,3-dimethyloctadecanoic acid. Branched-chain fatty acids can be further divided into the iso acids, in which the methyl group is in the penultimate, referred to as ω-1, position from the carboxyl group, and the anteiso acids, in which the methyl group is in the ω-2 position. The anteiso isomers are optically active. (*R*) and (*S*) are absolute-configuration labels assigned to any asymmetrical carbon atoms and are preferred to the L and D labels. (*R*) is derived from the Latin word *rectus*, meaning right, and (*S*) from the Latin word *sinister*, meaning left. It is to describe the spatial relationship among the groups around the

asymmetrical carbon (Cahn et al., 1956). The most common type of branch is a single methyl group, but sometimes there are longer branches or more than one branching methyl.

Iso and anteiso acids occur frequently but in small amounts in animal fats, waxes, and marine oils. For example, phytanic acid (3,7,11,15-tetramethylhexdecanoic acid) is an isoprenoid acid derived from the corresponding alcohol (phytol), and it is found in most land and marine fats. Also, phytenic acid is the corresponding C_{20} branched-chain acid with a double bond adjacent to the carboxyl group resulting from the corresponding alcohol phytol (Gunstone and Herslof, 1992). Although rare in plant lipids, branched-chain acids are major components of the lipids of gram-positive bacteria. More elaborate reviews of the branched-chain fatty acids are available (Shorland, 1956; Gensler, 1957; Hartman, 1957; Abrahamsson et al., 1964). Lipids of mycobacteria have also been found to contain a very complex mixture of branched-chain acids (Asselineau, 1966).

If a fatty acid contains two carboxyl groups, the suffix becomes -dioic; three carboxyls, -trioic; and so forth. The saturated dicarboxylic acids conform to the general formula $(CH_2)_x(COOH)_2$. This series of acids is frequently referred to as the oxalic acid series. Like the members of most other acid series, the various dioic fatty acids are best known by their trivial (common) names. In the present accepted method of nomenclature, the carbon atom of the carboxyl group is considered part of the chain, and the acids are named according to the number of carbon atoms in the hydrocarbon chain, that is, alkanedioic. Table 1.4 lists the chemical names, formulas, and common names for some of the more important saturated dicarboxylic acids. The lower molecular weight dioic acids are easily recognizable as fatty acid and tricarboxylic acid cycle intermediates involved in many of the biochemical pathways in metabolism. Of somewhat less frequent occurrence are the higher molecular weight dioic acids.

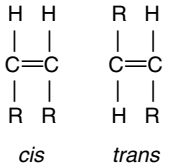
IV. UNSATURATED FATTY ACIDS

The unsaturated fatty acids may contain one or more double or triple bonds and so can be separated into monounsaturated, polyunsaturated, and acetylenic fatty acids. If an unsaturated fatty acid contains only double bonds, it is called an alkenoic, ethenoic, or olefinic acid. If it contains only triple bonds, it is called an alkynoic, ethynoic, or acetylenic acid. If the unsaturated fatty acid contains a number of double bonds in the hydrocarbon chain, the prefix di-, tri-, tetra-, and so forth, is inserted before "-enoic" to indicate the number of such double bonds (Table 1.1). An unsaturated fatty acid

TABLE 1.4
Chemical and Trivial Names of Dicarboxylic Acids

Chemical Name	Formula	Trivial Name
Ethanedioic	$(COOH)_2$	Oxalic
Propanedioic	$CH_2(COOH)_2$	Malonic
Butanedioic	$(CH_2)_2(COOH)_2$	Succinic
Pentanedioic	$(CH_2)_3(COOH)_2$	Glutaric
Hexanedioic	$(CH_2)_4(COOH)_2$	Adipic
Heptanedioic	$(CH_2)_5(COOH)_2$	Pimelic
Octanedioic	$(CH_2)_6(COOH)_2$	Suberic
Nonanedioic	$(CH_2)_7(COOH)_2$	Azelaic
Decanedioic	$(CH_2)_8(COOH)_2$	Sebacic
Tridecanedioic	$(CH_2)_{11}(COOH)_2$	Brassylic
Hexadecanedioic	$(CH_2)_{14}(COOH)_2$	Thapsic
Heneicosanedioic	$(CH_2)_{19}(COOH)_2$	Japanic

with a double bond can have two possible configurations, either *cis* or *trans*, depending on the relative positions of the alkyl groups:



Most naturally occurring unsaturated fatty acids have the *cis* orientation. With systematic nomenclature, the prefix *cis*- or *trans*- precedes the unsaturation position. Some naturally occurring acids have trivial names that are sufficiently specific. For example, oleic and elaidic refer to the *cis* and *trans* forms, respectively, of 9-octadecenoic acid. In other trivial names containing "-oleic" and "-elaidic," the former denotes a *cis* form and the latter a *trans* form. Thus, myristoleic refers to *cis*-9-tetradecenoic acid and by convention myristelaidic to *trans*-9-tetradecenoic acid.

More than 100 naturally occurring monounsaturated fatty acids have been identified, but most of these are very rare compounds. In general, these fatty acids have an even number of carbon atoms and the double bond has the *cis* conformation. Table 1.5 lists some of the more important monounsaturated fatty acids. Petroselinic acid (18:1Δ6c) is a positional isomer of oleic acid and occurs widely in seed oils of the order Umbellifere, usually along with oleic acid and other saturated and unsaturated fatty acids. Oleic acid is one of the most widely distributed fatty acids. It is a major component of safflower, pecan, pistachio, and macadamia nut oils and occurs in fish oils and animal fats (Holman, 1966). Some important oils containing erucic acid (22:1Δ13c) are rape, mustard, and *Crambe abyssinica*.

TABLE 1.5
Some Important Monounsaturated Acids

Systematic Name	Abbreviation	Trivial Name
9-Decenoic	10:1Δ9 (9-10:1)	Caproleic
cis-4-Dodecenoic	12:1Δ4c (4c-12:1)	Linderic
cis-9-Dodecenoic	12:1Δ9c (9c-10:1)	Lauroleic
4-Tetradecenoic	14:1Δ4 (4-14:1)	Obtusilic
cis-4-Tetradecenoic	14:1Δ4c (4c-14:1)	Tsuzuic
cis-5-Tetradecenoic	14:1Δ5c (5c-14:1)	Physeteric
cis-9-Tetradecenoic	14:1Δ9c (9c-14:1)	Myristoleic
cis-9-Hexadecenoic	16:1Δ9c (9c-16:1)	Palmitoleic
trans-9-Hexadecenoic	16:1Δ9t (9t-16:1)	Palmitelaidic
cis-6-Octadecenoic	18:1Δ6c (6c-18:1)	Petroselinic
cis-9-Octadecenoic	18:1Δ9c (9c-18:1)	Oleic
cis-11-Octadecenoic	18:1Δ11c (11c-18:1)	Asclepic or *cis*-vaccenic
trans-11-Octadecenoic	18:1Δ11t (11t-18:1)	Vaccenic
cis-9-Eicosenoic	20:1Δ9c (9c-20:1)	Gadoleic
trans-9-Eicosenoic	20:1Δ9t (9t-20:1)	Gadelaidic
cis-11-Docosenoic	22:1Δ11c (11c-22:1)	Cetoleic
cis-13-Docosenoic	22:1Δ13c (13c-22:1)	Erucic
trans-13-Docosenoic	22:1Δ13t (13t-22:1)	Brassidic
cis-15-Tetracosenoic	24:1Δ15c (15c-24:1)	Selacholeic or nervonic
cis-17-Hexacosenoic	26:1Δ17c (17c-26:1)	Ximenic
cis-21-Triacosenoic	30:1Δ21c (21c-30:1)	Lumequic

Although most natural sources of unsaturated fats are of the *cis* configuration, *trans* fatty acids do occur in foods. Unsaturated fats are hydrogenated to produce solid fats for margarine and shortening product. Hydrogenation also improves the oxidative and flavor stability of the oils. However, this process can move double bonds from their naturally occurring positions and convert configurations from *cis* to *trans*, creating both positional and geometrical isomers. See Emken (1984) and Emken and Dutton (1979) for a review of geometrical and positional fatty acid isomers.

Polyunsaturated fatty acids, sometimes referred to as PUFAs or polyalkenoic acids, can be divided into a number of categories depending on the relative positions of the double bonds. When double-bonded carbon atoms alternate with single bonds, that is, (–C=C–C=C–), the acid is referred to as *conjugated*. If the double bonds are separated by one or more carbon atoms with only single bonds (–C=C–C, –C=C–), the acid is said to be *unconjugated*. Unconjugated fatty acids usually occur in a methylene-interrupted arrangement. A third group, frequently described as nonmethylene-interrupted dienes, has double bonds that are not entirely in a methylene-interrupted arrangement. If a single carbon atom has two double bonds, that is, (–C=C=C–), then it is called *allenic*. These acids are chiral by virtue of their allenic group. Examples are laballenic acid [$H_3C(CH_2)_{10}CH{=}C{=}CH(CH_2)_3COOH$] and lamenallenic acid [$H_3CCH{=}CH(CH_2)_8CH{=}C{=}CH(CH_2)_3COOH$] (Gunstone and Herslof, 1992).

The most common conjugated polyunsaturated acids are trienes such as octadecatrienoic acids, of which seven isomers occur naturally. Conjugated dienes do exist—for example, 2,4-hexadienoic or sorbic acid and *trans*-2-*cis*-4-decadeinoic, which is a known flavor component in the Bartlett pear (Jennings et al., 1964)—but do not occur extensively. Seven conjugated octadecatrienoic acids have been identified as natural compounds, including three 8,10,12-trienes and four 9,11,13-trienes. Systematic and trivial names and some indication of occurrence are given in Table 1.6. These acids are all believed to arise from linoleic acid (18:2Δ9,12) by oxidation and dehydration mechanisms. Reviews have been published by Smith (1971, 1979), Pohl and Wagner (1972), Hopkins (1972), Pryde (1979), and Badami and Patil (1980). Conjugated PUFAs are in limited abundance in animal fats and occur extensively in only a few seed oils.

The unconjugated and methylene-interrupted PUFAs are probably the most important PUFAs in terms of extent of occurrence. These acids have been arranged into families based on the number of carbons on which the methylene-interrupted double bonds begin. The acids are categorized into n-1 through n-12 families. These symbols indicate the carbon number counting from the methyl end. Formerly, the terms ω1 to ω12 were used. The most important families are the n-3, n-6, and n-9 acids. Monounsaturated acids can be included in this family system, with oleic being an important

TABLE 1.6
Natural Octadecatrienoic and Octadecatetraenoic Acids

Systematic Name	Trivial Name	Typical Source
Trienes		
8c,10t,12c-Octadecatrienoic	Jacaric	*Jacaranda minosifolia*
8t,10t,12c-Octadecatrienoic	Calendic	*Calendula officinalis*
8t,10t,12t-Octadecatrienoic		*Calendula officinalis*
9c,11t,13c-Octadecatrienoic	Catalpic	*Catalpa ovata*
9c,11t,13t-Octadecatrienoic	α-Eleostearic	Tung oil
9t,11t,13c-Octadecatrienoic	Punicic	*Punica granatum*
9t,11t,13t-Octadecatrienoic	β-Eleostearic	
Tetraenes		
9c,11t,13t,15c-Octadecatetraenoic	α-Parinaric	*Impatiens balsamina*
9t,11t,13t,15t-Octadecatetraenoic	β-Parinaric	

TABLE 1.7
Methylene-Interrupted Unsaturated Acids Arranged by Family

Family	Systematic Name	Abbreviation	Trivial Name
n-3	9,12,15-Octadecatrienoic	18:3(n-3)	α-Linolenic
	6,9,12,15-Octadecatetraenoic	18:4(n-3)	Stearidonic or moroctic
	5,8,11,14,17-Eicosapentaenoic	20:5(n-3)	
	4,7,10,13,16,19-Docosahexaenoic	22:6(n-3)	
	6,9,12,15,18,21-Tetracosahexanenoic	24:6(n-3)	Nisinic
n-6	9,12-Octadecadienoic	18:2(n-6)	Linoleic
	6,9,12-Octadecatroenoic	18:3(n-6)	γ-Linolenic
	8,11,14-Eicosatrienoic	20:3(n-6)	Dihomo-γ-linolenic
	5,8,11,14-Eicosatetraenoic	20:4(n-6)	Arachidonic
	7,10,13,16-Docosatetraenoic	22:4(n-6)	Adrenic
n-7	9-Hexadecenoic	16:1(n-7)	Palmitoleic
	11-Octadecenoic	18:1(n-7)	Vaccenic
n-9	9-Octadecenoic	18:1(n-9)	Oleic
	15-Tetracosenoic	24:1(n-9)	Nervonic or selacholeic
	5,8,11-Eicosatrienic	20:3(n-9)	Mead's

member of the n-9 family. Table 1.7 lists some of the more important natural fatty acids arranged by families. The acids within each family are biosynthetically related, being interconverted by enzymatic processes of desaturation, chain elongation, and chain shortening. One of the most familiar and widespread fatty acids is linoleic, which is also nutritionally essential. Natural linoleic acid is predominately the *cis–cis* isomer. It occurs in almost every vegetable fat, with rich amounts in corn, cottonseed, safflower, sunflower, and soybean oils. It is present in animal fats and fish oils at much lower levels. An important n-3 fatty acid is α-linolenic. This fatty acid is a major component of linseed oil and is valuable in paints because of its drying properties. Linolenic acid is the major fatty acid of plant leaves, stems, and roots, and is also a significant component of many photosynthetic organisms (Hilditch and Williams, 1964). The other two important n-3 acids are eicosapentaenoic acid and docosa-hexaenoic acid, which are primarily found in fish oils and marine algae. The C_{20} acid is also a precursor of some prostaglandins and thromboxanes. Arachidonic acid, an n-6 acid, is best known as a precursor to the prostaglandins, thromboxanes, and leukotrienes. It is present in animal fats and at lower levels in many fish oils.

There are also a number of PUFAs in which the unsaturation is not completely methylene interrupted. These acids are thought to occur by insertion of an additional double bond into the more common poly- and monounsaturated compounds. These fatty acids are seen in some seed oils, certain microorganisms, and marine lipids. Table 1.8 lists natural nonmethylene-interrupted PUFAs.

The acetylenic acids are composed of a series of straight-chain carboxylic acids characterized by the presence of one or more triple carbon–carbon bonds (–C≡C–) in the hydrocarbon chain. They are therefore conveniently divided into those with only one triple bond and those with more than one. In the former group of acids, one or more double bonds can occur, and unsaturation frequently appears in the conventional methylene-interrupted pattern. Alkenoic acids containing a triple bond must be named with the positions of the double and triple bonds in mind; for example, 11-octadecene-9-ynoic is an 18-carbon fatty acid with the double bond at position 11 and the triple bond at position 9. In general, most natural acetylenic acids are C_{18} compounds with conjugated unsaturation involving one or more acetylenic groups along with olefinic unsaturation (usually at C-9), which may be *cis* or *trans*. Table 1.9 lists some of the more common acetylenic acids. Acetylenic acids are rarely found in naturally occurring fats and oils; they are seen only in a number of rare seed oils and in some mosses. The first acetylenic compound identified in nature was the 18-carbon tariric acid found by

TABLE 1.8
Natural Nonmethylene-Interrupted Polyunsaturated Acids

Systematic Name	Abbreviation
5,9-Octadecadienoic	18:2Δ5,9
5,11-Octadecadienoic	18:2Δ5,11
2t,9,12-Octadecatrienoic	18:3Δ2t,9,12
3t,9,12-Octadecatrienoic	18:3Δ3t,9,12
5t,9,12-Octadecatrienoic	18:3Δ5t,9,12
5,9,12-Octadecatrienoic	18:3Δ5,9,12
5,11,14-Octadecatrienoic	18:3Δ5,11,14
3t,9,12,15-Octadecatetraenoic	18:3Δ3t,9,12,15
5,9,12,15-Octadecatetraenoic	18:3Δ5,9,12,15
5,11-Eicodadienoic	20:2Δ5,11
5,13-Eicosadienoic	20:2Δ5,13
7,11-Eicosadienoic	20:2Δ7,11
7,13-Eicosadienoic	20:2Δ7,13
5,11,14-Eicosatrienoic	20:3Δ5,11,14
7,11,14-Eicosatrienoic	20:3Δ7,11,14
5,11,14,17-Eicosatetraenoic	20:4Δ5,11,14,17
5,11-Docosadienoic	22:2Δ5,11
5,13-Docosadienoic	22:2Δ5,13
7,13-Docosadienoic	22:2Δ7,13
7,15-Docosadienoic	22:2Δ7,15
7,17-Docosadienoic	22:2Δ7,17
9,13-Docosadienoic	22:2Δ9,13
9,15-Docopadienoic	22:2Δ9,15

TABLE 1.9
Acetylenic Acids

Systematic Name	Abbreviation[a]	Trivial Name
10t-Heptadecene-8-ynoic	17:2Δ8a,10t	Pyrulilc
6-Octadecynoic	18:1Δ6a	Tariric
9-Octadecynoic	18:1Δ9a	Stearolic
11t-Octadecene-9-ynoic	18:2Δ9a,11t	Ximenynic or santalbic
8-Hydroxy-11t-octadecene-9-ynoic	18:2Δ9a,11t-8OH	Ximenynolic
17c-Octadecene-9,11-diynoic	18:3Δ,9a,11a,17c	Isanic or erythrogenic
8-Hydroxy-17c-Octadecene-9,11-diynoic	18:3Δ9a,11a,17c-8OH	Isanolic
9c-Octadecene-12-ynoic	18:2Δ9c,12a	Crepenynic
13t-Octadecene-9,11-diynoic	18:3Δ13t,9a,11a	Exocarpic

[a]The presence of "a" in the abbreviation denotes the triple bond.

Arnaud in a fatty oil in 1892. Compounds up to the pentyne stage have been isolated, but from triyne onwards some of the fatty acids become increasingly unstable and even explosive. Bohlmann et al. (1973) described crepenynic (18:2Δ9c12a), isolated from the seed oil of *Crepis foetida*, as one of the most important monoacetylenic acids. See Meade (1957) for a review on the naturally occurring acetylenic acids.

V. OXYGENATED FATTY ACIDS

The naturally occurring oxygenated fatty acids include the hydroxyl, keto, and epoxy groups, of which the hydroxy-substituted acids are most common. As mentioned previously, if one or more hydrogen atoms of the hydrocarbon chain are replaced with some other atom or group, such as a hydroxyl or keto, the acid may retain its specific name and be prefixed by the name and position of the substituent atom or group, such as 2-hydroxybutanoic acid and 9,10-dihydroxyoctadecanoic acid.

The hydroxy fatty acids are composed of a series of straight-chain carboxylic acids that contain one or more hydroxyl groups substituted on the hydrocarbon portion of the molecule. Since the hydroxy fatty acids possess at least one asymmetrical carbon atom, they are capable of being resolved into their optical isomers. Most of the naturally occurring hydroxy acids are optically active. The hydroxy fatty acids may be saturated, or they may contain one or more unsaturated bonds in the hydrocarbon chain. Those hydroxy acids that contain an ethylenic bond exhibit geometrical isomerism and can be obtained in either the *cis* or *trans* form. Natural monohydroxy acids that are either saturated or contain unconjugated unsaturation are listed in Table 1.10. The hydroxy position precedes the name of the substituted hydrocarbon chain and is followed with any stereochemical designations. Ricinoleic acid (12-hydroxyoleic) is the best known, being the major acid in castor oil. In all of these 18- and 20-carbon acids, the hydroxy groups are in the 3 and/or 4 positions from the methyl end. Hydroxy acids with conjugated unsaturation (*cis* and/or *trans* olefinic and/or acetylenic) are listed in Table 1.11.

TABLE 1.10
Hydroxy Acids without Conjugated Unsaturation

Systematic Name	Abbreviation	Trivial Name
12-Hydroxydodecanoic	12:0-12OH	Sabinic
6-Hydroxytetradecanoic	14:0-6OH	Butolic
11-Hydroxytetradecanoic	14:0-11OH	Convolvulinic
11-Hydroxyhexadecanoic	16:0-11OH	Jalapinolic
16-Hydroxyhexadecanoic	16:0-16OH	Juniperic
16-Hydroxy-7c-hexadecanoic	16:1Δ7c-16OH	Ambrettolic
12**D**(R)-Hydroxy-9c-octadecanoic	18:1Δ9c-12OH	Ricinoleic
9**D**(S)-Hydroxy-12c-octadecanoic	18:1Δ12c-9OH	Isoricinoleic
12**D**(R)-Hydroxy-9c,15c-octadecanoic	18:2Δ9c,15c-12OH	Densipolic
14**D**(R)-Hydroxy-11c-eicosanoic	20:1Δ11c-14OH	Lesquerolic
14-Hydroxy-11c,17c-eicosadienoic	20:2Δ11c,17c-14OH	Auricolic
22-Hydroxydocosanoic	22:0-22OH	Phellonic

TABLE 1.11
Hydroxy Acid with Conjugated Unsaturation

Systematic Name	Abbreviation	Trivial Name
8-Hydroxy-11t-octadecene-9-ynoic	18:2Δ9a,11t-8OH	Ximenynolic
8-Hydroxy-17c-octadecene-9,11-diynoic	18:3Δ9a,11a,17c-8OH	Isanolic
9-Hydroxy-10t,12t-octadecadeinoic	18:2Δ10t,12t-9OH	Dimorphecolic
9-Hydroxy-10t,12c-octadecadeinoic	18:2Δ10t,12c-9OH	Helenynolic
13-Hydroxy-9c,11t-octadecadienoic	18:2Δ9c,11t-13OH	Coriolic or artemesic
18-Hydroxy-9c,11t,13t-octadecatrienoic	18:3Δ9c,11t,13t-18OH	Kamlolenic

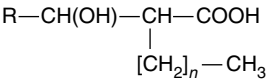
TABLE 1.12
Some Common Polyhydroxy Fatty Acids

Systematic Name	Abbreviation	Trivial Name
3,11-Dihydroxytetradecaenoic	14:0-3,11-diOH	Ipurolic
threo-12,13-Dihydroxyhexadecanoic	16:0-12,13-diOH	Dihydroxystearic
2,15,16-Trihydroxyhexadecanoic	16:0-2,15,16-triOH	Ustilic
9,10,16-Trihydroxyhexadecanoic	16:0-9,10,16-triOH	Aleuritic
threo-9,10-Dihydroxyoctadecanoic	18:0-9,10-diOH	
threo-12,13-Dihydroxy-9c-octadecenoic	18:1Δ9c-12,13-diOH	Dihydroxyoleic

The polyhydroxy fatty acids are also a distinctive group that range in chain length from C_{14} to C_{24}. The acids with 2–5 hydroxy groups are usually saturated, and the C_{16} and C_{18} acids are the most common. Several long-chain polyhydroxy acids that contain adjacent hydroxy groups produce asymmetrical centers and have been isolated from natural sources (Hilditch and Williams, 1964). The prefixes *cis*- and *trans*- used by many investigations in naming these open-chain compounds should be replaced by *threo*- and *erythro*-. By definition, *threo* compounds result by *trans* addition to a *cis*-ethylenic compound or by *cis* addition to a *trans*-ethylenic compound, and the *erythro* isomers are the products of *cis* addition to a *cis*-ethylenic or *trans* addition to a *trans*-ethylenic compound. Table 1.12 lists some of the most common polyhydroxy fatty acids.

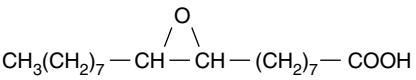
Natural hydroxy acids do not occur extensively, although they are very important industrial sources of synthetic oils. They occur in sphingolipids, stillingia oil, leaf waxes, and fungal and bacterial lipids. Unsaturated oils often contain small amounts of hydroxy and epoxy acids after prolonged storage, probably as a result of oxidation. See Downing (1961) for a review of the naturally occurring aliphatic hydroxy acids found in animals, plants, and microorganisms.

Mycolic acids are a series of very complex hydroxy fatty acids with high molecular weights. These acids occur in a wide range of microorganisms. They are generally 2-alkyl-3-hydroxy acids with the structure shown below.



These fatty acids may contain up to 80 carbons and may even contain one or more cyclopropane units (Polgar, 1971).

The epoxy fatty acids are characterized by the presence of a cyclic bond between two carbons in the chain and one oxygen atom. The epoxy carbons are counted in the longest hydrocarbon chain, and the acids are named accordingly. The name of the acid is prefixed by the two positions of the epoxy carbons attached to the prefix "epoxy." Epoxy carbons also allow *cis* or *trans* conformations to occur, and this is also designated; for example, *cis*-9,10-epoxyoctadecanoic acid describes an 18-carbon acid with an epoxy group at positions 9 and 10 of the *cis* configuration.

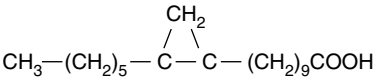


The epoxy fatty acids are another group of acids known to develop in some seed oils after prolonged storage (Gunstone et al., 1986). Natural epoxy acids occur in seed oils as triacylglycerols and in cutins as polymers of hydroxy acids. The best known and most widely occurring of the natural epoxy acids is vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecenoic), which was first investigated as the characteristic epoxy acid of *Vernonia anthelmintica*. Coronaric acid (*cis*-9,10-epoxy-*cis*-12-octadecenoic), a positional isomer of vernolic acids, is the second most common epoxy acid, and sometimes the two acids occur together.

The keto fatty acids comprise a group of straight-chain carboxylic acids that contain one or more carbonyl groups in the hydrocarbon portion of the molecule. The hydrocarbon chain may be either saturated or unsaturated. The naming of keto acids is similar to that of the hydroxy acids, with the keto position prefixing the fatty acid; for example, licanic acid or 4-keto-9,11,13-octadecatrienoic (18:3Δ4-oxo-9c, 11t, 13t) and lactarinic acid (6-oxo-octadecanoic acid). The properties of specific keto acids depend on the position of the keto group relative to the carboxyl group. Keto acids are rarely found in naturally occurring fats and oils.

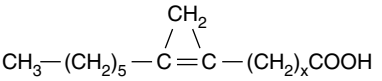
TABLE 1.13
Cyclic Fatty Acids

1. The cyclopropanes



10-(2-Hexyl-cyclopropanyl)decanoic, 11,12-methyleneoctadecanoic (lactobacillic or phytomonic)

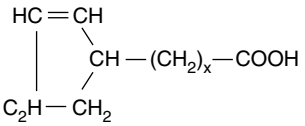
2. The cyclopropenes



x	Cyclopropene	Trivial Name
7	8-(2-Octyl-cyclopropenyl)octanoic	Sterculic
6	7-(2-Octyl-cyclopropenyl)heptanoic	Malvalic

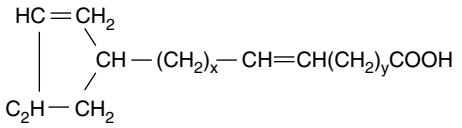
3. The cyclopentenes

a. Monoene acid



x	Cyclopropene	Trivial Name
0	3-(2-Cyclopentenyl)methanoic	Aleprolic
2	3-(2-Cyclopentenyl)propanoic	Alepramic
4	5-(2-Cyclopentenyl)pentanoic	Aleprestic
6	7-(2-Cyclopentenyl)heptanoic	Aleprylic
8	9-(2-Cyclopentenyl)nonanoic	Alepric
10	11-(2-Cyclopentenyl)undecanoic	Hydnocarpic
12	13-(2-Cyclopentenyl)tridecanoic	Chaulmoogric
14	15-(2-Cyclopentenyl)pentadecanoic	Hormelic

b. Diene acids



x	*y*	Cyclopentene	Trivial Name
4	4	11-(2-Cyclpentenyl)-4-undecenoic	Manaoic
6	4	13(2-Cyclopentenyl)-6-tridecenoic	Gorlic
6	6	15-(2-Cyclopentenyl)-6-pentadecenoic	Oncobic

VI. CYCLIC FATTY ACIDS

The cyclic fatty acids may contain a cyclic unit with three (cyclopropane and cyclopropene acids), five (prostaglandins and cyclopentene acids), or even six carbon atoms (cyclohexane acids). Most of these acids are known by their trivial names. The systematic name is derived again from the hydrocarbon chain including the carboxyl but not the cyclic structure. The cyclic structure and any R groups prefix the fatty acid name, with their position designated; for example, 8(-2octyl-cyclopropenyl) octanoic describes an eight-carbon fatty acid attached with a terminal cyclopropene that contains an eight-carbon alkyl group. Table 1.13 lists some of the more common cyclic fatty acids with their structures.

The cyclopropane fatty acids occur frequently in bacterial membrane phospholipids. They also generally accompany the cyclopropene acids in seed oils. Although other chain lengths have been reported, the most common cyclopropane acids are the *cis* 17-carbon and *cis* 19-carbon (lactobacillic acid) compounds. The cyclopropene acids have been identified in seed oil triacylglycerols. They are found mainly among Malvales, especially Sterculiaceae, Malvalaceae, Bombaceae, and Tiliaceae. In addition to the seeds, the cyclic acids are found in leaves, stems, and roots and are present mainly as glycerides. See Badami and Patil (1980), Christie (1970), and Lie Ken Jie (1979) for information with respect to these fatty acids.

VII. CONCLUSION

This chapter describes a number of naming systems, some of which are more popular than others. The IUPAC system for naming chemical compounds is the most comprehensive for naming fatty acids. This system permits the unambiguous naming of all the fatty acids. The abbreviated nomenclature, with carboxyl-reference or n-/ω-reference, found in the tables is aimed to provide a coherent way to substitute the trivial names or chemical names. The abbreviated terminology for naming fatty acids, when standardized, will be of great value in presentation and written formats for both laymen and professionals.

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2 Chemical and Physical Properties of Fatty Acids

John M. deMan

CONTENTS

I. INTRODUCTION

Fatty acids in foods occur mainly in the form of esters with the alcohol glycerol. The predominant esters are triacylglycerols accompanied by minor amounts of mono- and diacylglycerols and free fatty acids. In fact, most fats and oils can be classified as mixtures of mixed glycerides. To understand the chemical and physical properties of these food fats and oils, we have to go back to the properties of the component fatty acids. However, many of the properties of food fats and oils are not simply a reflection of the properties of the component fatty acids but are also influenced by the nature of their incorporation into the acylglycerols. In this chapter, therefore, both the properties of fatty acids and their behavior in the form of acylglycerols will be covered.

II. REACTIVITY OF SATURATED AND UNSATURATED FATTY ACIDS

Saturated fatty acids, being composed of paraffinic hydrocarbon chains, are generally considered to have a very low reactivity. Introduction of one or more double bonds into a fatty acid provides an active center that can be the site of a variety of reactions—undesirable, as in the case of oxidation, or desirable for industrial purposes, such as hydrogenation, or for analytical purposes, such as reactions with halogens or mercuric compounds.

The relative stability of oxidation at 100°C has been given by Pardun (1976) as follows: saturated fatty acids 0.8, oleic acid 1.1, linoleic acid 13.7, and linolenic acid 25.5. Since fatty acids occur in food fats and oils as mixtures of mixed glycerides, the interactions between these different fatty acids must be taken into account. Raghuveer and Hammond (1967) studied the rate of autoxidation of mixtures of triunsaturated glycerides and tridecanoin and found that randomization with sodium methoxide decreased the rate of autoxidation. They suggested that the concentration of unsaturated fatty acids in the 2-position of the glycerides stabilizes a fat toward autoxidation.

Autoxidation of unsaturated fatty acids involves attack on the α-methylene group, resulting initially in a number of unsaturated hydroperoxides. Saturated fatty acid oxidation occurs at high temperatures (100°C–120°C) and is mainly a β-oxidation. Stearic acid yields lower fatty acids with an even number of carbon atoms and in addition small amounts of oxalic acid and methyl ketones with odd carbon numbers. The latter results from decarboxylation of β-keto acids. Even the position of double bonds in a polyunsaturated fatty acid may affect its oxidation rate. Zhang and Chen (1998) found that conjugated linoleic acid oxidized considerably faster than linoleic acid.

III. AUTOXIDATION

The unsaturated bonds present in all fats and oils represent active sites that can react with oxygen. This reaction leads to the formation of primary, secondary, and tertiary oxidation products that may make the fat or fat-containing food unsuitable for consumption.

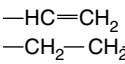
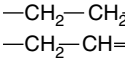
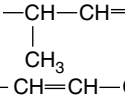
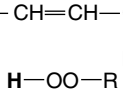
The process of autoxidation and the resulting determination in the flavor of fats and fatty foods are often described by the term *rancidity*. Lundberg (1961) distinguishes several types of rancidity. Common oxidative rancidity can be seen in fats such as lard and is characterized by sweet but undesirable odor and flavor that progressively become more intense and unpleasant as the reaction progresses. *Flavor reversion* is the term used for the objectionable flavors that develop in oils containing linolenic acid such as soybean oil. This type of oxidation is produced with considerably less oxygen than common oxidation.

Among the factors that affect the rate of autoxidation are the amount of oxygen present, degree of unsaturation, presence of antioxidants, presence of prooxidants (especially copper and some organic compounds such as heme-containing molecules and lipoxidase), storage temperature, and exposure to light. The autoxidation reaction can be divided into three stages: initiation, propagation, and termination. In the initiation part, hydrogen is abstracted from an olefinic compound to yield a free radical:

$$RH \rightarrow R^{\bullet} + H^{\bullet}$$

The removal of hydrogen takes place at the carbon atom next to the double bond and can be brought about by the action of, for instance, light or metals. The dissociation energy of hydrogen in various olefinic compounds has been given by Ohloff (1973) and is listed in Table 2.1. Once a free radical has been formed, it will combine with oxygen to form a peroxy free radical, which can in turn abstract hydrogen from another unsaturated molecule to yield a peroxide and a new free radical,

TABLE 2.1
Dissociation Energy for the Abstraction of Hydrogen from Olefinic Compounds and Peroxides

Compound	ΔE (kcal/mol)
	103
	100
	85
	77
	65
	90

Source: Ohloff, G. (1973). Fats as precursors. In *Functional Properties of Fats in Foods* (J. Solms, ed.), Forster Publishing, Zurich.

thus starting the propagation reaction. This reaction may be repeated up to several thousand times and has the nature of a chain reaction.

$$R^{\bullet} + O_2 \rightarrow RO_2^{g}$$
$$RO_2^{g} + RH \rightarrow ROOH + R$$

The propagation can be followed by termination if the free radicals react with themselves to yield nonactive products, as shown here:

$$R^{\bullet} + R^{\bullet} \rightarrow R{-}R$$
$$R^{\bullet} + RO_2^{\bullet} \rightarrow RO_2R$$
$$nRO_2^{\bullet} \rightarrow (RO_2)n$$

The hydroperoxides formed in the propagation part of the reaction are the primary oxidation products. The hydroperoxide mechanism of autoxidation was first proposed by Farmer (1946). These oxidation products are generally unstable and decompose into the secondary oxidation products, which comprise a variety of compounds, including carbonyls, which are the most important. The peroxides have no importance to flavor deterioration, which is wholly caused by the secondary oxidation products. The nature of the process can be represented by the curves of Figure 2.1 (Pokorny, 1971). In the initial stages of the reaction, the amount of hydroperoxides increases slowly; this stage is termed the *induction period*. At the end of the induction period, there is a sudden increase in peroxide content. As peroxides are easily determined in fats, the peroxide value is frequently used to measure the progress of oxidation. Organoleptic changes are more closely related to the secondary oxidation products, which can be measured by various means, including the benzidine value, which is related to aldehyde decomposition products. As the aldehydes are themselves oxidized, fatty acids are formed; these free fatty acids can be considered tertiary oxidation products. The length of the induction period, therefore, depends on the method used to determine oxidation products.

Although even saturated fatty acids can be oxidized, the rate of oxidation greatly depends on the degree of unsaturation. In the series of 18-carbon-atom fatty acids—18:0, 18:1, 18:2, 18:3—the relative rate of oxidation has been reported to be in the ratio of 1:100:1200:2500. The reaction of

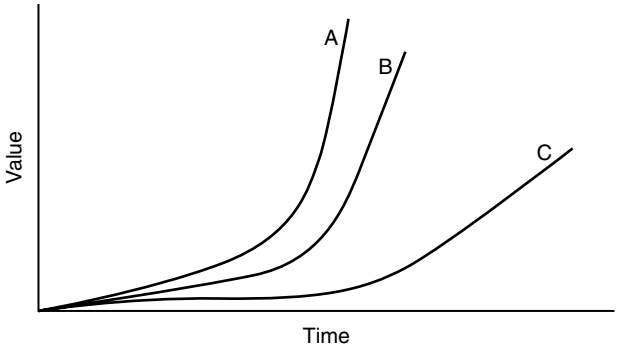
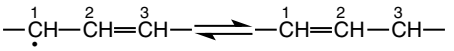
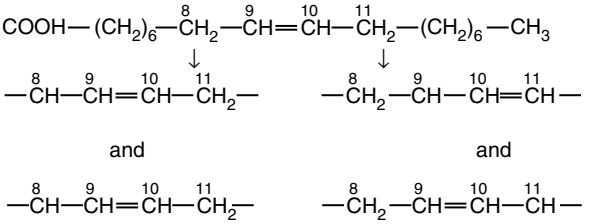


FIGURE 2.1 Autoxidation of lard. A, peroxide value; B, benzidine value; and C, acid value. (From Pokorny, J. (1971). *Can. Inst. Food Sci. Technol. J. 4*: 68–74.)

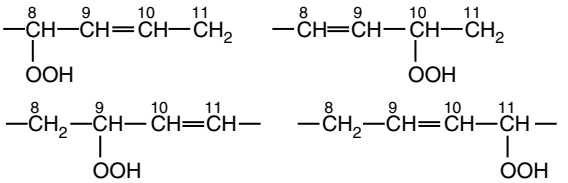
unsaturated compounds proceeds by the abstraction of hydrogen from the α-carbon, and the resulting free radical is stabilized by resonance as follows:



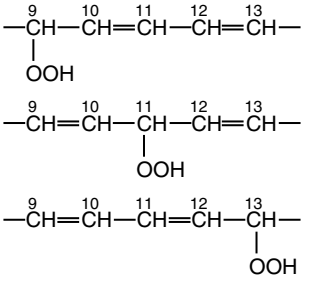
If oleic acid is taken as an example of monoethenoid compound (*cis*-9-octadecenoic acid), the reaction will proceed by abstraction of hydrogen from carbons 8 or 11, resulting in two pairs of resonance hybrids.



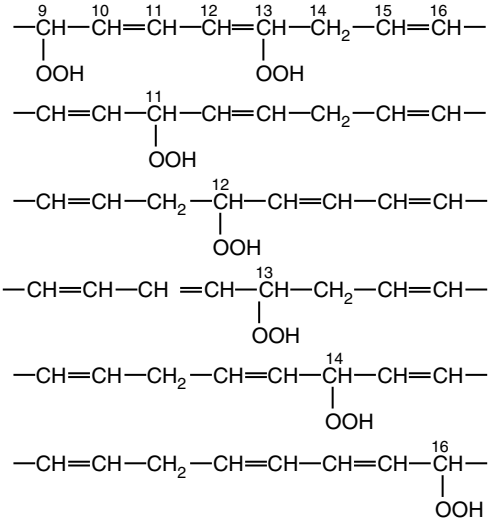
This leads to the formation of four isomeric hydroperoxides:



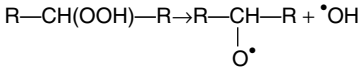
In addition to the changes in double-bond position, there is isomerization from *cis* to *trans*, and 90% of the peroxides formed may be in the *trans* configuration (Lundberg, 1961). From linoleic acid (*cis–cis*-9,12-octadeadienoic acid), three isomeric hydroperoxides can be formed:



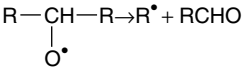
In this mixture of 9-, 11-, and 13-hydroperoxides, the conjugated ones occur in greater quantity because they are the more stable forms. The hydroperoxides occur in the *cis–trans* and *trans–trans* configurations, the content of the latter being greater with higher temperature and greater extent of oxidation. From the oxidation of linolenic acid (*cis–cis–cis*-9,12,15-octadecatrienoic acid), six isomeric hydroperoxides can be expected according to theory:



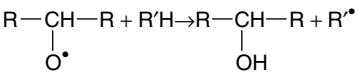
Hydroperoxides of linolenate decompose more readily than those of oleate and linoleate, because active methylene groups are present. The active methylene groups are the ones located between a single double bond and a conjugated diene group. The hydrogen at this methylene group could readily be abstracted to form dihydroperoxides. The possibilities here for decomposition products are obviously more abundant than with oleate oxidation. The decomposition of hydroperoxides has been outlined by Keeney (1962). The first step involves decomposition to the alkoxy and hydroxy free radicals:



The alkoxy radical can react to form aldehydes:



This reaction involves fission of the chain and can occur on either side of the free radical. The aldehyde that is formed can be a short-chain volatile compound, or it can be attached to the glyceride part of the molecule; in the latter case, the compound is nonvolatile. The volatile aldehydes are in great part responsible for the oxidized flavor of fats. The alkoxy radical may also abstract a hydrogen atom from another molecule to yield an alcohol and a new free radical:



The new free radicals formed may participate in propagation of the chain reaction. Some of the free radicals may interact with themselves to terminate the chain, and this could lead to the formation of ketones as follows:

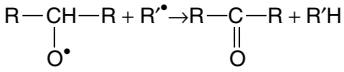


TABLE 2.2
Hydroperoxides and Aldehydes (with Single Oxygen Function) That May Be Formed in Autoxidation of Some Unsaturated Fatty Acids[a]

Fatty Acid	Methylene Group Involved	Isomeric Hydroperoxides Formed from the Structures Contributing to the Intermediate Free Radical Resonance Hybrid	Aldehydes Formed by Decomposition of the Hydroperoxides
Oleic	11	11-Hydroperoxy-9-ene	Octanal
		9-Hydroperoxy-10-ene	2-Decenal
	8	8-Hydroperoxy-9-ene	2-Undecenal
		10-Hydroperoxy-8-ene	Nonanal
Linoleic	11	13-Hydroperoxy-9,11-diene	Hexanal
		11-Hydroperoxy-9,12-diene	2-Octenal
		9-Hydroperoxy-10,12-diene	2,4-Decadienal
Linolenic	14	16-Hydroperoxy-9,12,14-triene	Propanal
		14-Hydroperoxy-9,12,15-triene	2-Pentenal
		12-Hydroperoxy-9,13,15-triene	2,4-Heptadienal
	11	13-Hydroperoxy-9,11,15-triene	3-Hexenal
		11-Hydroperoxy-9,12,15-triene	2,5-Octadienal
		9-Hydroperoxy-10,12,15-triene	2,4,7-Decatrienal
Arachidonic	13	15-Hydroperoxy-5,8,11,13-tetraene	Hexanal
		13-Hydroperoxy-5,8,11,14-tetraene	2-Octenal
		11-Hydroperoxy-5,8,12,14-tetraene	2,4-Decadienal
	10	12-Hydroperoxy-5,8,10,14-tetraene	3-Nonenal
		10-Hydroperoxy-5,8,11,14-tetraene	2,5-Undecadienal
		8-Hydroperoxy-5,9,11,14-tetraene	2,4,7-Tridecatrienal
	7	9-Hydroperoxy-5,7,11,14-tetraene	3,6-Dodecadienal
		7-Hydroperoxy-5,8,11,14-tetraene	2,5,8-Tetradecatrienal
		5-Hydroperoxy-6,8,11,14-tetraene	2,4,7,10-Hexadecatetraenal

[a] Only the most active methylene groups in each acid are considered.

Source: Keeney, M. (1962). Secondary degradation products, in *Lipids and Their Oxidation* (H.W. Schultz, E.A. Day, and R.O. Sinnhuber, eds.), AVI Publishing, Westport, CT.

As indicated, a variety of aldehydes have been demonstrated in oxidized fats. Alcohols have also been identified, but the presence of ketones is not identified. Keeney (1962) listed the aldehydes that may be formed from breakdown of hydroperoxides of oxidized oleic, linoleic, linolenic, and arachidonic acids (Table 2.2). The aldehydes are powerful flavor compounds and have very low flavor thresholds; for example, 2,4-decadienal has a flavor threshold of less than 1 ppb. The presence of a double bond in an aldehyde generally lowers the flavor threshold considerably. The aldehydes can be further oxidized to carboxylic acids or other tertiary oxidation products.

When chain fission of the alkoxy radical occurs on the other side of the free radical group, the reaction will not yield volatile aldehydes but will instead form nonvolatile aldehydoglycerides. Volatile oxidation products can be removed in the refining process during deodorization, but the nonvolatile products remain, and this could result in lower oxidative stability of oils that were already oxidized before refining.

IV. PHOTOOXIDATION

In addition to the free radical process of autoxidation of lipids, oxidation may be brought about by photooxidation. Light-induced oxidation, or photooxidation, results from the reactivity of an excited state of oxygen, known as singlet oxygen ($^1O_2$). Normal or ground-state oxygen is triplet

oxygen ($^3O_2$). The activation energy for the reaction of normal oxygen with an unsaturated fatty acid is very high: in the range of 146–273 kJ/mol. When normal oxygen converts to the singlet state, energy is taken up amounting to 92 kJ/mol. This conversion makes the oxygen much more reactive. Production of singlet oxygen requires the presence of a sensitizer. The sensitizer is activated by light and can react directly with the substrate (type I sensitizer) or activate oxygen to the singlet state (type II sensitizer). The wavelength of the light can be from the visible or ultraviolet (UV) spectrum. With both type I and type II sensitizers, unsaturated fatty acids are converted into hydroperoxides according to the following equations:

$$^3\text{Sens}^* + {}^3O_2 \rightarrow {}^1O_2 + {}^1\text{Sen}$$

$$^3O_2 + \text{RH} \rightarrow \text{ROOH}$$

Singlet oxygen is short-lived and reverts back to the ground state with the emission of light. This light is fluorescent, meaning its wavelength is higher than that of the light originally absorbed. The reactivity of single oxygen is 1500 times greater than that of normal oxygen. Compounds that can act as sensitizers are widely occurring food components, including chlorophyll, myoglobin, riboflavin, and heavy metals. These sensitizers are activated by light and then produce singlet oxygen. The singlet oxygen reacts directly with the double bond by addition and causes the double bond to shift away by one carbon atom. Singlet oxygen attack on linoleate produces four hydroperoxides. Photooxidation has no induction period, but the reaction can be stopped by compounds known as quenchers. These quenchers compete for the singlet oxygen and return it to the ground state. Carotenoids are widely occurring quenchers. Rahmani and Saari Csallany (1998) reported that in the photooxidation of virgin olive oil, pheophytin A functioned as sensitizer while β-carotene acted as quencher. Ascorbyl palmitate has been described as a quencher in the photosensitized oxidation of oils (Lee et al., 1997).

Food containing sensitizers should be protected from light exposure during storage and handling. One of the important characteristics of singlet oxygen oxidation is that phenolic antioxidants do not provide protection against this reaction (Yasaei et al., 1996).

V. THERMAL OXIDATION

The fatty acids in food lipids are exposed to heat during processing and also during cooking, baking, frying, and other treatments of foods. Great care is taken to minimize thermal oxidation of fats and oils during processing; for example, by applying vacuum to remove oxygen. Heating during processing mainly involves hydrogenation, physical refining, and deodorization. The temperatures used in these processes may range from 120°C to 270°C. At the high temperatures used in physical refining and deodorization, several chemical changes may take place. These include randomization of the glyceride structure, dimer formation, *cis–trans* isomerization, and formation of conjugated fatty acids (Hoffmann, 1989). The *trans*-isomer formation during high temperature deodorization has been described by Ackman (1994).

Conditions favoring breakdown of oils or fats exist during frying, whether practiced in the home, in food service, or in commercial frying operations. Frying temperatures are in the range of 160°C–195°C. Deep frying is a complex process involving both the oil and the food to be fried. Steam is given off and this removes volatile antioxidants, free fatty acids, and other volatiles. Contact with air leads to autoxidation and the formation of a large number of degradation products. The presence of steam results in hydrolysis with the production of free fatty acids and partial glycerides. Oxidation can result in the formation of oxidized monomeric, dimeric, and oligomeric triglycerides, as well as volatile compounds, including aldehydes, ketones, alcohols, and hydrocarbons. In addition, oxidized sterols may be formed. The polymerization reaction may take place by conversion of part of the *cis–cis*-1,4-diene system of linoleates to the *trans–trans* conjugated 1,3-diene. The 1,4- and

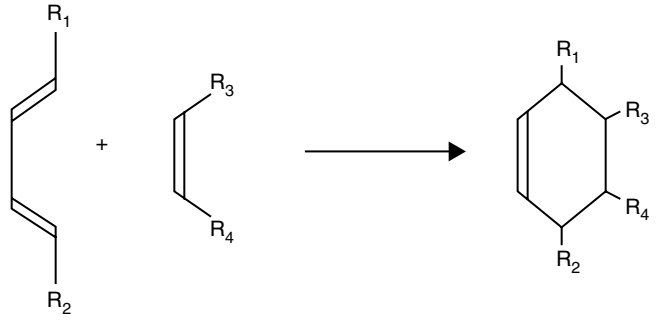


FIGURE 2.2 Dimer production of 1,4- and 1,3-dienes.

1,3-dienes can combine in a Diels–Alder-type addition reaction to produce a dimer (Figure 2.2). From these dimers, higher oligomers can be produced, the structure of which is relatively unknown.

Another class of compounds formed during frying is that of cyclic monomers of fatty acids. Linolenic acid can react at either the C9 or the C12 double bonds to give rings between carbons 5 and 9, 5 and 10, 8 and 12, 12 and 17, and 13 and 17. Cyclic monomers with a cyclopentenyl ring have been isolated from heated sunflower oil (Le Quéré and Sébédio, 1996). The loss of polyunsaturated fatty aids during deep frying has been reported by Tyagi and Vasishtha (1996).

VI. ENZYMATIC OXIDATION

Enzymatic oxidation reactions are important in both animal and plant systems. In animal systems, they involve mainly the oxidative transformation of arachidonic acid to prostaglandins, thromboxanes, and leukotrienes. These compounds are formed in all mammalian tissues and have a broad range of biological activities. The first product formed from arachidonic acid is prostaglandin G_2 (PGG_2), which contains hydroperoxide and cyclic peroxide functions. The enzyme involved in this reaction is cyclooxygenase. The 5-hydroperoxide reacts further to form leukotrienes. PGG_2 is the precursor of prostaglandins and thromboxanes. The 5-hydroperoxide and the 5,6-epxide are precursors of the leukotrienes (Gunstone, 1986).

Enzymatic oxidation in plant systems is mediated by a widely occurring group of enzymes that use molecular oxygen to catalyze the oxidation of lipids containing a *cis–cis*-1,4-pentadiene group. This reaction leads to the formation of conjugated hydroperoxides. The best known of these enzymes is soy lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12).

The major source of lipoxygenase is in legumes, soybeans, other beans, and peas. It is present in lesser amounts in peanuts, wheat, potatoes, and radishes. Lipoxygenase is a metalloprotein with an iron atom in its active center. Two types of lipoxygenase occur in plants: type I lipoxygenase peroxidizes only free fatty acids with a high stero- and regioselectivity; and type II, which is less specific for free linoleic acid, acts as a general autoxidation catalyst. Type I reacts only on free fatty acids after they have been released from triacylglycerols by lipase. Type II will act directly on triacylglycerols.

The mechanism of lipoxygenase activity has been reviewed by Gardener (1996). The enzyme has been reported (Georgalaki et al., 1998) as a component in virgin olive oil. The reaction is highly specific for the *cis–cis* methylene-interrupted group and forms the basis for an analytical method for measuring essential fatty acids. The reaction is shown in Figure 2.3 (Chism, 1985).

VII. HYDROGENATION

Hydrogenation of fats is a chemical reaction consisting of the addition of hydrogen at double bonds of unsaturated acyl groups. This reaction is of great importance to industry, because it permits the

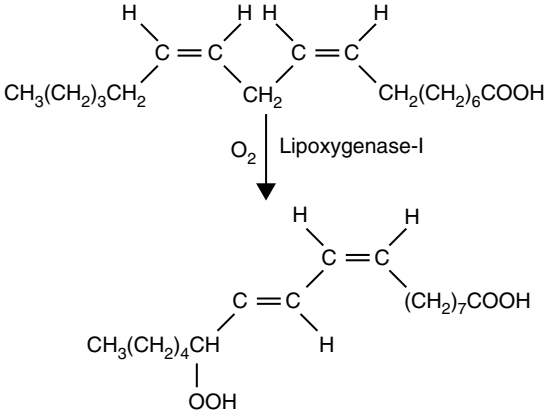
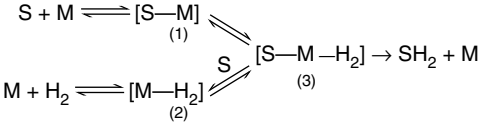


FIGURE 2.3 Lipoxygenase-catalyzed reaction of *cis–cis* methylene-interrupted compounds.

conversion of liquid oils into plastic fats for the production of margarine and shortening. For some oils, the process also results in a decreased susceptibility to oxidative deterioration. In the hydrogenation reaction, gaseous hydrogen, liquid oil, and solid catalyst participate under agitation in a closed vessel. Although most industrial processes use solid nickel catalysts, interest in organometallic compounds that serve as homogeneous catalysts has increased greatly. Frankel and Dutton (1970) represented catalytic hydrogenation by the following scheme, in which the reaching species are the olefinic substrate (S), the metal catalyst (M), and H_2:



Intermediates 1, 2, and 3 are organometallic species. If the reaction involves heterogeneous catalysis, the olefins and hydrogen are bound to the metal by chemisorption. If homogeneous catalysis takes place, the intermediates are organometallic complexes. The intermediates are liable and short-lived and cannot usually be isolated. In heterogeneous catalysis, the surface of the metal performs the function of catalyst, and the preparation of the catalyst is of major importance. When hydrogen is added to double bonds in a natural fat consisting of many component glycerides and component unsaturated fatty acids, the result depends on many factors if the reaction is not carried to completion. Generally, hydrogenation of fats is not carried to completion, and fats are hydrogenated only partially. Under these conditions, hydrogenation may be selective or nonselective. "Selective" means that hydrogen is added first to the most unsaturated fatty acids. Selectivity is increased by increasing the hydrogenation temperature and decreased by increasing pressure and agitation. Table 2.3 shows the effect of selectivity on the properties of soybean oil. The selectively hydrogenated oil is more resistant to oxidation because of the preferential hydrogenation of the linolenic acid. The influence of selectivity conditions on the fatty acids of hydrogenated cottonseed and peanut oils is demonstrated by the data presented in Table 2.4.

Another important factor in hydrogenation is the formation of positional and geometrical isomers. Formation of *trans*-isomers is rapid and extensive. The isomerization can be understood by the reversible character of chemisorption. When the olefinic bond reacts, two carbon-metal bonds are formed as an intermediate stage (represented by an asterisk in Figure 2.4). The intermediate may react with an atom of adsorbed hydrogen to yield "half-hydrogenated" compound, which remains attached by only one bond. Additional reaction with hydrogen results in the formation of the

TABLE 2.3
Differences in Selective and Nonselective Hydrogenation of Soybean Oil

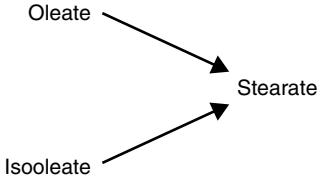
	Selective	Nonselective
Induction period, A.O.M. (h)	240	31
Micropenetration	70 (more plastic)	30
Capillary mp (°C)	39	55
Condition		
Temperature (°C)	177	121
Pressure (psi)	5	50
Ni catalyst (%)	0.05	0.05

Source: Lundberg, W.O. (1961). *Autoxidation and Antioxidants*, Wiley, New York.

TABLE 2.4
Fatty Acid Composition of Cottonseed and Peanut Oils Hydrogenated Under Different Conditions of Selectivity to Iodine Value 65

Oil	Hydrogenation Conditions	Fatty Acids		
		Saturated (%)	Oleic (%)	Linoleic (%)
Cottonseed	Moderately selective	31.5	64.5	4.0
Peanut	Moderately selective	27.5	72.5	—
Cottonseed	Nonselective	36.0	56.0	8.0
Peanut	Nonselective	30.0	67.0	3.0
Cottonseed	Very nonselective	39.5	48.5	12.0
Peanut	Very nonselective	33.0	61.0	6.0

saturated compound. There is also the possibility that the half-hydrogenated olefin may again attach itself to the catalyst surface at a carbon on either side of the existing bond, with simultaneous loss of hydrogen. Upon desorption of this species, a positional or geometrical isomer may result. The proportion of *trans* acids is high, because this is the more stable configuration. Double-bond migration occurs in both directions but probably more extensively in the direction away from the ester group. The hydrogenation of oleate can be represented as follows:



The change from oleate to isooleate involves no change in unsaturation but does result in a considerably higher melting point. Hydrogenation of linoleate first produces some conjugated

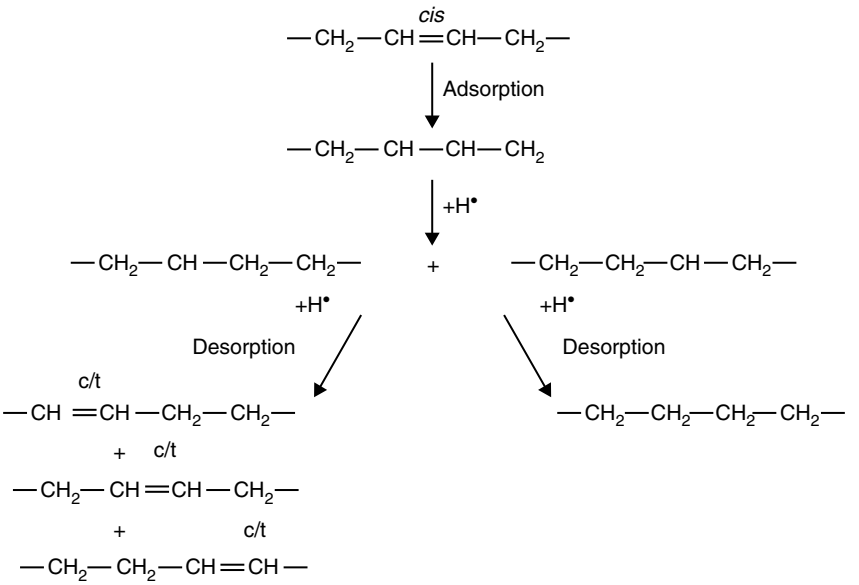
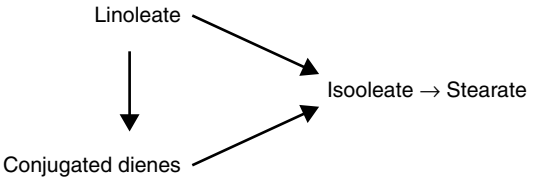
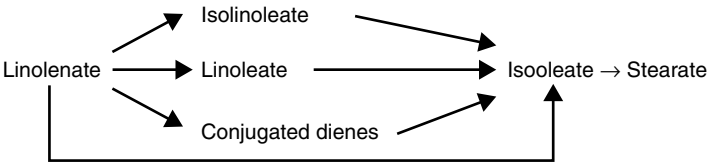


FIGURE 2.4 Hydrogenation of an olefinic compound.

dienes, followed by the formation of positional and geometrical isomers of oleic acid and finally stearate.



Hydrogenation of linolenate is more complex and is greatly dependent on reaction conditions. The reactions can be summarized as follows:



During hydrogenation, solid isomers of oleic acids are formed by partial hydrogenation of polyunsaturated acid groups or by isomerization of oleic acid. This has an important effect on the consistency of partially hydrogenated oils. For example, compare olive oil, which has an iodine value of 80 and is liquid at room temperature, with soybean hydrogenated to the same iodine value, which is a fat with a consistency similar to that of lard.

It is difficult to eliminate oxidation-sensitive polyunsaturated fatty acids by partial hydrogenation of fish oils. This has been demonstrated by Ackman (1973) in the progressive hydrogenation of anchovetta oil. The original eicosapentaenoic acid (20:5ω3) is not completely removed until an iodine value of 107.5 is reached. Even at this point, there are other polyunsaturated fatty acids present that may be susceptible to flavor reversion. In the nonselective hydrogenation of typical seed oils, polyunsaturated fatty acids are rapidly reduced and *trans*-isomer levels increase to high values; Figure 2.5 shows the hydrogenation of canola oil (deMan et al., 1982).

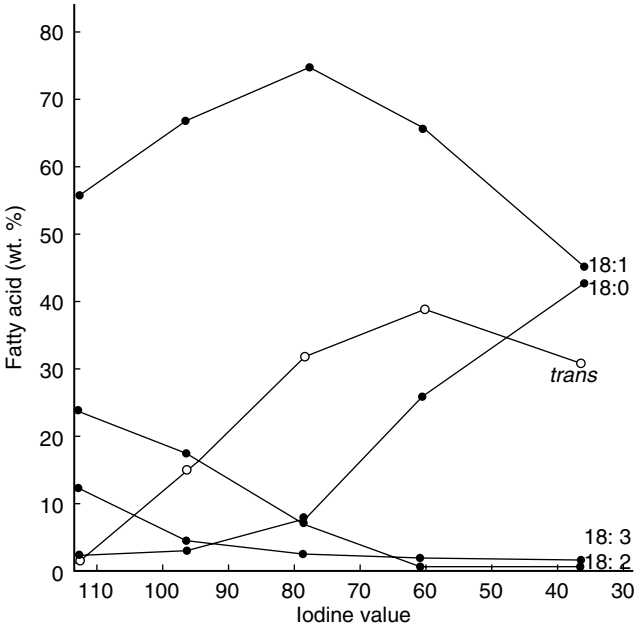


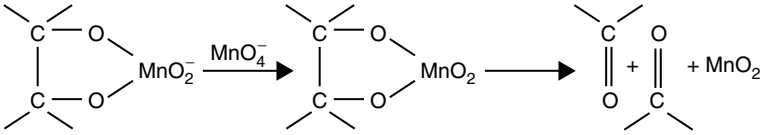
FIGURE 2.5 Change in fatty acid composition during hydrogenation of canola oil. (From deMan, J.M. et al. (1982). *Chem. Microbiol. Technol. Lebensm. 7*: 117–124.)

VIII. HALOGENATION

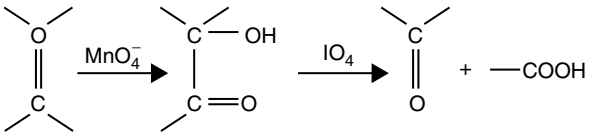
The reaction of halogens with unsaturated fatty acids is important, because it forms the basis for the measurement of the iodine value of fats and oils. This has traditionally served as a measure of total unsaturation. In part, this has been superseded by the gas–liquid chromatographic analysis of fatty acid composition. Chlorine, bromine, iodine monochloride, and iodine monobromide add to the double bonds of unsaturated fatty acids. The most widely used method, known as the Wijs method, employs iodine monochloride. The Hanus method employs iodine monobromide. The addition reaction can be speeded up greatly by using mercuric acetate as a catalyst. Conjugated fatty acids react only incompletely with halogens. Bromination of polyunsaturated fatty acids yields 9,10,12,13-tetrabromostearic acid (mp 116°C) from linoleic acid and 9,10,12,13,15,16-hexabromostearic acid (mp 186°C) from linolenic acid. These tetra- and hexabromides are insoluble in cold ether and petroleum ether. Brominated vegetable oils are used as clouding agents in soft drinks. Other uses for halogenated fatty compounds are as intermediates in the preparation of other industrial chemicals.

IX. OXIDATIVE FISSION

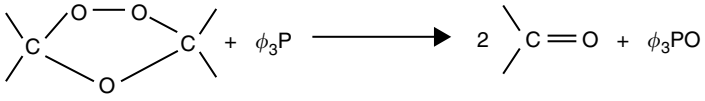
Fatty acid chains can be cleaved at the location of a double bond by two procedures: permanganate oxidation or ozonolysis. Both methods are used to determine the structure of unsaturated fatty acids.The reaction of olefinic compounds with $KMnO_4$ under neutral conditions results in carbon–carbon bond cleavage. The first product formed is a cyclic ester intermediate, which is oxidized to a neutral ester, that in turn is cleaved to two aldehydes. This is followed by oxidation to the corresponding acids.



An improvement to this procedure was suggested by von Rudloff (1956). In this reaction, a catalytic amount of permanganate is used with an excess of periodate. The permanganate oxidizes the olefin to a glycol or ketol. The periodate affects both the cleavage of the intermediate and the regeneration of the permanganate.



The yield of the expected dicarboxylic acid from the oxidation of unsaturated acids has been reported to be within 2% of the expected value. Ozone can add to the double bond to form a primary ozonide. On oxidation, the ozonolysis products give carboxylic acids and/or ketones. Mild reduction yields aldehydes and/or ketones. Reduction can result in clean oxidation products that can be used directly for analysis by gas–liquid chromatography. In the usual procedure, the ozonized sample is reacted with triphenylphosphine. The phosphine reduces the ozonide and is itself oxidized to triphenylphosphine oxide.



X. ESTER INTERCHANGE

It is possible to change the position of fatty acid radicals on the glycerides in a fat by the process known as interesterification, randomization, or ester interchange. This is because, in the presence of certain catalysts, the fatty acid radicals can be made to move between hydroxyl positions so that an essentially random fatty acid distribution results, according to the reaction pattern (Formo, 1954).

The reaction is used in industry to modify the crystallization behavior and physical properties of fats. The catalysts are usually alkaline and consist of sodium methoxide or alloys of sodium and potassium. Several procedures are available. A fat may be randomized by carrying out the reaction at temperatures above its melting point, several raw materials may be interesterified together so that a new product results, or a fat may be interesterified below its melting point so that only the liquid fraction reacts (this is called *directed interesterification*). The effect of randomization can be shown, for example, by considering the simple mixture of equal quantities of triolein and tristearin. After randomization, six possible triglycerides are formed in quantities that can be calculated (Figure 2.6). Industrially, interesterification is applied to lard. Lard has some undesirable physical properties: It has a narrow plastic range, creams poorly, and gives poor cake volume. Interesterification results in considerable improvement in these properties so that interesterified lard can be used as a shortening. Natural fats can be interesterified with simple glyceryl esters such as acetates and butyrates to give new mixed glycerides. Acetoglycerides prepared in this manner are translucent, waxy materials that can be used as edible protective coatings and plasticizers.

Interesterification may provide an alternative to hydrogenation for the production of margarine and shortening fats. Hydrogenation has the disadvantages of forming *trans*-isomers and losing essential fatty acids. Interesterification of liquid oils with highly saturated fats (obtained by complete

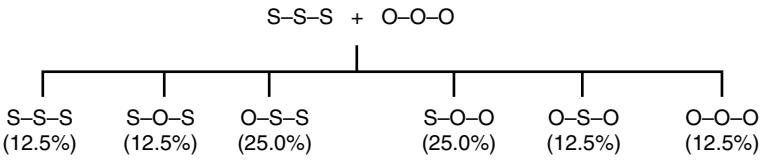


FIGURE 2.6 Interesterification of a mixture of equal quantities of triolein and tristearin.

hydrogenation or fractionation) may yield suitable fats with little or no *trans*-isomers and high levels of essential fatty acids.

Ester interchange of fats with a large excess of glycerol at high temperature, under vacuum, and in the presence of a catalyst results in an equilibrium mixture of mono-, di-, and triglycerides. After removal of excess glycerol, the mixture is called technical monoglyceride and contains about 40% 1-monoglyceride. Technical monoglycerides are used as emulsifying agents in foods. Molecular distillation yields products with well over 90% 1-monoglycerides; the distilled monoglycerides are also widely used in foods.

Interesterification is being adopted as an alternative to hydrogenation for the production of low-*trans* or *trans*-free margarines and shortenings. The physical and chemical properties of *trans*-free fats produced by chemical interesterification of vegetable oil blends have been described by Petrauskaite et al. (1998). Interesterification is used for producing commercial margarines and shortenings (Allen, 1998) and also for preparing structured lipids. These materials are triglycerides that contain mixtures of either short- or medium-chain fatty acids and long-chain fatty acids in the same glyceride molecule (Fomuso and Akoh, 1997). They can be tailor-made for specific disease or metabolic conditions and also serve as reduced- or low-calorie fats. To produce structured lipids, position-specific lipase catalysts are used to obtain the desired glyceride structure. Rodriguez et al. (2001) have manufactured a shortening using chemical interesterification of tallow-sunflower oil blends to replace fish oil in the formulation.

XI. HYDROLYSIS

In the presence of water, fatty acid esters can be hydrolyzed to free acids and alcohol. In industrial fat splitting, this reaction is carried out under conditions of high temperature and high pressure. Hydrolysis is catalyzed by acids. Lipolytic enzymes rapidly break down triglycerides under appropriate conditions. This occurs frequently in oilseeds and palm fruit during processing. Limiting the action of lipases is important, because removal of free fatty acids reduces the yield of neutral oil.

It is known that the lipases will promote the reverse of lipolysis, that is, esterification, under conditions of limited availability of water. These reactions include both esterification and transesterification (Lazar, 1985).

The catalytic activity of lipases is reversible and depends on the water content in the reaction mixture. At high water content, the hydrolytic reaction prevails, whereas at low water content, the synthetic reaction is favored. The lipases that have received attention for their ability to synthesize ester bonds have been obtained from yeasts, bacteria, and fungi. Lipases can be classified into three groups based on their specificity (Macrae, 1983). The first group contains nonspecific lipases; these show no specificity regarding the position of the ester bond in the glycerol molecule or the nature of the fatty acid. Examples of enzymes in this group are lipases of *Candida cylindracea*, *Corynebacterium acnes*, and *Staphylococcus aureus*. The second group contains lipases with position specificity for the 1- and 3-positions of the glycerides. 1,3 Specificity is common among microbial lipases. This positional specificity is the result of the inability of the sterically hindered ester bond of the 2-positon of glycerol to enter the active site of the enzyme. Examples of lipases in this group are obtained from *Aspergillus niger*, *Mucor javanicus*, and *Rhizopus arrhizus*. The third group of lipases shows specificity for particular fatty acids. As an example, the lipase from *Geotrichum candidum* has a marked specificity for long-chain fatty acids containing a *cis* double bond in the 9-position.

XII. MELTING AND CRYSTALLIZATION

The economic importance of the distinctive melting and solidification behavior of fatty acids and their esters was well described by Bailey (1950). Fatty acids and their derivatives constitute an almost infinite variety of long-chain compounds differing in carbon chain length, unsaturation, and isomerism, resulting in a complex and fascinating gamut of physical properties.

The transformation from the liquid to the solid state is accompanied by release of heat (latent heat of crystallization signifying an exothermic reaction); the reverse, transformation from solid to liquid, is accompanied by a negative heat effect (endothermic reaction). This forms the basis for the widely used technique of differential scanning calorimetry (DSC).

Another important phenomenon is melting expansion. Conversion from the solid state to the liquid state results in a melting expansion that is added to the normal thermal expansion. This phenomenon constitutes the basis for the determination of the solid fat index by dilatometry. Protons in the solid state of a fat behave differently from those in the liquid state when subjected to radiofrequency energy when the sample is contained in a magnetic field. This serves as the basis for the determination of the solid fat content in a product by wide-line or pulsed-nuclear magnetic resonance.

The internal structure of solid fats in the crystalline state is fairly well-known. Crystals are closely packed systems of molecules or atoms with a regular three-dimensional repeating order. The molecules or atoms are held together by strong forces, and crystals have well-defined spacings between repeating groups. These short and long spacings are characteristic of different fat crystals.

Although it can be assumed that the internal structure of liquids is represented by molecules in complete disorder, there is evidence that a limited degree of order does exist in liquids. According to Bailey (1950), long-chain compounds in the liquid state contain limited areas of orderly packing. However, these regions of orderly packing extend only over a very limited number of molecules, whereas in a crystal the lattice arrangement extends throughout the crystal. Bailey speaks of the quasicrystalline character of liquids as "short range order" and of crystals as "long range order."

Studies by Hernqvist (1984) shed light on the structure of triglycerides in the liquid state and how this affects crystallization. On the basis of x-ray diffraction and Raman spectroscopy studies, he suggested a gradual decrease in size with increasing temperature of the melt (Figure 2.7). The order in the melt is constant even at 40°C above the melting point. The order is related to chain length; a long chain is more disordered at the methyl end-group plane than a short one. When the temperature is decreased, the lamellar units increase in size until crystallization occurs (Figure 2.8).

Substances consisting of identical molecules have a well-defined sharp melting point. Complications arise in long-chain compounds such as fatty acids and their esters because of polymorphism and because fats and oils are mixtures of mixed glycerides. The molecules in these compounds, although of the same chemical structure, differ in chain length, unsaturation, and isomerism. Each component in these products has its own melting point. Fats, therefore, have a melting range. What is commonly known as the melting point of a fat is in reality the end of the melting range (deMan et al., 1983). There are a variety of methods for determining the melting points of fats, and the results may differ depending on the method chosen. Some of the better known methods are the capillary tube, slip point, Wiley melting point, and Mettler dropping point methods. It is also possible to determine the end of the melting range from a DSC heating curve.

Melting of a fat is an instantaneous reaction, whereas crystallization is usually a slow process. The driving force in crystallization is the degree of supercooling. Crystallization consists of two

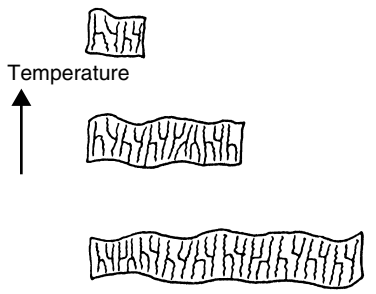


FIGURE 2.7 Reduced size of lamellar units with temperature. (From Hernqvist, L. (1984). *Fette Seifen Anstrichm. 86:* 297–300.)

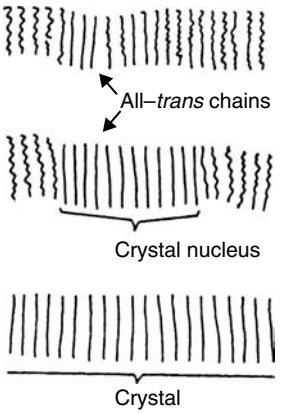


FIGURE 2.8 Proposed mechanism for the crystallization of a triglyceride melt. (From Hernqvist, L. (1984). *Fette Seifen Anstrichm*. *86*: 297–300.)

phases: nucleation and crystal growth. A high degree of supercooling will be conducive to nucleation, and many small crystals will be formed. At temperatures closer to the crystallization point, crystal growth will be favored and large crystals will be formed. Another result of a high degree of supercooling is the formation of mixed crystals, also known as solid solutions. Molecules with a range of melting points may crystallize together. As a result, rapidly cooled fats may have higher solid fat content than the same fats that cooled more gradually. These mixed crystals will partially melt when the fat is subjected to temperature variations below its melting point, a phenomenon known as tempering (Moziar et al., 1989).

Svenstrup et al. (2005) have shown that the number of melting points and the melting temperature are correlated with the cooling rate for pork fat, lard, and leaf fat in three different products: extracted fat, raw fat, and fat as an ingredient in liver pate, a rapid cooling leads to lowering of the melting point, assigned to the presence of unstable β' crystals, and that the melting points vary with the treatment of the fat. The findings suggest that the fraction of unsaturated fatty acids present in the fat is important for both crystallization rate and melting points of α and β' crystals in extracted lard, and is less pronounced in liver pate because of the presence of diverse components such as proteins. The identification of the various levels of structure present in fat crystal networks, and the development of analytical techniques to quantify these levels have been reviewed by Narine and Marangoni (1999, 2002). The types, formulations, functionality, and processing required for the production of lipid-shortening systems, as well as their crystallization, structural elucidation, and mechanical modeling of fat crystal networks have been reviewed by Ghotra et al. (2002). Also, Humphrey and Narine (2004) have compared the lipid-shortening functionality as a function of molecular ensemble and shear: crystallization and melting.

XIII. FRACTIONATION

Fats can be separated into fractions with different physical characteristics by fractional crystallization from a solvent or by fractionation from the melt. The former process gives sharply defined fractions but is only used for the production of high-value fats, and the latter process is much simpler and cost effective. Fractionation from the melt or dry fractionation is carried out on a very large scale with palm oil and also other fats, including beef tallow, lard, and milk fat.

There are several reasons for employing fractional crystallization (Hamm, 1995):

1. To remove small quantities of high-melting components that might result in cloudiness of oil. This can be either a triacylglycerol fraction or nontriacylglycerol compound. The

former happens when soybean oil is lightly hydrogenated to convert it to higher stability oil. The resulting solid triglycerides have to be removed to yield a clear oil. The latter occurs when waxes crystallize from oils such as sunflower oil. This type of fractionation is known as winterization.

2. To change a fat or oil into two or more fractions with different melting characteristics. In simple dry fractionation, a hard fraction, or stearin, and a liquid fraction, or olein, is obtained. This is by far the most common application of fraction.
3. To produce well-defined fractions with special physical properties that can be used as specialty fats or confectionery fats, often done by solvent fractionation.

The process of fractionation involves the controlled and limited crystallization of a melted fat. By careful management of the rate of cooling and the intensity of agitation, it is possible to produce a slurry of relatively large crystals that can be separated from the remaining liquid oil by filtration. By far the major application of fractionation is with palm oil.

The fractionation of palm oil can be carried out in a number of ways to yield a variety of products. In a multistage process, a palm mid fraction is obtained, which can be further fractionated to yield a cocoa butter equivalent (Kellens, 1994). Palm oil can be double fractionated to yield a so-called super olein with an iodine value of 65.

Milk fat fractionation has been described by Deffense (1993). By combining multistep fractionation and blending, it is possible to produce modified milk fats with improved functional properties. The use of anhydrous milk fat fractions in milk chocolate formulations has been described by Full et al. (1996). Dimick et al. (1996) reported the chemical and thermal characteristics of milk fat fractions isolated by a melt crystallization. Methods for determining thermal fat crystal properties for the fractionation of milk fat were developed by Breitschuh and Windhab (1996).

XIV. POLYMORPHISM

Polymorphism is the ability of long-chain compounds such as fatty acids and their esters to exist in more than one crystal form, and this results from different patterns of molecular packing in the crystals. Triglycerides may occur in three main forms, namely, α, β′, and β in order of increasing stability and melting point. When fats are cooled, crystals of a lower melting form may be produced. These may change slowly or rapidly into a more stable form. The change is monotropic, that is, it always proceeds from lower to higher stability. Polymorphism results in the phenomenon of multiple melting points. When a fat is crystallized in an unstable form and heated to a temperature slightly above its melting point, it may resolidify into a more stable form. This is demonstrated by the melting points of the polymorphic forms of triglycerides containing palmitic and stearic acids (Table 2.5).

TABLE 2.5
Melting Points of the Polymorphic Forms of Tristearin (SSS), 2-Palmitoyldistearin (SPS), and 2-Stearoyldipalmitin (PSP)

	Melting Point (°C)		
Form	**SSS**	**SPS**	**PSP**
α	54.7	51.8	47[a]
β′	64.0	69.0[b]	69.0
β	73.3	68.5	65.5[b]

[a] Softening point.
[b] Obtained with difficulty.

Source: Lutton (1972).

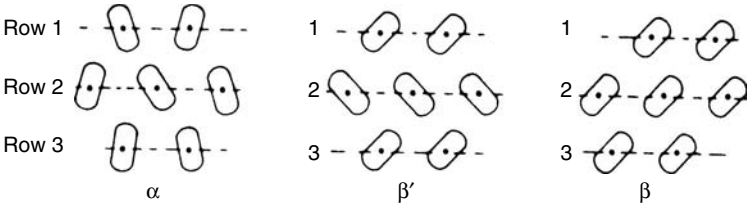


FIGURE 2.9 Cross-sectional structures of long-chain compounds. (From Lutton, 1972.)

The cross-sectional structures of long-chain compounds in the different polymorphic forms are shown in Figure 2.9. In the α-form, the chain axes are randomly oriented—the crystal is of the hexagonal type. In the β′-form, alternate rows are oriented in opposite directions and the crystal type is orthorhombic. In the β-form, the rows are oriented in the same direction and the crystal type is triclinic. The polymorphic forms are also distinguished by differences in the angle of tilt of the unit cell in the crystal. X-ray diffraction analysis enables measurement of the short spacings and long spacings of the unit cell.

The short spacing measured by x-ray diffraction can be used to characterize the polymorphic forms. Principal short spacings are as follows: α, 0.415 nm; β′, 0.42 and 0.38 nm; β, 0.46, 0.385, and 0.37 nm. Forms with identical x-ray patterns but with different melting points are described by a letter subscript, for example, α_1 and α_2.

The polymorphic behavior of cocoa butter is more complex than that of most other fats. There are six distinct polymorphic modifications that are usually designated by roman numerals as forms I to VI in the order of increasing melting point. The desirable form in chocolate is form V, which is a β modification. The polymorphism of cocoa butter has been studied by van Malssen et al. (1996) and Loisel et al. (1998). Narine and Humphrey (2004) have compared lipid-shortening functionality, including microstructure, polymorphism, solid fat content, and texture, as a function of molecular ensemble and shear.

XV. THERMAL PROPERTIES

The specific heat of a substance is the amount of heat required to increase the temperature of 1 g of material by 1°C. The specific heats of the solid and liquid states of fatty compounds are different. The specific heat of the liquid state is always higher. The transformation of solid to liquid releases the latent heat of crystallization. Transformation of a lower to a higher polymorphic form is also an exothermic reaction. The heat content curve of a fat covering the solid, melting, and liquid states is given in a schematic form in Figure 2.10 (Bailey, 1950). The specific heat and latent heat of fusion of saturated fatty acids are given in Table 2.6.

There is a similarity between heat effects and volume changes, as was pointed out by Bailey (1950). Calorimetric curves such as the one in Figure 2.10 represent heat content plotted against temperature. Dilatometric curves are plots of specific volume against temperature. Both methods can be used to establish the relative amounts of solid and liquid in a plastic fat. Newer methods have superseded the calorimetric and dilatometric procedures. DSC is now a widely used thermal method. A sample is subjected to a linear temperature program, either heating or cooling, and the heat flow rate is measured as a function of temperature (O'Neill, 1966). DSC has many advantages including the use of small samples; only a few milligrams are needed. In addition to the latent heat of crystallization, it is possible to measure crystallization rate constants (Yap et al., 1989), polymorphic changes (Kawamura, 1980), and melting points (deMan et al., 1989). Examples of DSC cooling curves and heating curves are given in Figures 2.11 and 2.12, respectively.

There are many methods available to test the physical properties and chemical changes of frying fats. Physical methods include density, viscosity, smoke point, color, refractive index, UV

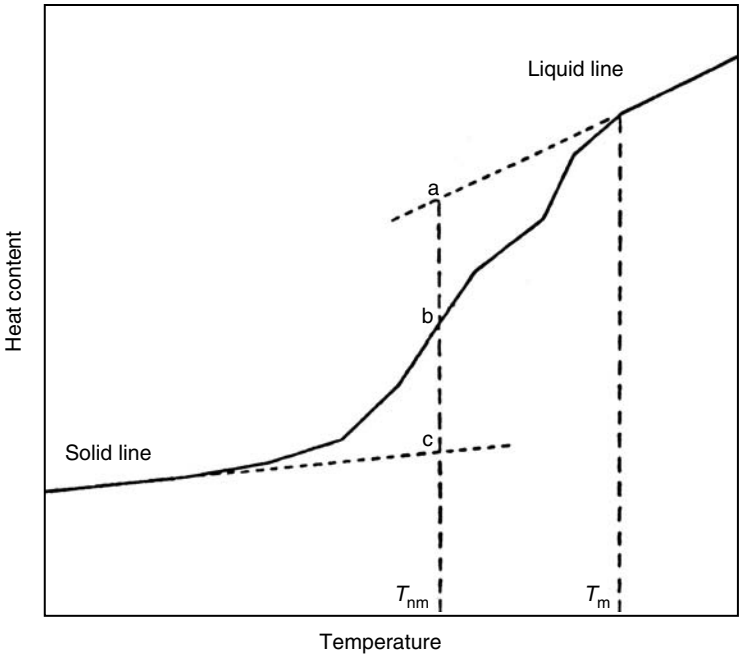


FIGURE 2.10 Schematic representation of the heat content curve of a plastic fat showing calculation of the heat of fusion at the point of half-fusion. (From Bailey, A.E. (1950). *Melting and Solidification of Fats*. Interscience, New York.)

TABLE 2.6
Specific Heat and Latent Heat of Fusion of Some Saturated Fatty Acids

Acid	Latent Heat of Fusion (cal/g)	Specific Heat (cal/g) (Temperature, °C)	
		Solid	Liquid
Caproic	31.2	0.4495 (–33 to –10)	0.5105 (0–23)
Caprylic	35.4	0.4650 (0–12)	0.5050 (18–46)
Capric	38.9	0.5009 (0–24)	0.4989 (35–65)
Lauric	43.7	0.5116 (19–39)	0.5146 (48–78)
Myristic	47.1	0.5209 (24–43)	0.5157 (to 84)
Palmitic	50.6	0.4920 (22–53)	0.5416 (to 68)
Arachidic	54.2	0.4597 (20–56)	0.5663 (to 100)
Behenic	55.1	0.4854 (18–71)	0.5556 (to 109)
Lignoceric	57.3	0.4656 (18–78)	0.5855 (to 109)

Source: Bailey (1979).

absorption, infrared spectroscopy, and dielectric constant, and chemical tests include free fatty acids (acid value), iodine value, anisidine index, saponification value, nonoxidized monomer fatty acids, polymerized triglycerides, fatty acids insoluble in petroleum ether, and total polar compounds can be utilized as quality indicators of frying oils (Gertz, 2000). The mechanical and thermal properties of a fat crystal network are determined by chemical composition, solid fat content, crystal habit (polymorphism and microstructure), and other factors. Campos et al. (2002) have shown that

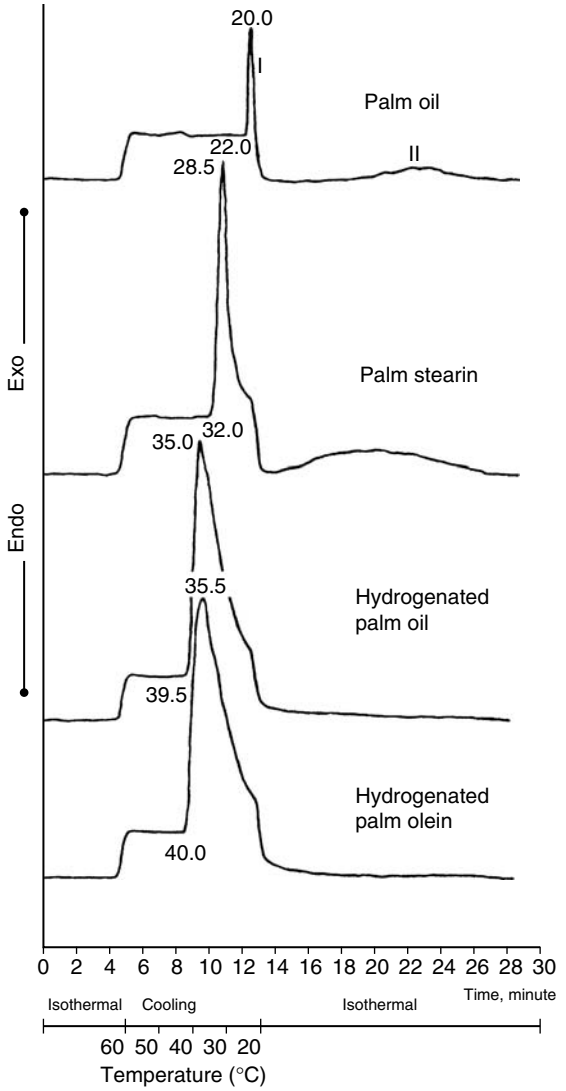


FIGURE 2.11 DSC crystallization curves of palm oil and hydrogenated palm oil IV 27.5. (From Yap, P.H. et al. (1989). *J. Am. Oil Chem. Soc*. *66*: 1792–1795.)

cooling rates affect the structure and mechanical properties of milk fat and lard. When crystallized rapidly, crystals are numerous and small, and when crystallized slowly, a smaller number of large crystals are found.

Tyagi and Vasishtha (1996) have shown that refractive index, specific gravity, color, viscosity, saponification value, and free fatty acids of soybean oil increased with frying temperature, whereas the iodine value decreased. The same trend was observed in vanaspati, a partially hydrogenated vegetable blend, but less markedly than in soybean oil, indicating a lesser degree of deterioration. Also, iodine values of soybean oil and vanaspati are decreased after frying, and polyunsaturated fatty acids are decreased in direct proportion to frying time and temperature.

XVI. OPTICAL PROPERTIES

Fatty acids and their esters crystallize in any one or several polymorphic forms in the hexagonal, orthorhombic, or triclinic crystal systems. Since all of these crystal systems are anisotropic, the

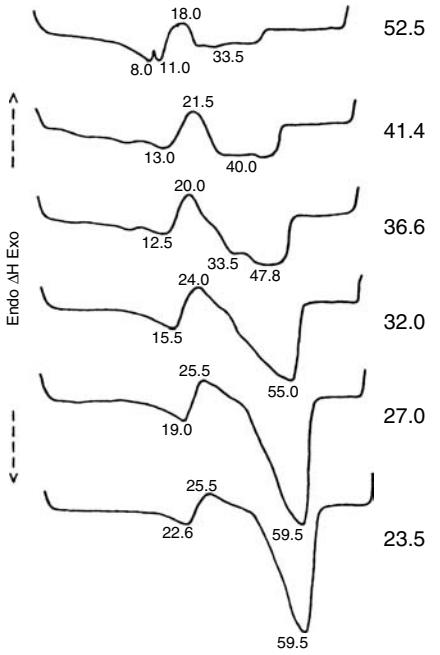


FIGURE 2.12 DSC heating curves of palm oil at various stages of hydrogenation. (Numbers at right indicate iodine values.)

crystals will rotate the plane of polarized light. This will make it possible for these crystals to be viewed in the polarizing microscope. A typical sample of shortening examined in the polarizing microscope shows a multitude of elongated crystal particles (Figure 2.13).

XVII. REFRACTIVE INDEX

The refractive index of a fat or fatty acid is a measure of the relative velocities of light in air, ν, and of the material being tested, ν′. If the angle of incidence of the light striking the surface of the material is *i* and the angle of the refracted beam in the oil is *r*, the refractive index *n* is expressed as

$$n = \frac{\nu}{\nu'} = \frac{\sin i}{\sin r}.$$

The refractive index of the fat or fatty acid is influenced by wavelength, temperature, density, and constitution. The measurement is usually carried out at the wavelength of 589 nm of monochromatic sodium light. As the temperature increases, the refractive index for an oil drops by 0.00035 per °C. Measurement of the refractive index is important in following the change in unsaturation during hydrogenation. This is possible because the refractive index and iodine value show a linear relationship. As an example of this relationship for the hydrogenation of canola oil, according to Patterson (1983), a drop of 10 units in the iodine value parallels a decrease in the refractive index of 0.00116, as shown in Figure 2.14.

XVIII. SPECTRAL PROPERTIES

UV spectroscopy has been used primarily to detect the presence of and type of conjugated double bonds in fatty acids. As conjugated double bonds are rarely found in fatty acids, the procedure is used after the treatment of polyunsaturated fatty acids with strong alkali (alkali isomerization). As the conjugation in a compound increases, the absorption changes to a higher wavelength. Chapman (1965) listed the absorption maxima for various conjugated chromophores (Table 2.7).

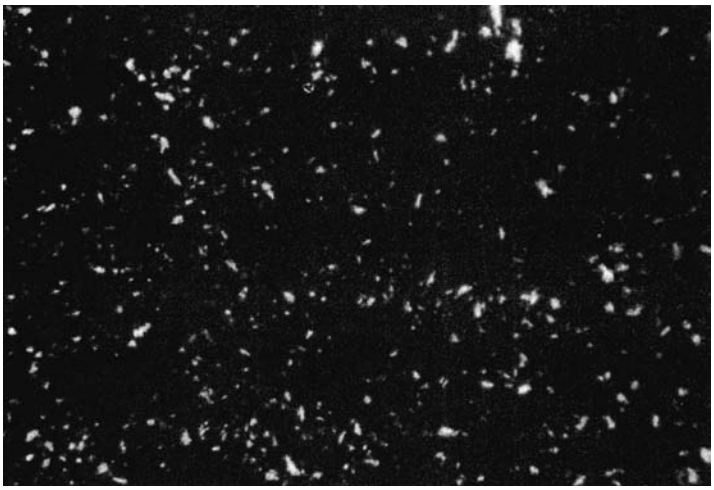


FIGURE 2.13 Crystal particles in a plastic fat as seen by using the polarizing microscope.

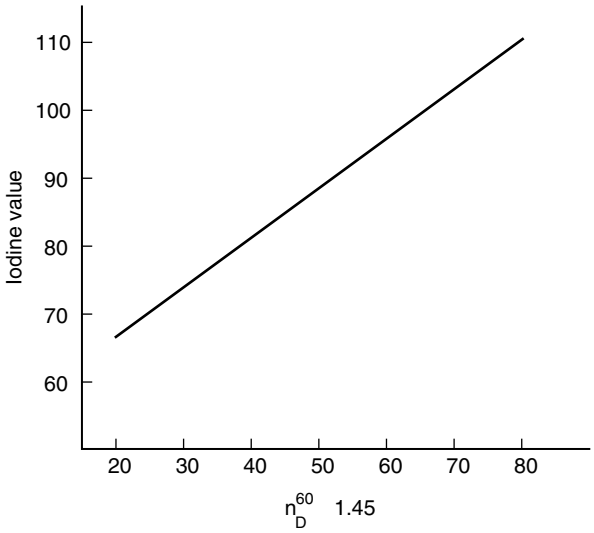


FIGURE 2.14 Relationship between refractive index and iodine value in the hydrogenation of canola oil.

Infrared spectroscopy has found important uses in the identification and quantitative analysis of isolated *trans* double bonds and conjugated systems with one or more *trans* double bonds. The *trans* group exhibits a characteristic maximum in the 10- to 11-μm range. The most widely used method of analysis for isolated *trans* double bonds is based on the absorption maximum at 10.35 μm. The absorption maxima for various *trans* compounds are shown in Figure 2.15 (Wolff and Miwa, 1965).

Recently, this technique has been improved by using attenuated total reflectance and Fourier transform principles. The resulting rapid methods have been used to determine *trans* fatty acids (Adam et al., 1998; de Greyt et al., 1998; Sedman et al., 1998). Other procedures include the determination of iodine value (Sedman et al., 1998) and free fatty acids (Che Man and Moh, 1998).

XIX. SOLUBILITY AND INTERFACIAL PROPERTIES

Fats and fatty acids are completely miscible with mot organic solvents at temperatures above their melting points. The solubility of fats in organic solvents decreases with increasing mean molecular

TABLE 2.7
Absorption Maxima of Various Chromophores in the UV Range

Chromophore	Type	Compound	λ max (nm)
—C=C—	Ethylenic	Octene-3	185
—C=C—C=O	Conjugated ene-al	Croton aldehyde	217
$(—C=C—)_2$	Conjugated diene	9,11-Linoleic acid	232
—C=C—C=C—C=O	Conjugated diene-al	Sorbaldehyde	263
$(—C=C—)_3$	Conjugated triene	α-Elaeostearic acid	270
$(—C=C—)_4$	Conjugated tetraene	Parinaric acid	302

Source: Chapman, D. (1965). *J. Am. Oil. Chem. Soc. 42*: 353–371.

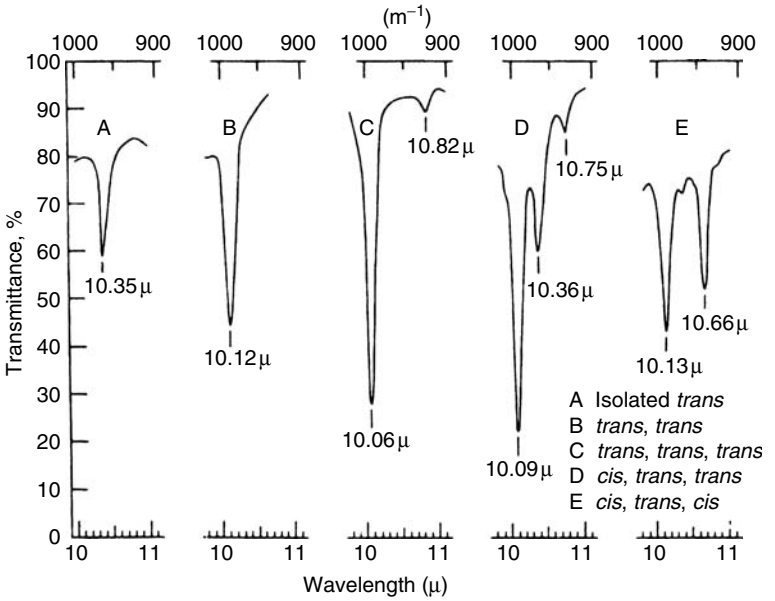


FIGURE 2.15 Absorption maxima for trans compounds in the infrared range. (From Wolff, I.A., and Miwa, T.K. (1965). *J. Am. Oil Chem. Soc*. 42: 208–215.)

weight and increases with increasing unsaturation. This is the basis for fractional crystallization of fats into a number of fractions based on molecular weight and unsaturation. Alcohols from methanol to octanol have the interesting property of being miscible with the liquid portion of plastic fats but not the solid. This has been used as a method for the separation of solid fat (Chawla and deMan, 1990).

Water is slightly soluble in oil at 0°C, with solubility amounting to about 0.07% and 0.14% at 32°C. Surface tension increases with increasing chain length and decreases with temperature. Surface and interfacial tensions against water for different oils are similar; Table 2.8 contains data reported by Feuge (1947).

Surface and interfacial tensions are greatly reduced when surfactants such as monoglycerides and phosphatides are added. The adhesion of edible oils to food contact surfaces is of importance in the cleaning of food-processing equipment and in product–package interactions. Theoretical models for oil adhesion to food contact surfaces have been presented by Michalski et al. (1998). This is of importance in deep frying. The effect of surfactants on the interfacial tension of frying fat has been described by Gil and Handel (1995).

TABLE 2.8
Surface and Interfacial Tension of Some Vegetable Oils

	Surface Tension (dyne/cm)		Interfacial Tension (dyne/cm)		
Temperature (°C)	Cottonseed	Coconut	Peanut	Cottonseed	Soybean
20	35.4	33.4	—	—	—
70	—	—	29.9	29.8	30.6
80	31.3	28.4	—	—	—
130	27.5	24.0	—	—	—

Source: Feuge, R.O. (1947). *J. Am. Oil Chem. Soc. 24:* 49.

TABLE 2.9
Viscosity of Some Oils

	Viscosity (cP)	
Oil	37.8°C	98.9°C
Olive	42.75	8.32
Rapeseed	46.15	9.41
Cottonseed	32.96	7.71
Soybean	26.29	7.01
Sunflower	30.67	7.07
Castor	282.22	19.31
Coconut	27.48	5.59
Palm kernel	28.42	5.97

XX. VISCOSITY

Viscosity or internal friction of fatty acids and their esters is relatively high because of the attraction between the long hydrocarbon chains. Viscosity increases with chain length and decreases slightly with unsaturation. The relatively high viscosity of high-erucic acid rapeseed oil can be related directly to its level of 22-carbon fatty acids. This is illustrated by the increasing viscosity of triglycerides of increasing fatty acid chain length (Table 2.9). Castor oil, because of its high content of ricinoleic acid, is a special case; its viscosity is exceptionally high. The other common oils range in viscosity from 20 to 50 cP at ambient temperature (Table 2.10). Except under conditions of high shear stress, oils behave as true Newtonian liquids. The viscosity of a fatty acid is higher than that of the corresponding methyl or ethyl ester because of hydrogen bonding between carboxyl groups.

Viscosity is an important parameter in the process of melt crystallization. The dry fractionation of oils to produce a solid (stearin) and a liquid (olein) fraction is greatly influenced by the viscosity of the oil at the fractionation temperature. Toro-Vazquez and Gallegos-Infante (1996) have described the relationship between viscosity and crystallization in a system-containing saturated triacylglycerols and liquid oil. The viscosities of fatty acids, triglycerides, and their binary mixtures are reported by Valeri and Meirelles (1997).

XXI. DENSITY

Apparent density equals mass per unit volume at the measurement temperature. Relative density or specific gravity is the ratio of the weight in air of a given volume to the weight of an equal volume of water. These measurements can be made at the same or different temperatures. In the American

TABLE 2.10
Viscosity of Triglycerides of Different Chain Lengths, Measured at 85°C

Triglyceride	Viscosity (cP)
Tricaprin	5.51
Trilaurin	7.22
Trimyristin	9.20
Tripalmitin	11.44
Tristearin	14.31

TABLE 2.11
Density of Some Commercial Fats and Oils

	Density (g/mL)		
Product	**20°C**	**40°C**	**60°C**
Cottonseed oil (IV = 110)	0.934	0.927	0.920
Soybean oil (IV = 130)	0.938	0.931	0.924
Hydrogenated vegetable oil, a lard (IV = 70)	0.928	0.821	0.914
Highly hydrogenated vegetable oil (IV = 10)	0.924	0.917	0.910

IV = iodine value.

Source: Formo, M.W. (1979). Physical properties of fats and fatty acids. In *Bailey's Industrial Oil and Fat Products* (D. Swern, ed.), vol. 1, Wiley, New York, pp. 233–270.

Oil Chemists' Society (AOCS) official method, a water temperature of 25°C is specified. For liquid oils, density can be determined as d_{25}^{25}. For fats, the usual temperature of measurement is 60°C, giving d_{25}^{60}. The density of fatty acids and their esters increases with decreasing molecular weight and increasing unsaturation. The densities of some commercial fats and oils are given in Table 2.11. A compilation of literature values for density of liquid oils as well as of other physical properties is given by Coupland and McClements (1997). They present a series of empirical equations to calculate the temperature dependency of these physical properties.

XXII. SMOKE, FLASH, AND FIRE POINTS

The smoke, flash, and fire points are important properties when oils and fats are heated in contact with air, as in frying operation.

The *smoke point* is the temperature at which smoke is first detected in a standardized test. The *flash point* is the temperature at which volatile substances are produced at a rate that permits them to be ignited but not to support combustion. When the latter point is reached, we speak of the *fire point*.

The smoke and flash points of fats and oils are greatly dependent on the content of free fatty acids and to a lesser degree on partial glycerides. The influence of the degree of unsaturation is minimal, but chain length has an important effect. Oils containing short-chain fatty acids (e.g., the lauric oils) have lower smoke and flash points than oils with predominantly longer-chain fatty acids. According to Bailey (1979), the smoke points of corn, cottonseed, and peanut oils vary from about 232°C at a free fatty acid content of 0.01% to about 94°C at 100% free fatty acid content. The flash points correspondingly decrease from about 329°C to 193°C and the fire points from 362°C to 221°C.

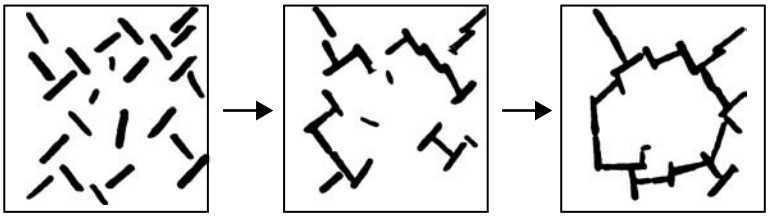


FIGURE 2.16 Schematic representation of network formation by crystals in a plastic fat.

XXIII. RHEOLOGICAL PROPERTIES

Partly solidified fats, also known as plastic fats, combine the physical properties of both solid and liquid. This is caused by the presence of a three-dimensional network of crystals in which a considerable amount of liquid oil is immobilized. A plastic fat is usually workable at room temperature when the solid fat content lies between 20% and 40%. Under the influence of weak attractive forces between crystals, mostly due to van der Waals forces, a three-dimensional structure is formed that lends the product a good deal of resistance to deformation. A schematic representation of such network formation is given in Figure 2.16.

As a result of the presence of a fat crystal network, plastic fats exhibit a yield value. The product behaves similar to a rigid solid until the deforming stress exceeds the yield value and the fat starts flowing like a viscous liquid. The application of a shear stress to such a product is accompanied by structural breakdown and a decrease in strength. This is also known as work softening.

The consistency of plastic fats is determined by a number of factors, including solid fat content, crystal size and shape, and polymorphic form. Rheological properties of fats can be measured by penetration and compression tests and by creep analysis. The latter method permits the separation of viscous and elastic components of the deforming force (deMan and Beers, 1987). The nature of the relationship between rheological properties and the crystal network has traditionally been explained by the postulated existence of weak attractive forces between crystal particles. In plastic fats, however, the crystals are present in such large numbers that a more likely hypothesis (deMan, 1997) is that the mechanical properties are the result of entanglement of mostly rod shaped crystals. Humphrey et al. (2004) have reviewed the phase behavior, from molecular to rheology, of a binary lipid shortening system.

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3 Application of Gas–Liquid Chromatography to Lipid Separation and Analysis: Qualitative and Quantitative Analysis

Robert G. Ackman

CONTENTS

I. ISOLATION AND ANALYSIS OF FATTY ACIDS

Almost all foods contain "fat," which is very loosely defined as material that is soluble in any of several organic solvents such as hexane, diethyl ether, or chloroform. The major lipid classes commonly encountered in foods are shown in Figure 3.1. For this discussion, pigments, sterols, hydrocarbons, tocopherols, waxes, and so on will be ignored, although they are coextracted along with the major classes of lipids. The distinguishing feature of lipids in foods is the universal occurrence of medium- and long-chain (C_{14}–C_{22}) fatty acids. The hydrocarbon chains of these fatty acids are the main factor in the solubility already mentioned. As emphasized by the adjectives, the nomenclature is rather confused. Most nutritionists distinguish the C_8, C_{10}, and C_{12} fatty acids from the common C_{16} and C_{18} fatty acids by calling the former "short chain" and the latter "long chain." The former are readily digested, and on absorption they pass directly to the liver via the portal vein, whereas the latter tend to transfer into the lymph in chylomicrons (Nelson and Ackman, 1988). For most foods, C_{22} fatty acids are the longest chain length present in any quantity, and in this chapter, "long chain" will mean C_{20} and C_{22}.

A further group of fatty acids of very short chain lengths (C_1–C_6) were historically called the volatile fatty acids (VFAs), primarily because they can be steam distilled at atmospheric pressure.

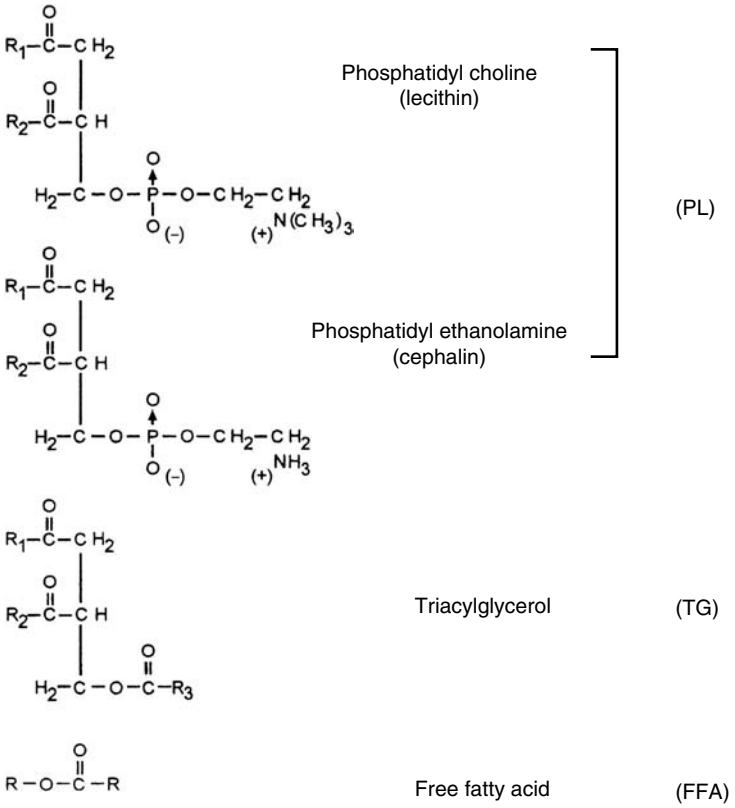

FIGURE 3.1 Principal classes of acyl lipids found in fish and shellfish. Free fatty acids are not initially important but develop postmortem.

Basically, these fatty acids are also quite water soluble, whereas fatty acids of C_8 and longer chains become progressively less water soluble. The VFAs will be considered separately from the longer-chain fatty acids, although it is intriguing to recollect that gas–liquid chromatography (GLC) was in fact invented expressly to determine low levels of VFAs (James and Martin, 1952).

II. DRAWBACKS OF SAMPLE ABUSE

Unless one is handed a bottle of salad oil, it is usually necessary to consider that the lipid classes of Figure 3.1 are dispersed in some sort of food matrix. At one time, a sausage was considered one of the toughest food items on which to perform an extraction, although a chicken pot pie is a close second (Sheppard et al., 1974; Hubbard et al., 1977). Historically, analysts regarded oven drying as an essential first step in sample "proximate analysis," because moisture content was needed in any event and a dry sample could be conveniently ground and homogenized. Unfortunately, the oven drying created two problems. One was that the fats or lipids might be encased in dry protein or some similar matrix that solvent could penetrate only with difficulty (Happich et al., 1984; de Koning and Mol, 1989). The second difficulty created was the potential for oxidation of polyunsaturated fatty acids. Until modern chromatographic methods were introduced, it was not appreciated that not only did oxidation create polar compounds of relatively low solubility in many organic solvents (Sebedio et al., 1987; Hara et al., 1989) but also these compounds could react with other fatty acids to produce relatively insoluble polymers or with amino acids or carbohydrates to give a more permanent and insoluble binding of fat or lipid (Allen and Hamilton, 1983).

Yet another form of sample abuse is freeze drying. This is often used by biologists and some biochemists, but more rarely by food scientists. The opportunity for oxygen damage is obvious. Solvent extraction of freeze-dried fatty tissues can be done with chloroform alone (Firth et al., 1985), because water is no longer a problem.

The rules for avoiding sample abuse in lipid analyses are simple. Leave the sample in the original matrix as long as possible, but store it frozen at the lowest possible temperature, preferably at –30°C or lower, to avoid enzymatic lipolysis (Hardy et al., 1979). An oxygen-impermeable wrap or a container flushed with nitrogen should be considered. The next best thing is to do a *total* extraction immediately and then store the extract in solution, or partially dried by solvent removal with nitrogen, at –30°C or lower.

With larger samples or packages of foods, it may be necessary to use a Hobart silent cutter or equivalent equipment to produce a uniform homogenate for subsampling and analysis of an economic or otherwise rational portion. The same technique would apply to "averaging" several units for one determination of lipid content. These critical points are basic to good practice in laboratory quality control plans (Malanoski, 1990).

III. TOTAL LIPID RECOVERY

Most of the organic solvents already mentioned will dissolve the triacylglycerols and free the fatty acids of Figure 3.1. The difficulty in total lipid extraction is twofold: (1) Some of the solvents are immiscible with water and (2) some are poor solvents for the polar lipids (mostly phospholipids). The triglycerides may be obvious in fatty streaks or layers, or they may exist in the form of adipocytes (fat cells) scattered within muscle tissue. A very small fraction will be found in the cell membrane bilayers, which are mostly phospholipids. It is likely that the latter do not exist in the neat arrangements usually shown but are instead hydrated to some extent because of the affinity for water of both the phosphoric acid and amino acid molecules on the 3-position of the glycerol.

Methanol is a poor solvent for triacylglycerols but is fully miscible with water in all proportions. It can thus serve to strip away the hydrating water and allow chloroform; for example, to dissolve the phospholipids. This was the basis of the famous Folch et al. (1957) 2:1 chloroform–methanol solvent system, which became the basic lipid extraction unit for biochemists. The water in the system caused the chloroform to separate cleanly, with the methanol component retaining the water and suppressing emulsions. The original method included extensive procedures for the removal of nonlipid components that are probably ignored by most people citing the method.

Similarly, the utilization of a monophase based on exact proportions of chloroform, methanol, and water as developed by Bligh and Dyer (1959) tends to be bypassed for expediency, and there are thousands of scientific papers published annually in which the extraction of various samples is executed with "chloroform:methanol:2:1" with little attention paid to proportions, ratios, and water content variables. The key role of methanol has been emphasized by Areas (1985).

The Bligh and Dyer system can yield more lipid than most other methods, and Table 3.1 compares the recovery of lipid by three methods in the difficult case of oxidizing fishmeal, which is in essence cooked and dried fish muscle (de Koning and Mol, 1989). Food applications of solvent systems are reviewed by Sheppard et al. (1974), Hubbard et al. (1977), and Sahasrabudhe and Smallbone (1983). Particular cases, such as recovery of all the free fatty acids formed in frozen stored fish, may require modification of "standard" techniques such as the Bligh and Dyer chloroform–methanol system (Hardy et al., 1979). Incomplete lipid recovery is sometimes not a problem if the material recovered is a representative sample but can be misleading if food fatty acid composition data are needed. Fish phospholipids include substantial proportions of polyunsaturated fatty acids (Table 3.2), and in lean fish samples, failure to recover phospholipids can give low values of 20:5n-3 and 22:6n-3. In red meat, 18:2n-6, 18:3n-3, and 20:4n-6 would be affected. (This shorthand notation is based on chain length, number of ethylenic *cis*-methylene-interrupted bonds [if more than one], and position counted from the terminal methyl group.)

TABLE 3.1
Amount of Lipid Extracted by Three Different Procedures form Anchovy Fishmeal, Untreated with Antioxidant, vs. Storage Time at 25°C (Expressed as g per 100 g Dry Meal)

	Storage Time (days)									
Extraction Method	**0**	**31**	**90**	**133**	**181**	**226**	**320**	**371**	**455**	**672**
Bligh and Dyer	13.12	12.22	11.49	11.36	10.76	10.51	10.30	10.28	9.78	8.54
Hexane	11.54	10.45	8.80	7.97	7.61	6.86	5.89	5.70	4.99	3.84
EEC, modified	13.57	12.13	10.84	10.29	9.98	9.46	8.72	8.10	7.49	6.02

Source: Adapted from de Koning, A.J., and Mol, T.H. (1989). *J. Sci. Food Agric. 46*: 259–266.

TABLE 3.2
Proportions of Principal and Biochemically Interesting Fatty Acids of Muscle Lipids (Triglyceride and Phospholipid) from a Retail Farmed Atlantic Salmon (*Salmo salar*) Compared to Beef and Three Common Vegetable Oils

	Salmon[a]		Lean Beef[b]	Vegetable Oils[c]		
	TG	**PL**	**Total Lipid**	**Canola**	**Sunflower**	**Corn**
14:0	8.62	3.46	3.36	—	—	—
16:0	16.13	23.13	26.72	3.1	5.5	10.4
18:0	2.03	1.87	12.16	1.5	3.4	2.8
Σ Saturated	26.78	28.46	42.40	5.2[d]	9.3[d]	14.1[d]
16:1n-7	8.10	2.39	5.44	—	—	—
18:1n-9	11.80	5.23	47.52	60.0	48.4	26.9
18:1n-7	2.56	1.80	—[e]	—[f]	—[f]	—[f]
20:1	11.14	2.11	—	1.3	—	0.4
22:1	10.07	1.05		1.0		0.1
Σ Monoenoic	43.67	12.58	52.96	62.3	48.4	27.4
18:2n-6	4.67	1.55	3.36	20.2	42.3	56.2
18:3n-3	0.91	0.46	0.64	12.0	—	2.1
18:4n-3	1.44	0.35	—	—	—	—
20:4n-6	0.27	0.51	0.64	—	—	—
20:4n-3	0.93	0.65	—	—	—	—
20:5n-3	5.26	10.65	—	—	—	—
22:5n-6	0.06	0.29	—	—	—	—
22:5n-3	1.78	2.98	—	—	—	—
22:6n-3	6.60	33.18				
Σ Polyenoic	21.92	50.62	4.64	32.2	42.3	58.3
Others	7.59	8.33	0.16	0.3	—	0.2

[a] S. M. Polvi and R. G. Ackman, unpublished data.

[b] U.S. Department of Agriculture, *Agricultural Handbook* 8–13, May 1990.

[c] Adapted from Ackman, R.G., and Ratnayake, W.M.N. (1989). Lipid analyses. Part 1. Properties of fats, oils and lipids: recovery and basic compositional studies with gas-liquid chromatography and thin-layer chromatography. In *The Role of Fats in Human Nutrition* (A.J. Vergroesen and M. Crawford, eds.), 2nd ed., Academic, London, pp. 441–514.

[d] Includes minor amounts (<1%) of each, 20:0 and 22:0.

[e] Both *cis*- and *trans*-18:1n-7 are included in 18:1n-9.

[f] Trace only, included in 18:1n-9.

IV. TOTAL FATTY ACID RECOVERY

The objective of lipid extraction is varied. Increasingly, it develops from requests for specific information on cholesterol and fatty acids. There is thus very little reason to bother with lipid extraction. Instead, total lipid recovery may be bypassed, and the sample may be digested under nitrogen to destroy the protein food matrix. Traditionally, as in the Association of Official Analytical Chemists' (AOAC) method 18.043 (Williams, 1984), concentrated HCI was used to digest carbohydrates and so on, but in practice alcoholic alkali will do this job more efficiently (Kovacs et al., 1979).

A problem with the concentrated HCI, in addition to the obnoxious fumes, is that the glyceride lipids of Figure 3.1 are only partially hydrolyzed. Thus the free fatty acids will be increased, but the proportions of these are unpredictable. With alcoholic alkali digestion there is another problem that may be overlooked. The saponification reaction in the presence of ethanol and water will produce the free acids as alkali salts but may also produce a small proportion of ethyl esters of fatty acids, because alkali-catalyzed transesterification is a very fast equilibrium-type reaction. If separate recovery of sterols and fatty acids is an objective, some small part of the fatty acids bound as ethyl esters may be lost if the sterols are removed as "unsaponifiables" from the water–alcohol solution of soaps. Usually these esters are a representative part of the whole fatty acid mixtures, but on occasion they may be specific to some part of the lipid. Similarly, the addition of a fatty acid as an internal standard may require that formation of esters be avoided at this stage of fatty acid recovery (Ackman et al., 1990) unless acid–ester equilibrium is attained.

Digestion is accelerated by providing a high proportion of ethanol to the matrix, but in view of the ethyl ester formation, additional water may be added as the reaction proceeds. This is usually required in any event to promote recovery of the unsaponifiable material from the alkaline solution and of the free acids after subsequent acidification with HCI. For these extractions, the use of diethyl ether usually reduces emulsion formation but may contribute damaging peroxides if the ether is not freshly distilled. *n*-Hexane or light petroleum ether is an acceptable alternative. Usually one extraction of free acids is about 90% efficient, and thus two extractions are required to complete adequate (99%) recovery of the free acids. If shorter chain (C_8–C_{12}) fatty acids are present, a third extraction is recommended. Christie (1982) covers some of the above problems. If a total lipid extract is to be later saponified and the fatty acids recovered, much the same principles apply.

The U.S. Food and Drug Administration has now defined total fat in a food as the sum of all fatty acids, expressed as triacylglycerols. A compendium issued by the AOAC (Sullivan and Carpenter, 1993) explains all the steps for gas chromatography-fatty acid methyl ester (GC–FAME) analysis clearly and it has been recently confirmed for ground beef and oilseeds that the GC–FAME method provides equivalent lipid recovery to supercritical fluid extraction with ethanol-modified CO_2 (Taylor et al., 1997). However, gravimetric results were less satisfactory. It should be noted that supercritical fluid chromatography itself may not be ideal as a replacement for gas–liquid or high-performance liquid chromatography (HPLC) in analytical separations for all classes of lipids (Borch-Jensen and Mollerup, 1996).

V. ANALYSIS OF FATTY ACIDS BY GAS–LIQUID CHROMATOGRAPHY

The objectives of fatty acid recovery having been met, it is usual to convert the fatty acids to derivatives for analysis. Although HPLC can be used, appropriate derivatives are required, usually those absorbing strongly in the ultraviolet (Ratnayake and Ackman, 1989). If that is the case, an advantage is that the only thing measured is the derivative molecule itself; this will give the composition in moles of fatty acid and not in weight units of fatty acids unless converted.

Probably 99% of the analyses of fatty acid compositions in foods are executed by GLC with the methyl esters. In principle, any ester can be used; for example, the isopropyl esters used for combined extraction and esterification by Peuchant et al. (1989). The reason for preferring esters to

free acids is twofold. The acids may "associate" in pairs in the vapor phase, a long-known phenomenon adversely affecting distillation of free acids, but they will also absorb on any convenient surface, resulting in tailing and/or "ghosting" (Ackman, 1972a; Cochrane, 1975). This problem can be reduced with flexible fused silica wall-coated open-tubular (WCOT) columns (Hordjik et al., 1990). Generally much higher temperatures are required for the GLC of free acids than for methyl esters, which is an advantage for the latter. The decade 1980–1990 marked a major shift in GLC technology. The packed GLC column became obsolete with the introduction of the flexible fused silica column (FFSC). Packed columns will therefore be omitted from further discussion, but the principles and column packings are well described elsewhere (Ackman, 1969, 1984; Lie Ken Jie, 1980).

The FFSC is a modern version of the WCOT column introduced in a practical commercial form about 1965 and the subject of a major review two decades ago (Ackman, 1972b). Glass WCOT columns were also available but were more widely used in Europe than in North America (Ackman, 1986). The modern GLC unit remains an assembly of the three basic parts and one essential auxiliary. These are as follows:

1. *Injection port*. This should be of the split type, because there are usually plenty of samples available to food scientists. Biochemists and environmentalists are the main users of on-column injection. An autosampler is a very useful, if rather expensive, accessory. A split ratio of 30:1 to 50:1 is compatible with 1- to 2-μL injections of 1%–5% solutions of methyl esters.
2. *Column.* Quality control departments may be able to standardize on a short (10 m) FFSC for the minimal separations required with numerous samples of common vegetable oil fatty acids. Times of 5 min or less are feasible. However, with "bonded" liquid phases (see below), the 30 m × 0.32 (or 0.25) mm i.d. FFSC may as well be used, because a small elevation in temperature will bring the sample off in the same time frame but with superior peak resolution. Fatigue or accidents usually break the FFSC near the end. With the longer column, the end can usually be reinstalled and the column continues in use. Sometimes with dirty samples a 1-m length of inert silica forecolumn may be useful to prolong column life.
3. *Detector*. This is universally the flame ionization detector (FID). The FFSC is usually fed through the base of the FID into the hydrogen gas stream, and often through the flame jet, with the tip almost in the flame itself. This elimination of postcolumn connectors is a major benefit of FFSC technology. The FID is tolerant of a wide range of sample sizes and column gas flows, but operating gas (air and hydrogen) flows should be checked regularly and follow the manufacturer's recommendation.
4. *Electronic integrator.* This indispensable auxiliary unit is now offered as an integral part of modern GLC equipment. A variety of options on data recording and processing are usually available, but generally peaks are identified by time printouts in a digitally regenerated chromatographic chart and then a matching area percent table is printed out.

VI. OPERATIONS—IDENTIFICATION AND QUANTIFICATION

For most analyses of food fatty acids, a polar column will be selected. The exception could be for gas chromatography–mass spectrometry (GC–MS) use, for which a methyl silicone liquid phase might be used, or for *cis–trans* analyses requiring a cyanosilicone column (see below). The term bonded column is not strictly accurate. The liquid phase contains a cross-linking agent so that the whole of the liquid phase layer becomes one supermolecule. Consequently, column bleed is minimal, with the advantages that the baseline is stable during temperature programming (compare Figures 3.2 and 3.3) and GC–MS operations are readily performed. The first successful polar bonded columns were based on Carbowax-20M (Union Carbide Corp., Danbury, CT) as the liquid phase, and they have excellent

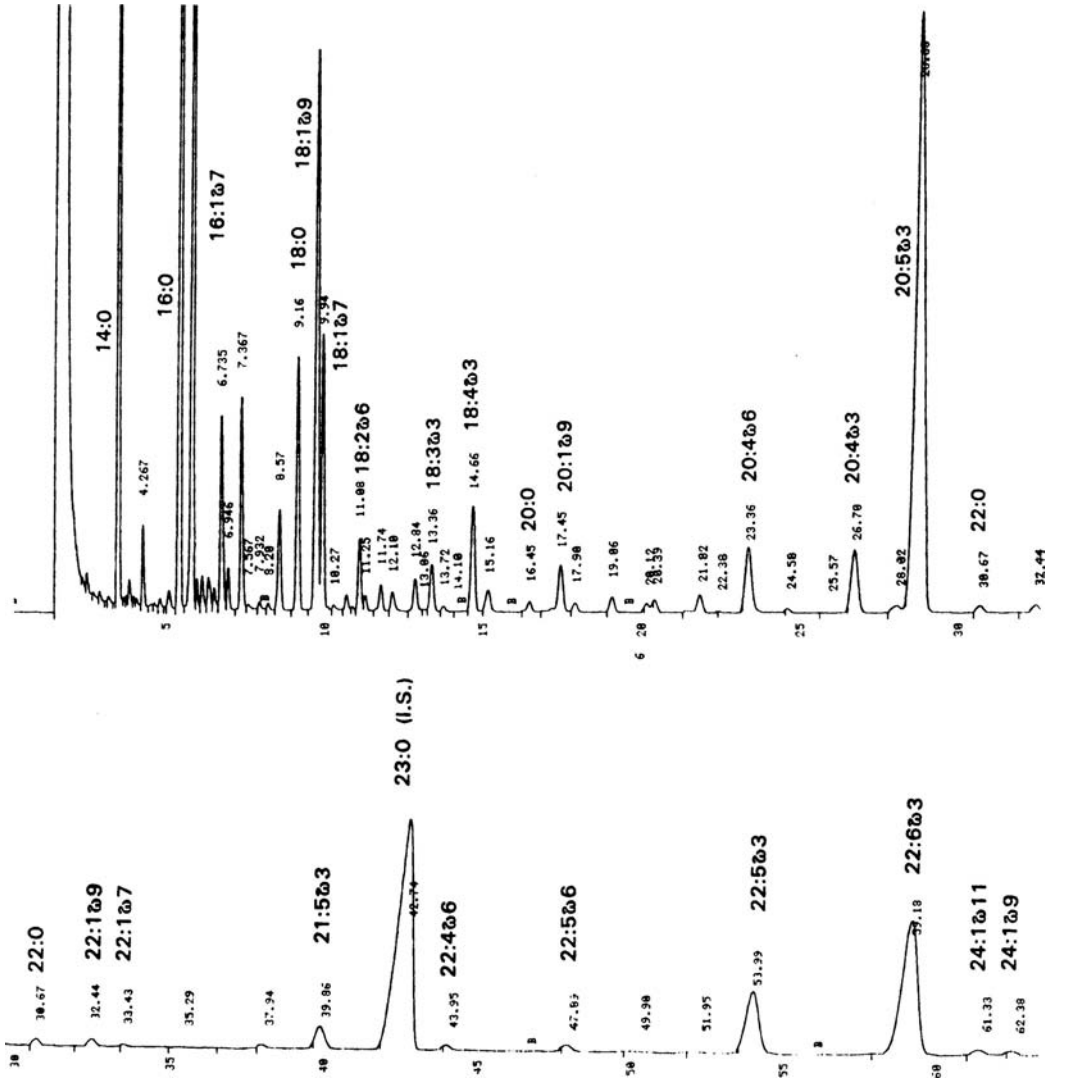

FIGURE 3.2 Isothermal analysis of methyl esters of fatty acids of menhaden oil, a fish oil produced in quantity in the United States, with added 23:0 internal standard. Column, Omegawax-320 (Supelco Inc., Bellefonte, PA), 30 m × 0.32 mm i.d., operated at 190°C with helium carrier gas at 12 psig. Print numbers at baseline are minutes from injection, so 22:6n-3 emerges at close to 60 min elapsed time.

stability up to 260°C or 280°C. Originally, this polyethylene glycol was an industrial chemical with a formula weight of approximately 20,000 produced by Union Carbide Corp. (Danbury, CT). Several companies coat FFSC with this or similar polymers. The Supelco (Bellefonte, PA) 1999 catalogue offers their bonded columns prepared from Carbowax-20m as SUPELCO-Wax, or Omegawax-250 or Omegawax-320, and lists the names of similar liquid phases offered by other companies. Their use greatly facilitates interlaboratory comparisons of fatty acid methylester retention data (Ackman and Ratanayake, 1989). The retention characteristics for methylesters of fatty acids are also less temperature sensitive than those of the more polar cyanosilicone liquid phases (Sidisky and Ridley, 1991). Several cyanosilicone liquid phases (Sidisky et al., 1988; Cumbers et al., 1990) are at least partially stabilized, and all benefit from having a deoxygenation cartridge installed in the carrier gas line. Either hydrogen or helium is necessary as the carrier gas.

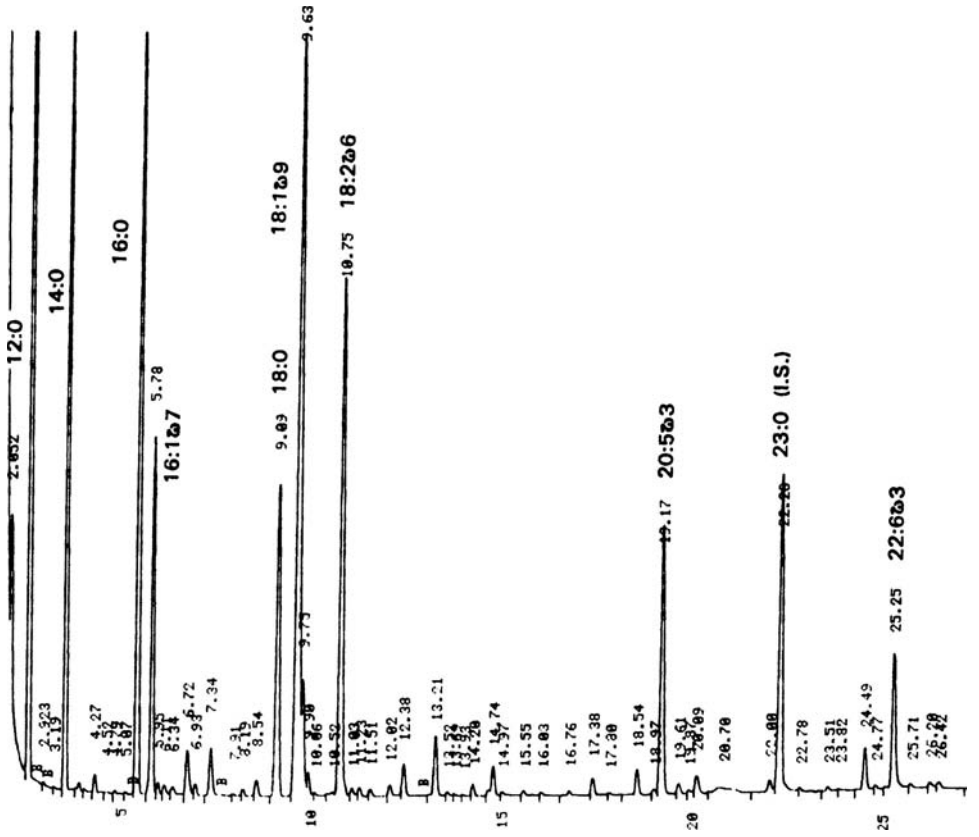

FIGURE 3.3 Temperature-programmed analysis of methyl esters of a mixture of menhaden oil (cf. Figure 3.2) and vegetable oils. Column and equipment as in Figure 3.2. Oven temperature: Hold at 190° for 8 min, program at 3°/min to 240°, hold. Note that elapsed time is reduced to 25 min. Features not observed in fish oils include the 12:0 and the high proportion of linoleic acid (18:2) relative to oleic acid (18:1). The presence of 12:0 and increases in 14:0, 16:0, and 18:0 suggest addition of a tropical oil or oil fraction.

A frequent injection problem is the failure to use a solvent flush (~1 μL) in a typical syringe. Drawn into the syringe prior to the actual solution of methyl esters, this small extra amount of solvent will help ensure complete injection of the sample (Berg, 1988). All of the solvent should be drawn back just out of the needle, the needle inserted through the septum quickly to its full length, and the syringe plunger smartly depressed. There is then no point in leaving the syringe needle in for more than one-tenth of a second.

The main problem in identification of peaks for fatty acids is the increased use of both programming and high-polarity columns. The GLC analysis of fish oils has long been recognized as being difficult (Ackman, 1969, 1972b), and for isothermal analyses tabulations of equivalent chain lengths (ECL values) are available (Ackman, 1984, 1986; Christie, 1988). One inexpensive standard solution of even-chain saturated fatty acids provides a series of reference points (14:0–24:0) from which most other fatty acids can be readily identified with ECL values. A virtue of the Carbowax-20M type of polyglycol FFSC is that the elution order will hardly change on going from isothermal operations to temperature programming. Figure 3.2 is an excellent example of the analysis of fish oil fatty acid methyl esters on columns of this type operated isothermally. With programming, the same analyses can be executed in a run time of about 20 min. Note that the only case of chain-length overlap is that 24:0 falls before and 24:1 after 22:6n-3. The liquid-phase DB-225 (J. & W. Scientific, Folsom, CA) is slightly less polar. With more polar liquid phases, chain-length overlap will appear in different chain lengths, for example, with possible coincidence of 20:1 and 18:3n-3, or of 22:1 with 20:4n-6 or 20:5n-3.

TABLE 3.3
Theoretical (Relative) Response Factors (TRF) for Fatty Acid Methyl Ester (FAME)

FAME	TRF	FAME	TRF	FAME	TRF	FAME	TRF
4.0	1.5396	14.1	1.0354	18.2	0.9865	22.1	0.9664
5.0	1.4009	15.0	1.0308	18.3	0.9797	22.2	0.9609
6.0	1.3084	15.1	1.0027	18.4	0.9730	22.3	0.9554
7.0	1.2423	16.0	1.0193	19.0	0.9919	22.4	0.9499
8.0	1.1927	16.1	0.0117	20.0	0.9846	22.5	0.9443
9.0	1.1542	16.2	1.0041	20.1	0.9785	22.3	0.9388
10.0	1.1233	16.3	0.9965	20.2	0.9724	23.0	0.9665
11.0	1.0981	16.4	0.9889	20.3	0.9663	24.0	0.9614
12.0	1.0771	17.0	1.0091	20.4	0.9603	24.1	0.9564
12.1	1.0670	17.1	1.0019	20.5	0.9452		
13.0	1.0593	18.0	1.0000	21.0	0.9780		
14.0	1.0440	18.1	0.0032	22.0	0.9720		

Atomic weights: carbon, 12.011; hydrogen, 1.0079; oxygen, 15.994 (*Handbook of Chemistry and Physics,* 64th ed., 1983–1984), (Chemical Rubber Publishing Co., Cleveland, OH). Factors are relative to 18:0, which has a factor of 1.0000 by definition. Factors for the following FAME have been verified experimentally: 4:0; 6:0; 8:0; 10:0; 12:0; 14:0; 16:0; 17:0; 20:0; c9–18:1; c9, c12–18:2; c9, c12, c15–18:3; c5, c8, c11, c14–20:4; c4, c7, c10, c13, c16, c19–22:6. Only one factor is given for all positional and geometrical isomers and for branced-chain FAME, as the factors are dependent only on the content of carbon to which hydrogen is bonded. Calculation by Craske, J.D., and Bannon, C.D. (1998). *J. Am. Oil. Chem.. Soc. 65*: 1190–1191.

The area percentage from the FID response represents a close approximation of weight percentage for simple C_{18} vegetable oils but is still only an approximation. To convert to true weight percentage for these VFAs, appropriate response factors (Table 3.3) must be employed, especially if coconut or similar oils are involved. It should be noted that these peaks may not be representative of the weight content of fatty acids in the sample. For example, a fish oil deodorized under very severe conditions contained 12% dimeric acid and 17% trimeric acid (Change, 1988). These would not appear in the chromatogram, and the analyst could report that the fatty acids of the sample contained, for example, 25% 22:6n-3, whereas in fact the true figure would be less. For this reason, in critical cases or if lipid or fat samples contain nonvolatile or inert material, an internal standard should be used to give the sample content of a particular fatty acid in milligrams per gram of sample. The procedure was outlined by Einig and Ackman (1987) and is now an Official Method (Ce-lb-89) of the American Oil Chemists' Society if fish oils or concentrates are involved (Ackman et al., 1990). This could become important to the food industry, because fish oils are receiving serious consideration as food additives (Garcia, 1998). An interlaboratory trial of this technology was also successful in satisfying the requirements of the AOAC (Joseph and Ackman, 1992). If the sample consists of shorter chain lengths, other appropriate internal standards such as 13:0, 15:0, and 17:0 may be used with suitable FID correction factors applied (Ackman, 1986; Craske and Bannon, 1988).

In the oils and fats industries, it is common to specify iodine values, and computer methods for converting GLC area percentages to weight percent fatty acids can be extended and adapted to calculate iodine values. This provides an excellent check on quantification if the calculated and Wijs iodine values are compared (Ackman and Eaton, 1978).

VII. *TRANS* FATTY ACIDS

A special problem in fatty acid analysis is that some labels require accurate figures for the content of saturated, polyunsaturated, and *trans* fatty acids. The analysis of saturated fatty acids by GLC is easy and accurate, and the total *trans* bonds in fatty acids have always been feasible using infrared spectroscopy.

The problems created by label requirements some years ago are also factors in the analysis of the polyunsaturated fatty acids. The soybean lipoxidase assay (Beare-Rogers and Ackman, 1969; Firestone, 1984) is specific for fatty acids with polyethylenic *cis* bonds in the n-6 and n-9 positions. An excessively narrow nutrition viewpoint that only the ω6 *cis–cis*-18:2n-6 was "essential" meant that *cis–cis–cis*-18:3n-3, which reacts equally well, could not be included. The C_{20} and C_{22} fish oil fatty acids have ω3 structures (n-3, n-6, n-9), and would also react (Beare-Rogers and Ackman, 1969). More recently, the ω3 fatty acids have been "rehabilitated" (Lands, 1986; Bjerve et al., 1987; Hunter, 1990) as "essential," a belated and long-overdue recognition (Holman, 1998).

For a decade or more, packed-column GLC (Conacher and Iyengar, 1978) was poorly suited for the specific C_{18} fatty acid types, with *trans*-isomers resulting from partial hydrogenation even with the cyanosilicone liquid phases (Ackman, 1986; Lercker et al., 1987; Ackman and Ratnayake, 1989), but more recently, the introduction of the FFSC has stimulated the use of capillary GCL (Lercker et al., 1987; Ackman and Ratnayake, 1989). The presence of simple geometrical isomers of all-*cis*-18:3n-3 (and of all-*cis*-18:2n-6) in deodorized vegetable oils was documented with capillary GLC over a decade ago (Ackman et al., 1974). These may have hitherto unforeseen biochemical

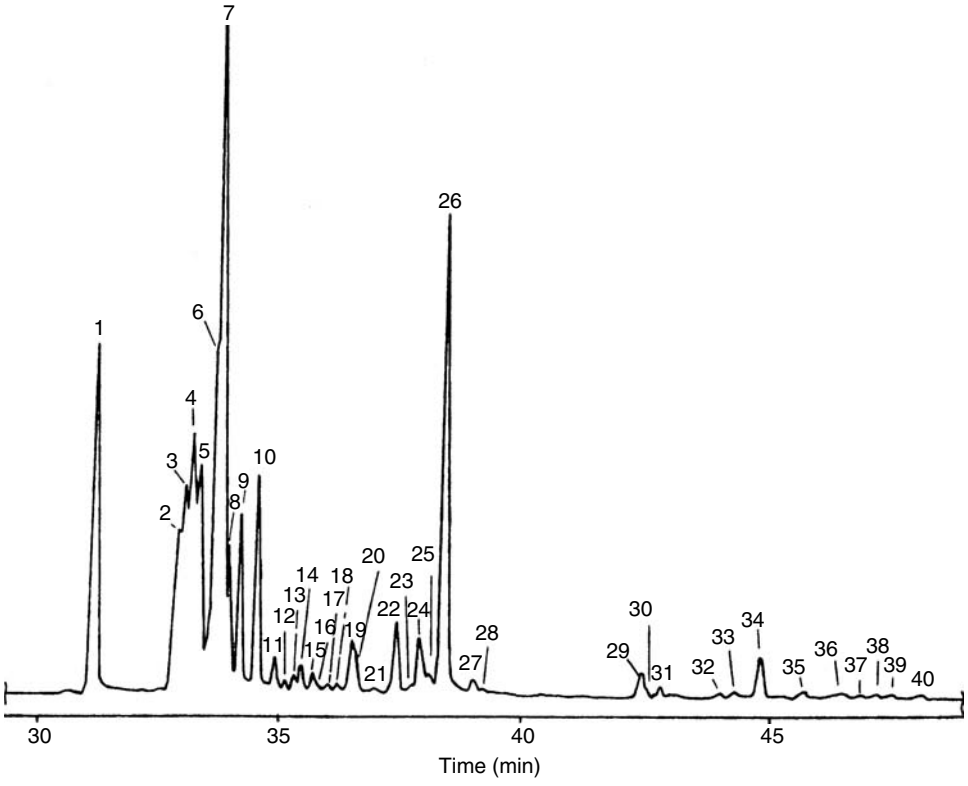

FIGURE 3.4 The C_{18} region of the gas chromatogram of the fatty acid methyl esters of soybean oil margarine. Analysis on an SP-2560 FFSC column (100 m × 0.25 mm i.d.). Numbers correspond to 1, 18:0; *2*, 18:1Δ6–8t; *3*, 18:1Δ9t; *4*, 18:1Δ10t; *5*, 18:1Δ11t; *6*, 18:1Δ12t; *7*, 18:1Δ9c + 18:1Δ13t + 18:1Δ14t; *8*, 18:1Δ10c; *9*, 18:1Δ11c; *10*, 18:1Δ12c + 18:1Δ15t; *11*, 18:1Δ13c; *12*, 18:2tt; *13*, 18:1Δ14c; *14*, 18:2tt; *15*, 18:1Δ15c; *16*, 18:2tt; *17*, *18*, *20*, *21*, 18:2tc/ct; *19*, 18:2Δ9t, 12t; *22*, 18:2Δ9c, 12t; *23*, ?; *24*, 18:2Δ9t, 12c; *25*, ?; *26*, 18:2Δ9c, 12c; *27*, 18:2Δ9c, 15c; *28*, ?; *29*, 20:0; *30*, 18:3?; *31*, 18:3Δ9c, 12c, 15t; *32*, 18:3Δ9t, 12c, 15c; *33*, 18:3Δ9c, 12t, 15c; *34*, 18:3Δ9c, 12c, 15c; *35*, 20:1; *36*–40, 18:2 conjugated? (Reproduced from Ratnayake, W.M.N., et al. (1990). *J. Am. Oil. Chem. Soc*. *67*: 804–810. With permission.)

properties when extended to the C_{20} and C_{22} chain lengths (O'Keefe et al., 1990). An appropriate AOAC interlaboratory collaborative study of margarine materials has now been executed by de Palma (Firestone, 1990). Two papers by Ratnayake and Beare-Rogers (1990) and Ratnayake et al. (1990) provide in-depth reviews of all facets of the particular problems caused by the overlap of certain *cis*- and *trans*-isomers. Other comprehensive material on margarine analyses is provided by Mossoba et al. (1990, 1993).

The total analysis of methyl esters of fatty acids from partially hydrogenated fat samples by GLC requires a column of special polarity (Figure 3.4), usually a cyanosilicone such as a Supelco SP-2560 (Ratnayake et al., 1997) or chrompack CP-Sil-88 (Chrompack, Middleburg, the Netherlands) (Wolff et al., 1995). It is the length that often deters analysts, since 60 m is good but 100 m is even better (Wolff and Bayard, 1995). Unfortunately very detailed analyses then run to 2 h. Although much useful information on the sample can be extracted, a significant portion of late-eluting 18:1 fatty acid isomers will be hidden under early-eluting *cis*-isomers (Figure 3.5). Auxiliary fractionation is then required (Ratnayake, 1995; Wolff et al., 1995). Curiously, among the *trans* 18:1 isomers, the butyl esters of *trans*-14-18:1 and *trans*-15-18:1 coincide on a polyglycol-based column (Figure 3.6), but when the isopropyl or methyl esters are used, *trans*-13-18:1 and *trans*-14-18:1 coincide on the cyanosilicone CP-Sil 88 column (Figure 3.7).

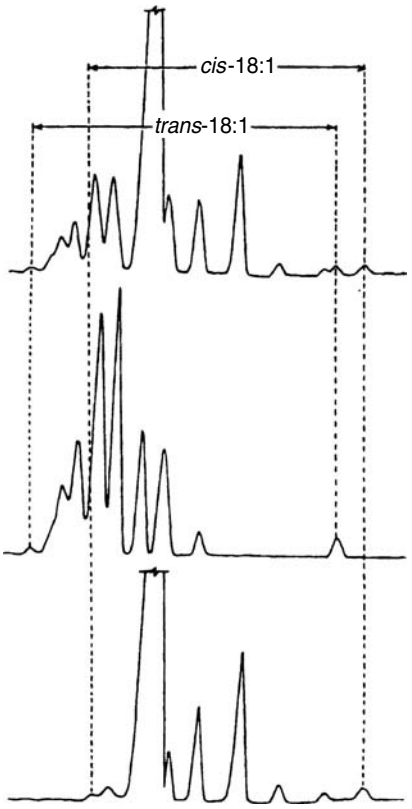

FIGURE 3.5 Partial chromatograms of the isopropyl esters of fatty acids of partially hydrogenated soybean oil (top). Included are the same esters after fractionation on silver nitrate TLC plates to give the *trans*-18:1 acids (center) and *cis*-18:1 acids (bottom), illustrating the extent of overlap. Column, 100 m × 0.25 mm, CP-Sil 88 coated, at 160°C. (Reproduced from Wolff, R.L., and Bayard, C.C. (1995). *J. Am. Oil Chem. Soc. 72*: 1197–1201. With permission.)

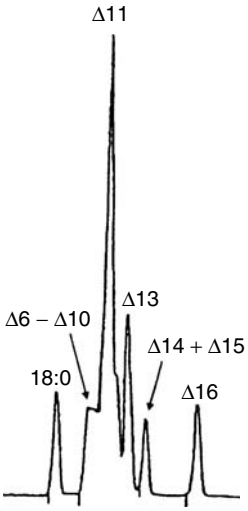

FIGURE 3.6 Partial gas–liquid chromatogram of butyl esters of *trans* monoethylenic fatty acids of butter fat as isolated by $AgNO_3$-TLC. Column 30 m × 0.25 mm, Omegawax-320; note coincidence of Δ14 and Δ15. (Adapted from Ackman, R.G., and Macpherson, E.J. (1994). *Food Chem. 50*: 45–52.)

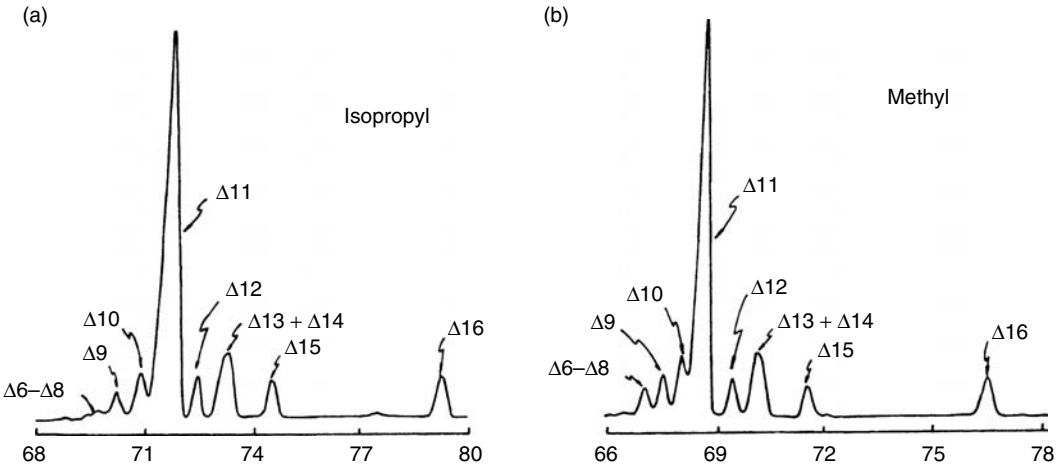

FIGURE 3.7 Comparison of chromatograms of isopropyl and methyl esters of *trans*-18:1 acids isolated from beef tallow (a and b, respectively). Analyses were done on a CP-Sil 88 fused-silica capillary column 100 M × 0.25 mm; note similarity of profiles. (Reproduced from Wolff, R.L., and Bayard, C.C. (1995). *J. Am. Oil Chem. Soc. 72*: 1197–1201. With permission.)

VIII. CONJUGATED LINOLEIC ACID

Recently, a spate of papers on conjugated linoleic acid (CLA) has appeared. One of the interesting aspects is that there are two major isomers (Figure 3.8) if CLA is produced chemically from linoleic acid, respectively, *cis*-9, *trans*-11- and *trans*-10, *cis*-12-octadecadienoic acids (Christie et al., 1997). However, with *in vivo* biosynthesis, in ruminants the *cis*-9, *trans*-11-octadecadienoic isomer dominates and is that commonly reported for the human body (Fritsche et al., 1998) because of our consumption of beef and dairy products. Synthetic and biological samples need to be distinguished (Ackman, 1997; Christie et al., 1997). CLA is not increased in the human body by adding a ready

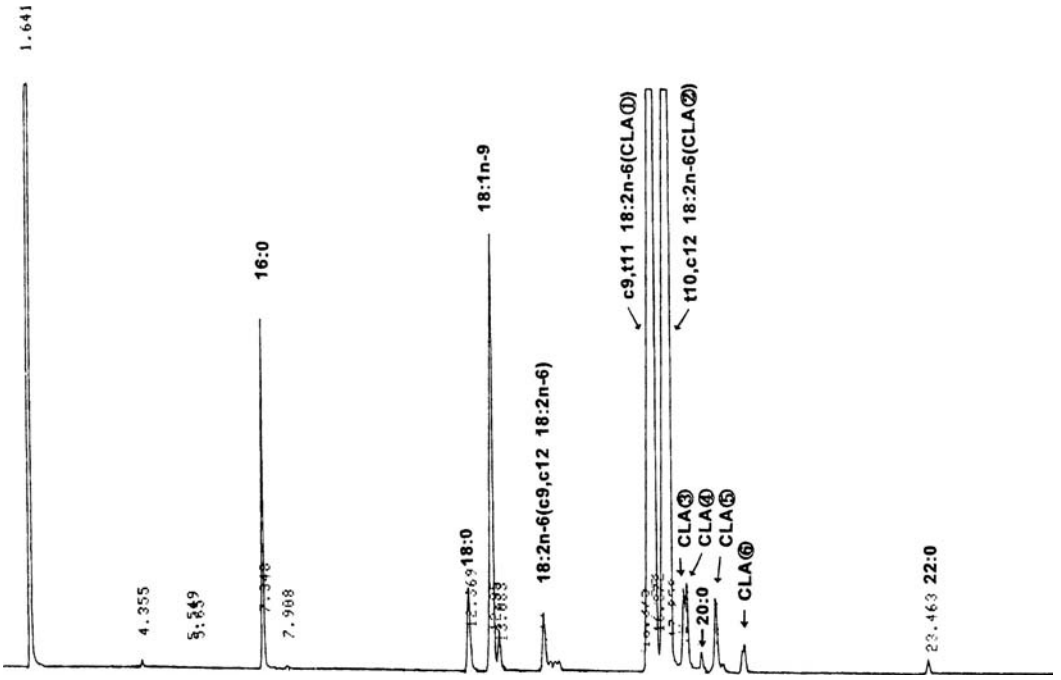

FIGURE 3.8 Gas–liquid chromatographic analysis of methyl esters of fatty acids of safflower oil after alkali isomerization to produce CLA from linoleic acid. Column, Omegawax-320. (Courtesy of S.J. Kang, Gyeongsang University, Korea.)

source of *cis*-9, *cis*-12 linoleic acid such as safflower oil to the diet (Herbel et al., 1998). Therefore, foods appear to be the principal sources of CLA. On GLC, they elute a little later than other C_{18} fatty acid esters (Figure 3.8). Overheating of homemade standards in alkali can produce many additional isomers (Mounts et al., 1970; Christie et al., 1997; Ackman, 1998), and careless saponification of lipids can also produce extra peaks of CLA (Ast, 1963). Special care is needed for methyl ester preparation from lipid samples if CLAs are suspected (Shantha et al., 1993; Kramer et al., 1997). In general, the analysis of CLA is difficult and should not be taken lightly (Sehat et al., 1998). This almost automatically includes all dairy products and human milks (McGuire et al., 1997; Jensen et al., 1998; Lavillonnière et al., 1998; Lin et al., 1998).

IX. OTHER LIPIDS

Food scientists may occasionally have a need to examine minor food ingredients—for example, mono- and diglycerides or even triglycerides—by using GLC. The modern FFSC is very well suited to these requirements, but because higher temperatures are involved, only "bonded" liquid phases should be considered. Many examples of this type of analysis have been published (Ackman, 1986), and it is only necessary to select an appropriate commercially available column because the GLC units manufactured in the past decade should already meet requirements. It is probable that thick liquid-phase column coatings should be avoided to keep temperatures as low as possible. In particular, peak asymmetry can indicate either too low a temperature or column overload.

The basic requirement for GLC analyses is that the analyte be volatile. Alternatives may have to be sought for materials of low volatility; an example of the combination of the sensitivity of the FID of GLC with the efficiency of thin-layer chromatography (TLC) is the Iatroscan-Chromarod combination (Ackman, 1981; Ackman and Ratnayake, 1989; Hara et al., 1989; Walton et al., 1989).

A recent development now widely used in this type of work is Fourier transform infrared (FT-IR) as an attachment to a gas chromatograph (Mossoba et al., 1993; Wolff and Sébédio, 1994). It can classify methyl esters of fatty acids as *cis* or *trans* (Mossoba et al., 1993), including those geometrical isomers of the *cis* bonds of polyunsaturated ethylenic fatty acids such as α-linolenic (Chardigny et al., 1996). The biochemical consequences of elongation products of such acids are almost unknown (O'Keefe et al., 1990; Grandgirard et al., 1994; Chardigny et al., 1995). The technology is of course in wider use with other types of samples (Mossoba et al., 1995).

To resolve problems of positional ethylenic bond identification, many laboratories can now benefit from quadrupole mass spectrometers fitted directly on the gas chromatograph. Nitrogen compounds at the carboxyl group such as picolinyl or pyrrolidide derivatives stabilize the bond so that the position can be identified (Harvey, 1992), and they were initially popular. The latter derivative has been applied to several foods, with preparation being accelerated by microwave irradiation (Dasgupta et al., 1992). Another nitrogen base, 4,4-dimethyloxazoline (DMOX), may give better gas chromatographic patterns, similar to that of the methyl esters, and is easy to work with (Lamberto and Ackman, 1994). As shown in Figure 3.9, a "gap" of 12 amu that usually fixes the bond position is *not* observed for fatty acids with an ethylenic bond in the 3-position of *cis* or *trans* esters. *Trans*-3-16:1 is found in photosynthetic parts of green vegetables and could be present in ruminant fats. Figure 3.10, of a *trans*-2-16:1 DMOX derivative, an artifact of the DMOX derivative preparation (Lamberto and Ackman, 1995), shows the expected 12 amu gap between fragments 98 and 110. This has been successfully applied with DMOX derivatives of polyunsaturated fatty acids and other structures in heated fats and oils (Mossoba et al., 1995).

Some lipid-type materials such as vitamins may be better analyzed by TLC or HPLC than by GLC. Progress in all such methods is erratic, but research on methods often gradually becomes commercial practice (Poole and Poole, 1989), much as the early flexible-fused silica GLC column held great promise but had "teething" problems (Ackman, 1981). Progress is inevitable, and food science has lagged behind some other fields in applying modern technology. There is no excuse for this in the application of GLC to fatty acids.

The preponderance of omega-3 fatty acids is going to be influenced slightly in the case of farmed fish. There is an apparent growing problem in the overfishing of the oceans, which creates some shortages in the feeds needed by the aquaculture industry. The GC in Figure 3.3 specified that it

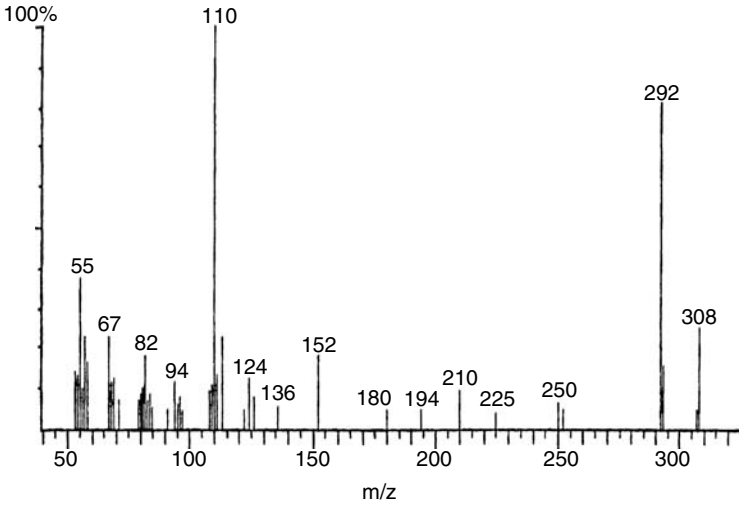

FIGURE 3.9 Electron impact mass spectrum of the GC peak of the 4,4-dimethyloxazoline derivative of natural *trans*-3-hexadecenoic acid. (Reproduced from Lamberto, M., and Ackman, R.G. (1995). *Anal. Biochem. 230*: 224–228. With permission.)

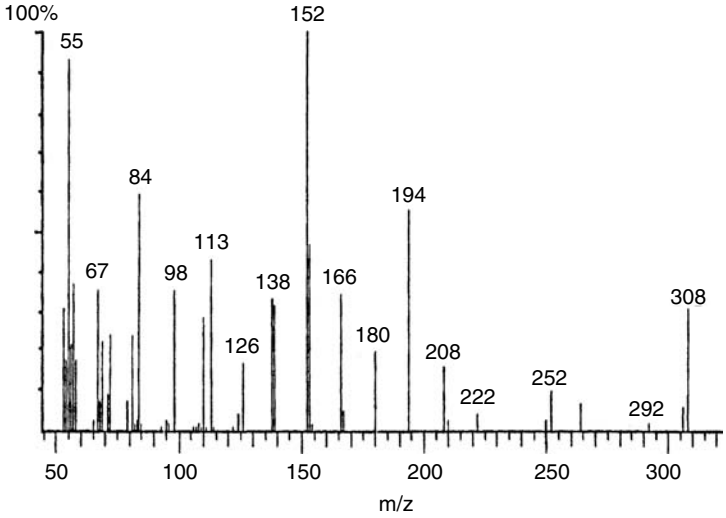

FIGURE 3.10 Electron impact mass spectrum of the GC peak of the 4,4-dimethyloxazoline derivative of an artifact 2-hexadecenoic acid. Fragments of 98 and 110 amu indicated the ethylenic bond position. (Reproduced from Lamberto, M., and Ackman, R.G. (1995). *Anal. Biochem. 230*: 224–228. With permission.)

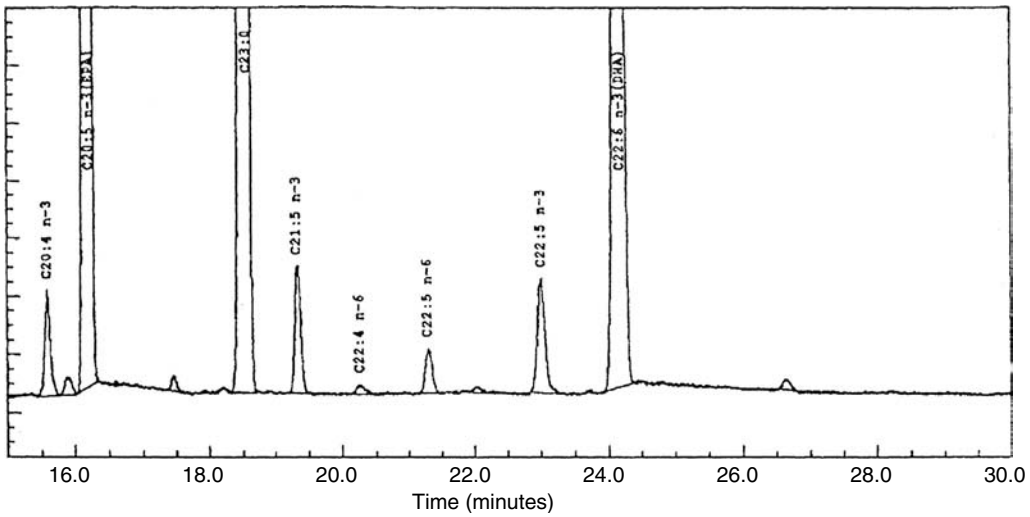

FIGURE 3.11 A contemporary gas–liquid chromatogram of the separations possible in a short time-frame of the methyl esters of typical fish oil of the anchovy or sardine type. The peak numbers identify specific fatty acids. The small peak marked 26 has been identified by the author as 24:1n-11, followed by the isomer 24:1n-9. In the explanation of the numbers EPA is 20:5n-3 and DHA is 22:6n-3. Adapted from European Pharmacopoeia 5.4 (2005).

was an analysis of (European) farmed salmon, presumably Atlantic salmon *Salmo salar*. Peaks in Figure 3.3 represent the fatty acids of an oil, but in fact salmon as a food may have a body rich in triacylglycerols, as it has always been ranked as an "oily" fish and therefore a desirable food source of omega-3 fatty acids. Unfortunately the high price of fish meal and scarcity of crude fish oil have led to increasing amounts of vegetable feed stuffs being added to the rations of farmed salmon (Pickova, 2006). Depending on the type of vegetable material fed, peaks 8 (18:2n-6) and 9 (18:3n-3) may be somewhat increased in farmed salmon lipids. Since these fatty acids are common in our diets

anyway, the effect may be only a slight dilution of the long chain fatty acids omega-3 present in wild salmon. There are at least five types of salmon to be considered (Ackman, 1996), but the general pattern for all fatty acids of edible fish should conform to that in Figure 3.11.

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4 Isotopic Methods for Assessing Lipid Metabolism

Joanne K. Kelleher, Carolina B. Cabral, and Jennifer M.K. Cheong

CONTENTS

I. INTRODUCTION

In recent years, new stable isotopic methods have been developed that enhance our capabilities to assess metabolism (Brunengraber et al., 1997). These newer methods complement and, in some cases, exceed the capabilities of more traditional radioisotopic methods. The major advantage of the new methods is the improved ability to estimate the enrichment of a precursor during biosynthesis leading to more accurate estimates of metabolic rates. To put this new information in perspective, this chapter presents a tutorial describing the underlying basis of isotopic methods for the analysis of lipid metabolism. We show how the new stable isotopic methods differ from radioisotopic methods and explain the rationale for choosing among various types of isotopic tracers in specific situations. The goal of this presentation is not limited to the fate of fatty acids in foods. We also include a discussion of methods for assessing the endogenous production of lipids *in vivo*. The literature citations offer examples of interesting applications but are not meant as an exhaustive

survey of all the noteworthy work in this field. We bring to this discussion the viewpoint of metabolic physiologists who think in terms of pathways. To deal with the complexities of dietary lipid metabolism, the classic definition of a pathway is expanded to include all processes from ingestion of food to oxidation. Thus, the major metabolic pathways of a dietary triglyceride involve many steps—oral ingestion, digestion, hydrolysis of the triglyceride, intestinal transport, chylomicron synthesis, export to plasma, flux through various lipoproteins, uptake by cells, recirculation through the liver, and ultimately oxidation. To lay the groundwork for the tutorial, we begin with a review of the key concepts underlying the isotopic methodology used in metabolic studies.

A. Compartments and Pathways

What is a pathway? A pathway is a set of fluxes related by precursor product relationships and regulated as a unit. A diagram, often called a metabolic network, defines the scope of the pathway under study (Figure 4.1). Note that a flux is simply the rate molecules travel along the routes designated by the arrows (mol/time). The metabolic network is important as the arrows represent allowable fluxes and the metabolites, shown enclosed, represent compartments. The classic discipline of compartmental analysis (Jacquez, 1985) provides a rigorous framework for analyzing a metabolic pathway. In this approach, a compartment is defined as a population of molecules that behave kinetically in a homogeneous manner. Each molecule in a compartment has the identical probability of participating in each of the reactions leading from the compartment. It is useful to contrast the definitions of compartment and the more commonly used term, *pool*. The definition of compartment is more precise than the definition of a pool. For example, we may speak of the total body triglyceride pool. However, this pool comprises a number of compartments, including, for example, plasma very low density lipoprotein (VLDL) triglyceride and hepatic intracellular triglyceride. Compartments may comprise more than one metabolite. For example, if lactate and pyruvate are rapidly interconverted because of high lactate dehydrogenase activity, lactate and pyruvate molecules may be equally likely to flow to lipid synthesis, and thus only one compartment may be needed for the lactate–pyruvate compartment. The key to defining a compartment is that all molecules in the compartment must be equally likely to leave by any of the paths permitted. By designating compartments and setting up the metabolic map, a hypothesis is provided for how the system interacts. This hypothesis may be then investigated experimentally. An important feature of compartmental models is that the fluxes in and out of the compartments describe the probability of a molecule from each compartment reaching any other compartment. For example, from Figure 4.1, it is clear that a molecule of A has a probability of 1.0 of reaching compartment B, a probability of 0.5 of reaching compartment C, and a probability of 0.25 of reaching compartment D. The use of probabilities in describing compartmental models is a powerful asset for obtaining information from the model. To obtain this information, all terms used to

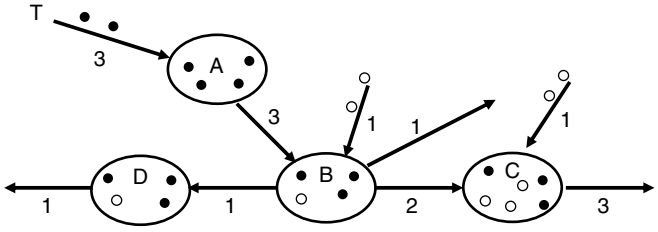

FIGURE 4.1 A metabolic network displaying essential features of compartmental model for tracer metabolism. Tracer atoms (filled circles) enter the pathway at compartment A. Natural molecules, not enriched with isotope (open circles), the tracee, enter the pathway at various points. Arrows indicate permitted fluxes and numbers indicate fluxes in moles/time. The isotopic distribution shown reflects metabolic and isotopic steady state, fluxes in equal fluxes out, and amount of tracer and tracee in each compartment is constant.

quantify the behavior of the model must be consistent with probability theory. Thus, throughout this chapter, we use fractions rather than percentages. Fractions sum to 1 and reflect probabilities.

Compartmental models may be used to examine metabolism in various kinetic states. The simplest kinetic state is that of the metabolic and isotopic steady state. In the metabolic steady state, the fluxes and amounts of each metabolite in each compartment are constant. End products may accumulate linearly. In the metabolic steady state, each arrow can be assessed a relative flux rate that does not change over the course of the time period we are studying. In the isotopic steady state, the amount of isotope and the amount of metabolite in each compartment are constant (see Figure 4.1). If the system reaches both the metabolic and isotopic steady state, the model is linear and the equations describing the behavior of isotopic tracers are algebraic, making the analysis relatively straightforward. This ideal may contrast with the real experience of researchers determining the fate of dietary lipids which rapidly flow into a system altering fluxes and concentrations and depart. A model describing these complex, more realistic, processes may be nonlinear and require differential equations to characterize the system. Thus, in designing isotopic tracer experiments, the investigator is faced with the dilemma of determining to what extent a real experiment may be depicted by a relatively simple steady state model. Additionally, it should be noted that compartmental models are most useful when pathways can be described by a series of discrete steps. Complications may arise when the composition of a metabolic pool changes gradually, as when a metabolite is removed from plasma across an organ to create a gradient. These more complicated situations require more sophisticated models that are beyond the scope of this chapter.

II. ISOTOPIC TRACERS

A. General Properties

By way of review it is useful to recall a few fundamental facts about isotopes as tracers. The word *trace* may have two meanings: to track a pathway or to be present in very small amounts. Both meanings are involved in the application of tracers to analyze metabolism. Tracers, stable or radioisotopes of specific compounds, offer an enticing approach to the quantitative metabolic pathways. They provide a simplified way to study complicated pathways and may replace the considerable effort of mass balance analysis. Tracers are normally assumed to behave identically to their naturally occurring counterpart, the "tracee." A tracer is a molecule containing isotopes of specific elements, which differ in atomic mass but not in the number of orbiting electrons. Isotopes may be stable at this higher mass (^{2}H, ^{15}N, and ^{13}C) or radioactive beta and gamma emitters (^{14}C, ^{131}I, and ^{3}H) decaying with a characteristic half-life. Radioactive compounds such as ^{11}C, ^{13}N, and ^{15}O (positron emitters) offer the advantage that they can be used for imaging studies. Offsetting this advantage is their short half-lives, less than 20 min, which necessitates specialized facilities for preparation and analysis. For lipid metabolism it is notable that metabolic tracers are available in either of two major elements, hydrogen and carbon, both as stable isotopes (^{2}H and ^{13}C) or as long-lived beta emitting radioisotopes (^{3}H and ^{14}C). Additional stable isotopes, including ^{18}O, and radioisotopes, such as ^{31}P, are also useful in assessing lipid metabolism. Metabolic tracers are assumed to be ideal, that is, to behave identically to the naturally occurring tracee in metabolic reactions. An ideal tracer then has the following characteristics. First, the amount of the tracer administered must not perturb the metabolic pathway such that the rates are changed. Thus, either the concentration of the tracer entering the metabolic pathway is low relative to the tracee concentration or the tracer substitutes for tracee. An example of this second situation is when tracer lipid is placed in a diet or total parenteral nutrition (TPN) solution and equivalent tracee removed. Second, the metabolic properties of the tracer molecules must be identical to those of the tracee. Thus, isotope effects must be insignificant. Many useful experiments are performed with tracers that are not ideal; however, the best experiments are those designed by investigators conscious of the shortcomings of the chosen tracer.

The very first isotopic studies of lipid metabolism illustrated the question of whether the tracer is ideal. In 1949, Stanely and Thannhauser investigated fat malabsorption using ^{131}I-labeled olive oil, chiefly triolein. The principle behind the isotopic approach was that absorbed ^{131}I from the radiolabeled lipid would be detected in urine and blood. Thus, a low level of ^{131}I in urine and blood indicated fat malabsorption. This isotopic procedure was, in principle, much simpler than the standard nonisotopic method of the day, which required a 3-day diet, quantitative stool collection, and fat analysis of these samples. However, this simple concept for the isotopic assay did not easily translate into a true quantitative method for detecting fat malabsorption in human subjects. The tracer atom, ^{131}I, is of course not a natural component of triolein. The fate of absorbed iodine as a component of iodinate lipids and the biochemical properties of iodinate lipids were not clearly understood at the time. Furthermore, the handling of iodine absorbed in this manner by the kidney was later found to be variable (Oddie et al., 1966). Clearly, iodinated fatty acids did not represent an ideal tracer. Despite the problems with the iodine tracer, this early method established the principle of using isotopes to evaluate fat absorption. In this chapter, we will assume that isotopic effects, such as the substitution of ^{2}H or ^{3}H for H, are negligible in determining fluxes.

B. Tracer Activity

To perform a tracer metabolic study, the investigator needs a method in hand for quantifying the amount of the tracer molecule in one or more key compartments. Consider as an example the fate of labeled palmitate triglyceride infused intravenously. By measuring the appearance of the tracer in various compartments and the total concentration (tracer + tracee), the investigator obtains a record of the fate not only of the labeled molecules given orally but also of the tracee traveling in parallel through the pathway. Tracee molecules generated endogenously may enter the pathway at various points, as shown in Figure 4.1. The relationship between the tracer and tracee molecules in a compartment is referred to as the *specific activity* (SA) in radioisotopic studies. Stable isotopic studies sometimes use the term *enrichment*, but we wish to define here the term *tracer mole fraction* (TMF), which may be more useful for metabolic studies using stable isotopes. In the past, relationship between the tracer and tracee has focused on the tracer to tracee ratio. Because our goal is to build a probability-based system for the analysis of metabolism, this ratio is not employed. Instead, we define for stable isotopes the TMF

$$\text{TMF} = \frac{\text{Tracer molecules}}{(\text{Tracer molecules} + \text{Tracee molecules})}$$

and for radioisotopes the SA

$$\text{SA} = \frac{k * \text{Tracer molecules}}{(\text{Tracer molecules} + \text{Tracee molecules})}$$

where k is a constant expressing the inherent activity of the tracer in either curies, becquerels, or disintegrations per minute (dpm)/per mole. For simplicity, we use dpm; 1 μCi = 2.22×10^6 dpm. The term TMF is used to indicate the relative amount of stable isotopic tracer molecules, independent of the amount of labeled atoms. We use these two terms to describe molecular equivalents for comparing radioisotopes and stable isotopes. At first glance, the two expression may not appear to be equivalent. The numerator of the SA relationship is dpm, a convenient measure of radioactivity, but not equivalent to tracer molecules, the numerator of the TMF equation. However, dpm can be converted to tracer molecules by dividing the SA of the tracer used. The SA of the infused tracer, in dpm/(mole tracer), is the link that relates SA to a true measure of tracer/(tracer + tracee). Thus, it is always important to perform this operation and present radioisotopic tracer data in terms of molecules in specific compartments. TMF or SA provides important insight into the rates of the

reactions in the pathway. Clearly, if tracee is present, the SA or TMF of the tracer will decrease as it travels through a pathway (see Figure 4.1). If no tracee is present, the SA or TMF at the exits from the pathway will equal that of the infusion.

The above definitions illustrate important properties of tracers as used in metabolic studies. First, the definitions are consistent with probability theory. TMF is the probability that a molecule selected randomly from a compartment is a tracer. SA is directly proportional to this probability. In contrast, the tracer-to-tracee ratio approaches the probability only if the tracer amount is very small relative to the tracee. Often in stable isotope studies, the tracer is of significant mass and tracer/tracee is not equivalent to probability. Second, the proposed definitions emphasize the fact that we are concerned with labeled molecules. An individual tracer molecule may contain one or more isotopically labeled atoms, but metabolic pathways concern the flow of molecules. Thus, definitions of tracer activity in terms of molecules are essential. Finally, before moving to specific types of tracers, we underscore that, in theory, either stable isotopes or radioisotopes may be used to trace metabolic pathways. The underlying tracer theory is equivalent. Yet, in practice, each type of tracer has distinct properties that are best for particular applications. To choose an appropriate tracer for a metabolic study, an investigator needs to understand these issues. To explain this to students approaching this subject for the first time, we stress that the differences between stable isotopes and radioisotopes arise on account of the way isotopically labeled molecules are detected. To continue this tutorial, we review the aspects of detection of radioisotopes and stable isotopes that lead to these differences.

C. Radioisotopes

Radioisotopes were the commonly used type of tracer in the growth of biomedical research in the 1950s and 1960s. Radioisotopes emit radiation in spontaneous nuclear reactions decreasing mass. Emitted radiation provides a powerful signal from each decaying atom. This signal provides a sensitive method for the detection of radiolabeled molecules and provides a major reason why radioisotopes have been preferred for many studies. For example, a liquid scintillation counter can detect 20 dpm above background for ^{14}C. Molecules with one ^{14}C-labeled atom are routinely available at 50 Ci/mol or 100×100^{12} dpm per mole. This means that an investigator can easily detect the presence of 0.02 pmol (100 dpm) of this ^{14}C-labeled compound. The purchaser of radioisotopes notices this sensitivity when calculating the amount of compound purchased. If 50 μCi of [1-^{14}C]palmitic acid is purchased at an SA of 50 Ci/mol, the amount of palmitic acid obtained is only 1 μmol. Thus, ^{14}C-labeled tracer lipids are often diluted in SA with natural compounds to perform studies at physiological concentrations without consuming large amounts of isotope. Table 4.1 illustrates a simple example, where an investigator wishes to expose cells to palmitic acid tracer and follow it into triglycerides. An important advantage of radioisotopes is that the detection of radioisotope tracers, using liquid scintillation counting or gamma counting, is not affected by the presence of unlabeled trace molecules. The natural abundance of radioisotopes is negligible; there is no background signal to consider, and thus the tracer can be detected even if the SA is very low. However, the offsetting disadvantage is that detection of radioisotope dpm does not confirm the identity of the labeled molecule. A second assay is required to obtain (tracer + tracee), the denominator in the SA equation. The accuracy of the resulting SA measurement is a function of the error in both methods.

When radioisotopes are used, ^{14}C-labeled lipids are often chosen over ^{3}H-labeled ones for metabolic studies, because ^{14}C is less likely to be lost in isotopic exchange reactions not related to the metabolic pathway. Also, ^{14}C is a stronger β-emitter and is less likely to be quenched, complicating the measurement of the dpm. On the other hand, an advantage of ^{3}H isotopes for some studies is that the maximum SA is higher for ^{3}H than for ^{14}C. The upper limit for SA is determined by the rate of decay or half-life. An interesting student exercise is to compare the maximum SA possible for a ^{14}C atom with a half-life of 5730 years to that of ^{3}H with a half-life of 12.3 years. The result for ^{14}C is that the maximum SA, with each atom ^{14}C labeled, corresponds to an SA of about 62 Ci/mol carbon. Thus, when [U-^{14}C]palmitate is purchased at 800 Ci/mol, about 80% of the palmitate C atoms are ^{14}C

TABLE 4.1
Calculation of ^{13}C and ^{14}C Palmitate Requirements for Isotopic Study

	^{14}C Study	^{13}C Study
Isotope	[U-^{14}C]palmitate	[1,2,3,4-^{13}C]palmitate
Activity	800 Ci/mol	99% ^{13}C
Amount of isotope	^{14}C 1 μCi = 1.25 nmol	150 nmol
Amount of natural palmitate	298.75 nmol	150 nmol
Total nanomoles palmitate/mL	300	300
Final SA or TFM of tracer	2.22×10^6 dpm/300 nmol	50%
Expected result in TG palmitate	444,000 dpm [U-^{14}C]palmitate	15% [1,2,3,4-^{13}C]palmitate
TG palmitate from tracer	4.44×10^5 dpm/(2.2×10^6 dpm/300 nmol)	30% TG palmitate from tracer * 200 moles
Amount of new TG palmitate from tracer	60 nmol TG palmitate	60 nmol TG palmitate

Conditions: Isolated cells to be suspended in 1 mL of medium containing isotopically labeled palmitate to determine incorporation into triglycerides (TG) over a 24-h period. Tracer concentration 0.3 mM fatty acid bound to albumin. Estimated incorporation: approximately 20% of label accounting for 30% of TG palmitate in the cells, since the cells contain 200 nmol of TG palmitate.

^{14}C *experiment:* Using 1 μCi [U-^{14}C]palmitate as tracer. Tracer is obtained at SA = 800 Ci/mol. Since 20% may be incorporated, adequate results should be obtained using 1 μCi; expected triglyceride incorporation will be $0.2^* 2.22 \times 10^6$ dpm = 444,000 dpm. However, 1 μCi of this tracer is only 1.25 nmol. For a 1 mL incubation, 300 nmol is required. Thus, add 298 nmol unlabeled palmitate for each milliliter of ^{14}C medium.

^{13}C *experiment:* Using [1,2,3,4-^{13}C]palmitate as tracer. Tracer is 99% ^{13}C at each labeled position. If incorporation labels 30% of TG palmitate in cells, tracer could be diluted 50% with natural palmitate to conserve isotope and still provide accurate detection of labeling.

rather than ^{12}C. It is easy to lose track of this fact, because the measurement of radioisotopes detects only those few atoms that decay in the scintillation counter. The shorter half-life of ^{3}H, greater dpm per labeled atom, is the key to understanding why ^{3}H-labeled compounds are available at SAs 100 times or more greater than ^{14}C. Thus, ^{3}H rather than ^{14}C-labeled tracers are often selected for radioimmunoassays and other sensitive assays that detect femtomoles or less of a compound.

D. Stable Isotopes

In the past decade, the use of stable isotopes as metabolic tracers has increased dramatically. Availability of specific stable isotopic tracers for metabolic studies now rivals that of radioisotopes. As with ^{14}C isotopes, ^{13}C-labeled tracer molecules are commercially available with high enrichments; routinely 99% of the specified carbon atoms of a molecule will be labeled. An advantage of stable isotopes in clinical studies is the absence of a radiation hazard and disposal issues. Although radioisotopes are detected only by decay of atoms, stable isotope may be detected by methods that report either the labeled atoms or labeled molecules. Stable isotopically labeled molecules may be combusted to atoms such as a mixture of $^{13}CO_2$ and $^{12}CO_2$. However, more information is available when stable isotopes are detected in organic mass spectrometry as molecules. Organic mass spectrometry for metabolic studies is typically accomplished by gas chromatography/mass spectrometry (GC/MS) or high-performance liquid chromatography/mass spectrometry (HPLC/MS). Organic mass spectrometry yields both the number of labeled molecules and the number of labeled atoms in each labeled molecule. To characterize this increased information, the term *isotopomer*, isotope isomer, is used to denote the number of labeled atoms in a molecule. In metabolic research, molecules with ^{12}C, ^{16}O, and ^{1}H at each position are represented by the term *mass + zero*, $m + 0$, or M_0. A molecule

with n heavy isotopes is designated mass + n, or M_n. Continuing with the example of palmitic acid as the tracer, mass + 0 corresponds to mass 270, the molecular weight of palmitate methyl ester. As the user of GC is aware, fatty acids are commonly derivatized to their methyl esters for GC. To simplify the mass distributions shown below, the methyl ester is omitted. Although 16 positional $m + 1$ isotopomers of palmitate may occur, mass spectrometry of the palmitate molecule detects each of them as M_1. In general, the detection of mass isotopomers by organic mass spectrometry does not directly report positional information. The exception to this generalization occurs when molecules are fragmented in the ionization process. Isotopomer patterns of these fragments may be used to provide positional information. An important advantage of organic mass spectrometry is the information indicating the number of labeled atoms in each molecule detected, the mass isotopomer distribution (MID) pattern.

A complication in interpreting the MID is the natural abundance of ^{13}C. Unlike ^{14}C and other radioisotopes, which are not present naturally, ^{13}C is both a useful tracer and a naturally occurring isotope. Approximately 1.1% of atmospheric carbon is ^{13}C rather than ^{12}C. Other stable isotopes appearing in lipids (^{17}O, ^{18}O, and ^{2}H) are much less abundant in nature, and thus the probability of isotopomers greater than M_0 in naturally occurring fatty acids is approximated by 0.011 times the number of carbon atoms. Natural palmitate produces a fractional MID of $M_0 = 0.84$, $M_1 = 0.15$, and $M_2 = 0.01$, all due to natural ^{13}C abundance. A comparison of the MID for natural palmitate (containing the 1.1% natural abundance of ^{13}C) and a mixture containing ^{13}C-enriched palmitate is shown in Figure 4.2. In describing the MID an important distinction is between "enrichment" and "abundance." Enrichment indicates an amount above the natural background, whereas abundance usually includes the natural background.

Investigators using enrichment subtract the natural abundance from the raw GC/MS data and then proceed to calculate. Although this process may simplify the appearance of the MID, we advocate retaining the natural abundance of molecules as part of the MID. In this way, the data may be fitted to models that have different assumptions about the natural abundance effect. Hence, to indicate the amount of each isotopomer, we prefer the term *fractional abundance*, the amount of a specific isotopomer over the sum of all isotopomers. Fractional abundance is directly obtained from the specific ion monitoring mass spectrometry data by dividing the signal intensity for each isotopomer by the sum. More formally, the fractional abundance of an isotopomer mass + i of a molecule M with a maximum number of heavy isotope positions of n is defined as the relative amount of M_1 detected by organic mass spectrometry over the sum of all isotopomers or,

$$\text{Fractional abundance} = \frac{M_i}{\sum_{i=0}^{n} M_i}$$

Again, it is important to note that this definition requires that the sum of the fractional abundances of all isotopomers equals 1 so that fractional abundance is equivalent to the probability of the occurrence of each mass isotopomer. This relationship with probability is not present when isotopomer amounts are expressed as atom or mole percent excess, a common terminology used in the past. Also, it should be noted that some investigators use the term *molar enrichment,* defined as the sum of the fractional abundances for each isotopomer multiplied by the number of labeled carbons or

$$\text{Molar enrichment} = \sum_{i=0}^{n} M_i * i$$

Molar enrichment is useful to predict the relationship between radioisotopes and stable isotopes.

The MID for the stable isotope tracer described in Table 4.1 is shown (Figure 4.2b) to indicate the fact that a stable isotope tracer is really a population of isotopomers with a specific MID that is not identical to that of the naturally occurring tracee. In other words, the simple depiction of the tracer as molecules designated by the filled circles in Figure 4.1 omits the important point that

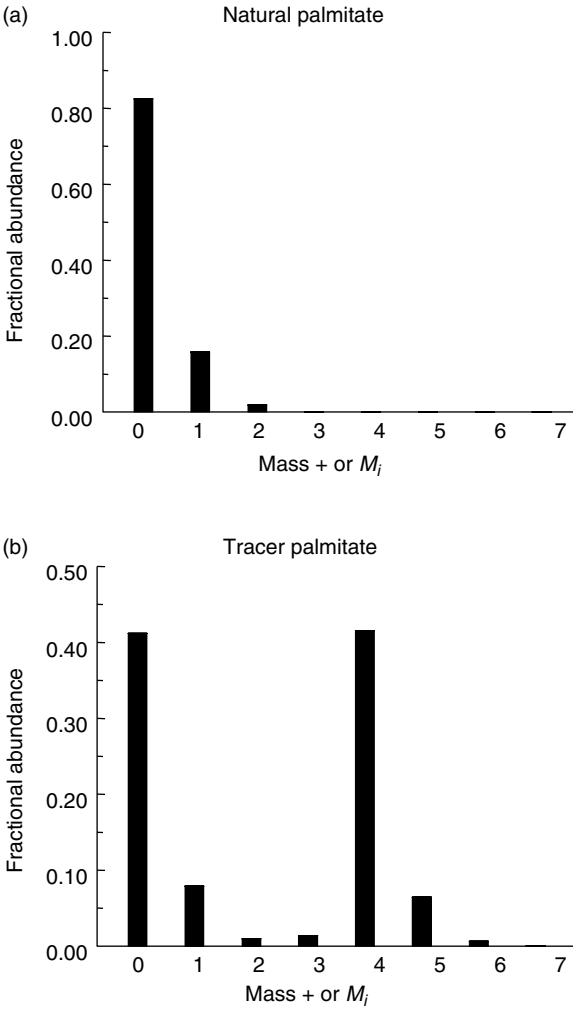

FIGURE 4.2 Mass isotopomer distribution profile for natural palmitate (a) and tracer formed as mixture of 50% [1,2,3,4-^{13}C]palmitate and 50% natural palmitate (b). Amounts are shown as fractional abundance and sum to 1.

individual tracer molecules are not identical because they may be in one of several isotopomers as indicated in Figure 4.3. The important point is that the population distribution of the isotopomers of the tracer is distinctly different from the population distribution of the tracee, which is characterized by natural abundance.

E. Selecting the Tracer

The MID profiles in Figure 4.2 represent only natural compound (a) and a specific tracer (b). In an experiment, it is expected that the tracer may be diluted by the flow of tracee into the pathway lowering the TMF, as shown in Figure 4.1. We now address this situation and consider the fractional abundance profile of a mixture of tracer and tracee. We consider two mixtures using ^{13}C palmitate as tracer. The examples representing palmitate sampled from a compartment illustrate the process of estimating the fraction of the total palmitate derived from the tracer (Figure 4.3). Both examples here are 25% tracer and 75% tracee, thus TMF = 0.25. If the tracer used is [1-^{13}C]palmitate, the

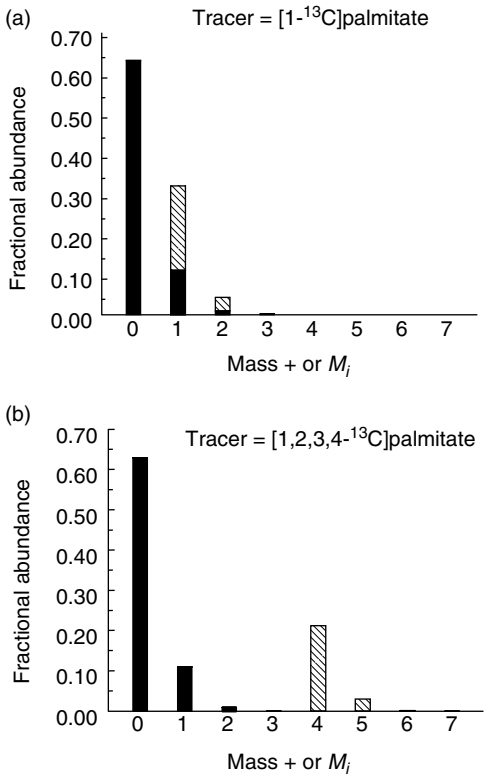

FIGURE 4.3 Mass isotopomer distribution profile for mixture of 25% [1-^{13}C]palmitate tracer and 75% natural palmitate (a). Profile of 25% [1,2,3,4-^{13}C]palmitate and 75% natural palmitate (b).

dominant mass will be M_1; however, higher masses will occur owing to the fact that the other 15 carbon atoms have a probability of 0.0108 of being ^{13}C rather than ^{12}C owing to natural abundance. In addition, the tracee will have significant abundance at M_1 for the same reason. The profile (Figure 4.3a) clearly indicates that the calculation of the TMF is not straightforward. The TMF cannot be determined without considering the contribution of the natural abundance to M_1. Alternatively, if [1,2,3,4-^{13}C]palmitic acid is the tracer (Figure 4.3b) used this time without any natural palmitate as part of the tracer, the entire MID of the tracer will be separated from the natural tracee compound so that:

$$\text{Tracer mole fraction (TMF)} = \frac{(M_3 + M_4 + M_5 + M_6)}{\sum M}$$

The process of identifying the contribution of the tracer to the total MID is straightforward when the tracer is sufficiently labeled to separate it from the isotopomers contributed by the natural form of palmitate, as in Figure 4.3b. For both examples shown, the TMF is 0.25 indicating that 25% of palmitate is derived from the tracer, but the ease of calculating the TMF for heavily labeled stable isotope tracer is clearly a convenience. Accordingly, researchers often consider it desirable to select a tracer three or more mass units greater than the base M_0 molecular weight of the tracee. The inconvenience of dealing with the overlap of tracer and tracee in the MID must be weighed against the usually increased expense of the heavily labeled tracer. If the singly labeled tracer is chosen, it is of course possible to account for the contribution of the tracee at M_1 from the natural abundance profile of the tracee alone. A linear regression least squares approach may be required for the most

precise estimates of TMF from the MID shown in Figure 4.2a. However, even when the presence of isotopomers greater than M_0 from the tracee is correctly considered, the signal from the natural abundance of ^{13}C decreases the precision of detection of the tracer. Thus, Figure 4.3 provides a view of the choices of the investigator when choosing a ^{13}C-labeled lipid for a metabolic study. For *in vivo* experiments, the activity of the tracer detected in plasma compartments will not be high and the investigator will have to determine whether the advantage of increased precision conferred by [1,2,3,4-^{13}C]palmitic acid outweighs its increased cost. Because organic mass spectrometry does not discriminate between increased mass due to ^{2}H vs. ^{13}C or other heavy isotopes, the effect of natural abundance of ^{13}C will also complicate the interpretation of experiments using ^{2}H as tracer.

III. PRACTICAL APPLICATIONS OF TRACERS IN LIPID METABOLISM

As indicated above, tracer can be used to trace the steps in a metabolic pathway if the pathway is not known. However, simple tracing experiments with tracers are rarely informative today. We know reasonably well the actual steps in most pathways but are lacking sorely in quantitative measurements of the true rates of pathways. Here we review a few simple types of tracer experiments to evaluate metabolic pathways of lipids.

A. Flux Ratios Determined in Isotopic and Metabolic Steady State

Returning to Figure 4.1, we now consider how tracers would be used to evaluate this metabolic pathway. We assume the system as shown has reached isotopic and metabolic steady state and that the compartments A, B, C, and D may be sampled. The fluxes indicated in the diagram are unknown and are to be investigated by the analysis of the TMF or SA of the tracer in various compartments. To make this more relevant, we may imagine that Figure 4.1 is a simplified diagram of dietary lipid metabolism. The tracer is a specific fatty acid and the compartments are the triglyceride of this fatty acid as chylomicrons (A), lipoprotein (B), adipocyte tissue (C), and endothelial cell (D). In this model, the chemical composition of the fatty acids is not altered by the metabolic pathway; that is, the structure of the fatty acid is not changed. Thus, the SA or TMF of the fatty acid in each compartment will be measured and the data used to estimate the relative fluxes of other sources of this fatty acid into the pathway. The major limitation of this type of steady state protocol is that SA or TMF alone at steady state cannot yield true flux rates, mol/(kg-min). They provide relative fluxes into each compartment. For example, from the fact that SA or TMF of B is 0.75 relative to A, the flux of tracer to tracee into this compartment must be 3:1. Likewise with compartment C, we can deduce that the flux ratio is 2:1, and we can deduce that no tracee flux enters D. For the steady state model to determine actual fluxes, some reference fluxes must be determined independently.

B. Flux Rates Relative to Pool Size: Metabolic Steady State with Isotope Filling Pools

From this simple model, more complex ones can be developed. For example, a model in metabolic but not isotopic steady state provides a method to estimate the size of each compartment relative to the fluxes through it. Imagine beginning with metabolic steady state but no tracer in the system. We may use the pathway described in Figure 4.1. Perhaps a natural source of the tracee feeds the system with initial flux into compartment A so that the size of each compartment is not altered when the tracer infusion is begun. Thus, the experiment begins with each pool with the same number of molecules as shown in Figure 4.1, except that tracer flux through the compartments has not yet begun.

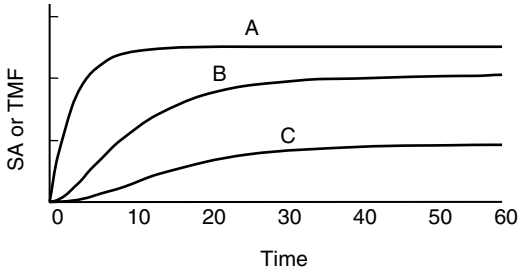

FIGURE 4.4 Tracer labeling profiles for hypothetical compartments from Figure 4.1 in an experimental protocol where tracer infusion was initiated in a system already in metabolic steady state.

With the start of tracer infusion, the size of the pools remains constant but each pool increases in SA or TMF until the steady state is attained. The equation describing the SA or TMF of the first pool A is

$$\mathrm{SA} = k\left[1 - \mathrm{e}^{-(F/N)\mathrm{time}}\right] \tag{4.1}$$

where F is the flux rate of the tracer into the pool and N is the pool size in moles. The equation for each of the successive pools is more complex, but the basic relationship holds that the filling of each pool is determined by the pool size and the rate of flux of tracer into the pool. The pools tend to reach steady state in the order they receive tracer, as indicated in Figure 4.4. These profiles can be used to estimate *F/N*, and if the pool size is determined independently, flux may then be estimated. Thus, the type of tracer experiment can be varied from the very simple isotopic and metabolic steady state to more complex situations to obtain additional information about the system. This equation for the filling of a pool with label will return as the equation for the fractional synthesis rate (FSR) in the analysis of de novo synthesis below.

C. Rate of Appearance

In metabolism of lipids, a primary measurement is the rate of appearance of a substance in plasma or Ra. Isotope dilution is the standard method for measuring Ra. The Ra for a specific compound is measured with a constant infusion of a tracer for that compound (Figure 4.5). The tracer is infused until the enrichment in plasma reaches a constant value, a steady state, and this value of the tracer enrichment is used in the following equations. Analogous equations are provided for both stable and radioisotopes. Using radioisotope terminology:

$$\mathrm{Ra} = \text{tracer infusion (moles/time)} \times [(\text{infused tracer SA/plasma tracer SA}) - 1].$$

Using stable isotope terminology:

$$\mathrm{Ra} = \text{tracer infusion (moles/time)} \times [(\text{infused tracer TMF/plasma tracer TMF}) - 1].$$

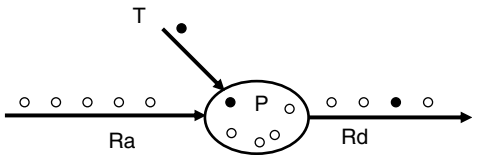

FIGURE 4.5 Rate of appearance of endogenous metabolite determined by tracer dilution. In steady state, rate of appearance into plasma (Ra) plus tracer infusion (T) equals rate of disappearance (Rd). Ra in this simple model is equivalent to endogenous production.

Both equations result in an estimate of Ra in moles/time where TMF and SA are as defined above. This equation simply indicates that the appearance of tracee in the plasma is detected by the finding that the SA of the tracer in the plasma at steady state is less than the SA of the injected material. Usually the tracer infusion (T) is a small percentage of the Ra to avoid perturbing metabolism. In a steady state system, the Ra will equal the rate of disappearance, Rd minus the trace infusion (Figure 4.5). Thus, the Ra of compounds in plasma will be measured to determine the rate at which a dietary or endogenous lipid enters the plasma. Another example is to determine the rate at which a lipid in the plasma is taken up by cells. These definitions of Ra and Rd assume that the distribution of the compound of interest in plasma is that expected of a substance in a homogeneous compartment.

One of the first isotopic studies of Ra using fatty acids was performed by Armstrong and coworkers (Watkins et al., 1982) using ^{14}C palmitate. To measure the plasma Ra of free fatty acids, the protocol required a constant intravenous infusion of ^{14}C palmitate followed by sampling of blood to measure the SA. A review of issues raised in the analysis of isotopic data from this study illustrates concerns that apply to investigations conducted today. The first issue is how far one can extend the behavior of a single fatty acid such as palmitate to represent the behavior of other related compounds. The answer depends on the situation and the level of accuracy required. The Ra of a single common fatty acid such as palmitate may be useful as an indicator of the behavior of plasma fatty acids. However, when examined in detail, individual fatty acids each have properties not identical to that of palmitate. Another issue raised by investigations of plasma Ra is the effect of the site of injection and sampling on the values determined. In theory, the Ra calculations above require that plasma behave like a single well-mixed compartment for tracer and tracee equilibration, as shown in Figure 4.1. Analysis of free fatty acid kinetics indicated differences in the values obtained depending on the sites of tracer injection and sampling. These differences indicate that plasma does not equilibrate fast enough to be a perfect single compartment for free fatty acid kinetics. It appears that the most physiological results are obtained with venous infusion of free fatty acids and arterial sampling (Jensen et al., 1988).

IV. ESTIMATING DE NOVO BIOSYNTHESIS OF LIPIDS

In the previous section, the discussion focused on "isotope dilution" methods for estimating Ra. A key feature of isotope dilution is that the tracer is identical to the tracee, except for the isotopic substitutions (Figure 4.5). Although the Ra measures the endogenous rate of flux of a tracee molecule into the plasma it does not discriminate between de novo synthesis and flux of preexisting molecules into the plasma. To quantify de novo biosynthesis, "isotope incorporation" methods are used. When more than one precursor tracer molecule is incorporated into a product, stable isotopes have distinct advantages over radioisotopes for estimating biosynthesis. The reason is that the incorporation of multiple labeled precursor molecules into a product can be used to estimate the precursor enrichment when measured by mass spectrometry. Precursor enrichment is required for estimating biosynthesis by isotope incorporation. The procedure for calculating precursor enrichment from the product isotopomer distribution is the basis of the MIDA (Hellerstein and Neese, 1992) and ISA methods (Kelleher and Masterson, 1992). A common application of this technique for lipid biosynthesis utilized ^{13}C acetate as the tracer. When products such as cholesterol and fatty acids are analyzed as polymers of acetate, the precursor enrichment can be estimated (Kharroubi et al., 1992; Neese et al., 1993). The development of MIDA and ISA demonstrated the power of stable isotope studies for estimating biosynthesis. The use of ^{13}C acetate and other acetyl CoA generating tracers to estimating de novo lipogenesis was valuable but had the drawback of requiring constant infusions of tracer. Recently, an increasing number of studies have replaced ^{13}C acetate as discussed in the following section.

A. Deuterium Incorporation Approach

H atoms from water are incorporated into lipids during biosynthesis. The incorporation may be directly as H^+ ions or from the H of NADPH, which has exchanged with water. The theoretical

basis for the use of labeled water as deuterium, ^{2}H, to estimate lipid biosynthesis follows from classic studies using tritium, ^{3}H (Wadke et al., 1973; Lowenstein et al., 1975; Dietschy and Spady, 1984). Most investigators now choose ^{2}H over ^{3}H, to eliminate the use of radioactivity. This is especially important because the precursor, H_2O is present at 55M resulting in a large requirement for ^{3}H isotope (Curies) to attain sufficient SA (Curies/mole) for detecting the label when incorporated into lipids. $^{2}H_2O$ may be preferred over other stable isotopes such as ^{13}C acetate because the isotope can be administered orally, is low cost, and can be used for multiple simultaneous assays (Hellerstein, 2004; McCabe and Previs, 2004). However, it should be noted that $^{3}H_2O$ continues to be used and has one advantage over $^{2}H_2O$ in terms of the signal-to-noise. When mass spectrometry of multicarbon lipid molecules is performed, a large signal is observed at M_1 is due to the natural abundance of ^{13}C (Figure 4.2a). In basic mass spectrometry protocols ^{13}C and ^{2}H provide identical signals at M_1. Thus, if only a small fraction of a specific lipid is newly synthesized, most of the M_1 signal will be due to the natural abundance of ^{13}C. The deuterium incorporated from water must be detected above the natural abundance ^{13}C. This may limit the ability of $^{2}H_2O$ to detect lipogenesis when the water enrichment is low as in most *in vivo* studies. In contrast, there is no comparable endogenous signal for radioisotopes. Therefore, $^{3}H_2O$ may be the tracer of choice when an experiment is designed to measure the synthesis of a small fraction of a large depot. Newer mass spectrometry methods may overcome some of these obstacles and are discussed below. Overall, the use of $^{2}H_2O$ to estimate lipid biosynthesis is a technique with great promise for enhancing our understanding of lipid synthesis and interconversions. The section that follows provides an overview of the use of $^{2}H_2O$ for estimating the biosynthesis of lipids using palmitic acid as an example.

An important difference between the two commonly used stable isotope tracers for lipid biosynthesis, ^{13}C acetate and $^{2}H_2O$, relates to the precursor enrichment. To determine the rate of biosynthesis, the following must be known, the precursor enrichment and the number of precursor units incorporated into the product during biosynthesis. Considering the first requirement, the precursor for biosynthesis from ^{13}C acetate is the cytosolic lipogenic acetyl CoA pool. The precursor for biosynthesis from $^{2}H_2O$ is cytosolic water and the NADPH equilibrated with water. Lipogenic acetyl CoA enrichment is unknown, and difficult to measure. This limitation also applies to ^{14}C acetate (Dietschy and Brown, 1974). As discussed above in reference to MIDA and ISA, with ^{13}C acetate, the precursor enrichment must then be calculated from the isotopomer distribution of the product. Alternatively, when the tracer is $^{2}H_2O$, the isotopic enrichment of all compartments is assumed to be equal. Water enrichment is estimated from a sample from an accessible compartment such as plasma. Thus, an advantage of $^{2}H_2O$ over ^{13}C acetate is the availability of the enrichment of the precursor for biosynthesis. However, the second requirement, the number of precursor units incorporated into product, is straightforward for ^{13}C acetate and problematic for $^{2}H_2O$. When ^{13}C acetate is used as the tracer for palmitic acid biosynthesis, it is well known that 8-acetyl CoA or malonyl CoA subunits are incorporated per molecule of the 16-carbon palmitic acid synthesized. However, when $^{2}H_2O$ is the tracer, the equilibration of water with NADPH may not be complete. Additionally, some ^{2}H may be incorporated into the acetyl units prior to biosynthesis. Consequently, the number of H atoms that may be exchanged with the labeled water may not be constant for all situations. For example, *in vivo* studies have found that n is approximately 21 for palmitate and 27 for cholesterol, but lower values are found *in vitro* (Diraison et al., 1996). In many cases, the small differences due to different values of n may not be significant. However, for novel biosyntheses it would be valuable to carry out initial studies to estimate n. When it is feasible to follow the labeling of a product molecule until 100% of the molecules are newly synthesized, the value of n may be calculated from the isotopic enrichment of the product at the plateau (Figure 4.6).

The actual estimation of n involves mathematical models for each isotopomer detected. The models predict the plateau values for each isotopomer at each successive value of n. The best-fit model is found, which most accurately predicts the various isotopomers of the product at each of several values for the water enrichment. Once the value of n is determined for a $^{2}H_2O$ experiment, the FSR may be determined without following the experiment to the plateau level using the assumption

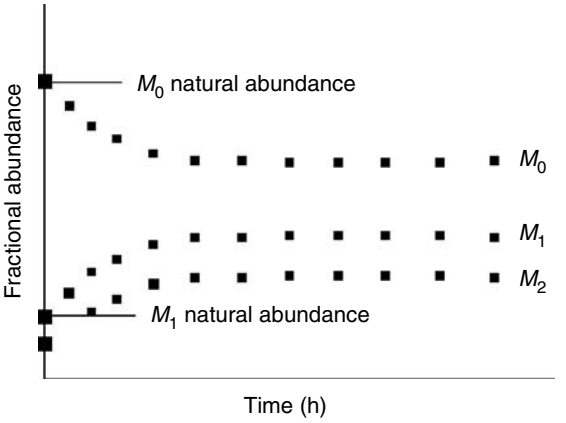

FIGURE 4.6 Tracer labeling profiles for isotopomers of palmitic acid incubated in 5% ^2H_2O. Data were collected over time until 100% labeling occurs and a plateau value is reached. Note that the incorporation of ^2H_2O leads to a decline in the M_0 isotopomer and an increase in M_1 and M_2.

that the product is behaving as a single pool labeled from the tracer. This situation is comparable to that described in Equation 4.1. Except that this time the label is reaching the product by isotope incorporation. To calculate the fractional synthesis, the concept of a single pool filling with a labeled metabolite is rewritten so that *F/N* is fractional synthesis (FSR). The result estimates the FSR from the fractional abundance of the M_1 isotopomer with Ap representing the plateau value of the isotopic enrichment.

$$M_1 = \mathrm{Ap}\left[1 - e^{-(\mathrm{FSR})\mathrm{time}}\right]$$

and

$$\mathrm{FSR} = -\ln\left[(M_1/\mathrm{Ap}) - 1\right]/\mathrm{time}$$

Finally, to illustrate the dependence of the isotopomer fractional abundances on the enrichment of water used in the study, a mathematical model was used to calculate the values for the isotopomers 0 through 7 for palmitic acid synthesized in the presence of ^2H_2O at various enrichment values (Figure 4.7). This model assumed that $n = 21$. The distribution of isotopomer values can provide much information for testing the assumptions of the model. If the water enrichment is near the higher end of those used here, the abundance of additional isotopomers above M_0 and M_1 is observed. These additional isotopomers allow the model to be "overdetermined," that is, they provide the information that allows the model to be thoroughly tested. In summary, the theory and practice for the use of ^2H_2O for estimating biosyntheses are now mature and should be very accessible to investigators.

V. METHODS FOR DETECTING ^{13}C ATOMS RATHER THAN MOLECULES

One of the problems with the use of ^2H_2O for estimating the biosynthesis of lipids as described above is the large natural abundance of ^{13}C that creates a large background noise. ^2H enrichment in newly synthesized lipid must be detected above this background. This situation applies when the entire lipid molecule is detected as the parent mass ion. Below, we briefly review methods for avoiding this situation and increasing the sensitivity of isotopic detection.

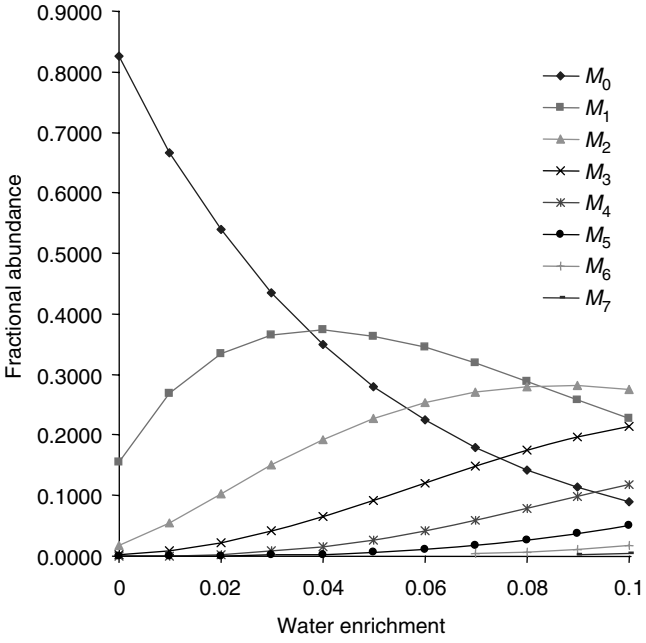

FIGURE 4.7 Calculated values of Ap for palmitate at different water enrichment levels. For each water enrichment value a mathematical model calculated each isotopomer value.

A. Gas Isotope Ratio Mass Spectrometry

Gas isotope ratio mass spectrometry (GIRMS) is theoretically akin to radioisotopic methods in that it detects labeled atoms. However, GIRMS instruments accept only pure gas as input and report the ratio of mass 45 (largely $^{13}C^{16}O_2$) to 44 ($^{12}C^{16}O_2$). If a ^{13}C-labeled lipid is combusted to CO_2, a signal proportional to the number of ^{13}C atoms will be reported by GIRMS. This is analogous to the dpm reported for a radioisotopic study. Both provide useful information, the number of labeled atoms recovered, but no details of the location of ^{13}C within the molecule.

Organic mass spectrometry provides the fractional abundance of various isotopomers of a molecule, but it does not provide the highest precision in the detection of ^{13}C or other stable isotopes. For highest precision, a method is required that detects labeled atoms rather than molecules and avoids the problems created by the natural abundance of multicarbon compounds. The best known of these is GIRMS first developed in the 1940s. Current instruments can detect very small amounts of $^{13}CO_2$: 10 parts per million or less. GIRMS is important for the analysis of the oxidation of dietary lipids. To detect fat absorption and metabolism, ^{13}C-enriched lipids is administered orally and breath samples are analyzed for $^{13}CO_2$. In detecting $^{13}CO_2$ enrichment resulting from the oxidation of ^{13}C-labeled lipids, GIRMS is much more precise than standard organic mass spectrometry. Typically, GC/MS may detect ^{13}C with a precision of about 1% relative standard deviation. In contrast, GIRMS machines are routinely available that detect ^{13}C at 10 parts/million. GIRMS data may be reported as atom percent excess, a percentile notation equivalent to percentage of enrichment or alternatively as the delta value, δ. The rationale behind the δ notation is that the ^{13}C:^{12}C ratio, $R_{(\text{sample})}$, is measured by GIRMS of CO_2 and compared to this ratio for standard, $R_{(\text{standard})}$, thus

$$\delta\ ^{13}C(\%) = [R_{(\text{sample})} - R_{(\text{standard})}]/R_{(\text{standard})} \times 1000.$$

By international convention, the standard is a specific calcium carbonate, which has a $^{13}C/^{12}C$ ratio of 0.012.

Two important applications of GIRMS in dietary lipid metabolism are the gastric emptying and fat malabsorption tests. Gastric emptying is measured indirectly by monitoring the appearance of $^{13}CO_2$ in breath using GIRMS following ingestion of a test meal containing ^{13}C-octanoic acid. The medium-chain free fatty acid is readily oxidized and its appearance as breath $^{13}CO_2$ correlates well with other measures of gastric emptying (Ghoos et al., 1993). This method was first developed using ^{14}C tracer, but with the advent of GIRMS, ^{13}C has become the method of choice. To measure fat malabsorption, ^{13}C-enriched triolein is administered orally and $^{13}CO_2$ enrichment in breath CO_2 is measured by GIRMS (Watkins et al., 1982). A more specific assay assessing pancreatic exocrine insufficiency focusing on cholesterol ester hydrolase deficiency has been developed recently (Ventrucci et al., 1998). This test analyzes the oxidation of cholesteryl[1-^{13}C]octanoate measuring breath $^{13}CO_2$ enrichment using GIRMS. This test clearly indicates severe pancreatic insufficiency but has not yet been proven effective in discriminating moderate pancreatic insufficiency from healthy controls. It serves, however, as an interesting example of a new variety of ^{13}C-labeled precursors that may be used to indicate very specific enzyme deficiencies.

B. Gas Chromatography Combustion Isotope Ratio Mass Spectrometry

To overcome the limitations of GIRMS due to its dependency on CO_2 and the large amount of sample required, new methods have been developed that first separate molecules using GC/MS and then perform combustion. Current versions of these GC combustion isotope ratio mass spectrometry (GCC-IRMS) instruments focusing on lipid metabolism have been pioneered by Brenna and coworkers (Goodman and Brenna, 1992, 1995). These investigators have been able to document the consistency of fatty acid $^{13}C/^{12}C$ ratios in human subjects (Rhee et al., 1997b). They developed methods for using tracer doses of dietary ^{13}C fatty acids (Rhee et al., 1997a) and found that an oral dose of only 30 mg of [U-^{13}C]palmitic or stearic acid is required to detect ^{13}C enrichment in the corresponding monounsaturated fatty acids in blood for 1 week. Other investigators using the GCC-IRMS methods have found that the minimum tracer dose of [U-^{13}C]palmitate to measure the palmitate Ra in human subjects was reduced to two orders of magnitude using GCC-IRMS compared with GC/MS (Demmelmair et al., 1997). This rapidly developing technique offers exciting possibilities for the analysis of the fate of fatty acids in foods without using large amounts of tracer.

An important carbon isotope effect with implications for the study of dietary fatty acids is fractionation of the natural abundance of ^{13}C in photosynthesis. The two major photosynthetic pathways discriminate against ^{13}C in carbohydrate synthesis. The preference for ^{12}C is greater for C3 plants (wheat) than for C4 plants (corn, sugarcane). This difference can be resolved by GIRMS techniques and has been the basis of interesting approaches to the analysis of dietary fatty acids. For example, a study of European infants switched from breast milk or formula to a corn oil diet detected a sufficient change in $^{13}C/^{12}C$ of plasma arachidonic acid and allowed researchers to estimate the ability of infants to synthesize arachidonic acid (Demmelmair et al., 1995). This study employed GCC-IRMS to obtain the $^{13}C/^{12}C$ of the lipids. The method is dependent on the fact that fats available in breast milk in Europe are derived largely from C3 plants such as wheat. These few examples of recent developments in the use of GCC-IRMS clearly indicate that the use of stable isotope tracers at concentrations that do not perturb metabolism is now becoming a reality.

VI. LOOKING TO THE FUTURE

A. Mass Spectrometry as a Key to New Lipid Methodologies

Mass spectrometry is widely used in the food industry to identify and quantify lipids. Stable isotopes are of value here as internal standards for quantification. In addition, the mass fragmentation of specifically labeled lipids provides detailed structural information. Most importantly, once techniques

have been validated for identifying novel lipids, the stage is set for future work quantifying fluxes. We highlight here a few interesting developments. In the fish industry, GC/MS has been used to identify chlorinated fatty acids (Zhuang et al., 2004), to clarify the structure, and to determine carbon chain length as well as the degree and position of double bonds in esterified fatty acids (Zhuang et al., 2004; Dayhuff and Wells, 2005). Mass fragmentation patterns confirmed the chemical structures of fatty acids. Based on these studies, it was found that all the polyunsaturated fatty acids in the fish that were examined had methylene-interrupted double bonds of *cis* geometry. The application of GC extends to other industries, including the infant formula industry where GC is used to determine lipid hydroperoxides and to study the stability of the lipid fraction in infant formula (Lagarda et al., 2003). The emerging biotechnology interests of the oil industry uses GC to identify the unique presence of unusual fatty acids such as ricinoleic, vernolic, and cyclopropenoid fatty acids in seed oils of oil-rich plants such as *Ochrocarpus africanus* (Hosamani and Ganjihal, 2003). The bakery products industry routinely utilizes GC for the identification and quantification of *trans* fatty acids and is now combining this technique with novel methods for isolating lipids (Ruiz-Jimenez et al., 2004). Liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization has been used to identify unusual very long chain fatty acids up to C47, both saturated and unsaturated, with two positional isomers (omega-9 and omega-26) in green alga *Chlorella kessleri* (Rezanka, 2002). The ratio of saturated to unsaturated fatty acids changes with the number of carbons, with saturated fatty acids predominating in the C29 acids and the unsaturated acid predominating in the C30 acids. In yet another method, a combination of HPLC techniques was used to isolate and identify conjugated isomers of linolenic acid and arachidonic acid in cheese (Winkler and Steinhart, 2001). In summary, the wide use of mass spectrometry in industry provides a wealth of techniques for identifying and quantifying lipids in food. These techniques lay the groundwork for new era of tracer-based studies quantifying the biosynthesis and metabolism of novel lipid products.

B. Isotopic Flux Studies as a Tool in the Genomics Era

Isotopic tracers have played a fundamental role in developing our understanding of metabolism over the past half century. Both the actual steps in pathways and the rates of the pathways have been elucidated with the aid of tracers. Currently, the Human Genome Project and other molecular techniques are providing massive amounts of information not only about the genome but also the protein sequences coded by the genome, "the proteome" and more recently "the metabolome." Lipids are clearly an important component of the metabolome (German et al., 2003). A first metabolomic goal may be to quantify lipids both those endogenously produced and those supplied in food. Eventually, this will lead to questions about the rates of biosynthesis and flux of lipids into various compartments. A static view will not suffice for long. This brings us again to the use of isotopes and tracers to quantify and understand a new level of control of metabolism. Looking toward the future, it may be relevant to focus on more complete networks rather than on isolated biosynthesis. In the past, we have learned how to accurately estimate de novo lipogenesis and fluxes of food-derived lipids across the plasma. In the future, we hope to integrate entire metabolic networks that include many reactions such as those that supply the NADPH and other cofactors required for lipogenesis to gain a deeper understanding of metabolic control.

We may ask how isotopic tracer studies specifically may contribute to advances in metabolic studies in this new environment. In the field of metabolism, quantitative tools for evaluating metabolism such as "metabolic control analysis" (Fell, 1997) have already provided a framework for organizing this new information. Isotopic tracers may be key to quantifying how changes in gene expression alter metabolic pathways, because they provide a quantitative measure of changes in fluxes and flux ratios. Consider the hypothetical case based on the metabolic network shown in Figure 4.1, where an investigator wants to increase flux to compartment D rather than compartment C to prevent disease. The investigator may hypothesize that this desired change may be accomplished by increasing a specific

enzyme catalyzing a key step in the network or by adding to the system a molecule that is an effector stimulating a specific reaction. The ultimate test of whether the goal of increasing flux has been accomplished may best be determined by isotopic studies that actually measure the flux changes. To study the effect of genetic manipulations on a larger, more complex network, one may select key compounds in the network as reporters for the flux through specific branches. The isotopic enrichment in these molecules offers a quantitative tool for the understanding of the behavior of the network. Clearly, the variety of metabolic alterations that may be feasible with the developing molecular technologies will challenge metabolic researchers to bring the isotopic methods and analytical skills to this new arena. Hopefully, this tutorial has provided a framework for understanding the methodology underlying tracer analysis and will enable more researchers to utilize these powerful tools.

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5 Fatty Acids in Meat and Meat Products

J.D. Wood, M. Enser, R.I. Richardson, and F.M. Whittington

CONTENTS

I. INTRODUCTION

Meat has an important place in a healthy diet, providing protein with a good balance of amino acids, iron in a readily available form, vitamins, especially of the B group and other essential minerals such as zinc (Williamson et al., 2005). Meat also contributes a significant amount of fat to the human diet and it is this component that has been most under the spotlight in recent years in relation to the healthiness of people consuming meat. Meat contains relatively high amounts of saturated fatty acids (SFA) and ruminant meats (beef and lamb) are low in polyunsaturated fatty acids. This balance, if replicated in the whole diet, predisposes people to a range of diseases including cardiovascular disease (CVD). However, research papers show that the fatty acid composition of meat can be greatly modified by production factors such as animal diet, age, weight, sex, and breed. Fatty acid composition also varies between species and tissue sites in the body. All these variations provide the meat industry with the tools to supply meat containing a healthy balance of fatty acids to the consumer, either in the form of fresh meat or meat products.

The fatty acids in meat are located mainly in adipose tissue, commonly termed "fat." This has a role in product quality, contributing toward texture (tenderness and mouthfeel) and juiciness in both fresh meat and meat products. The softness/hardness of fat, which is greatly influenced by fatty acid composition, affects various properties such as the sliceability of bacon and the stability of

sausage emulsions (Teye et al., 2006b). Fatty acids in adipose tissue and in muscle membranes also contribute to meat flavor, providing volatile degradation products during cooking. Changes in fatty acid composition can affect all these aspects of meat quality.

The aim of this chapter is therefore to review current research findings on the factors affecting the fatty acid composition of meat. Much of the information presented comes from research on cattle, sheep, and pigs conducted at the University of Bristol.

II. TYPES OF FATTY ACIDS IN MEAT

The fatty acids in meat are mainly of medium to long chain length, that is, they have 12- to 22-carbon atoms in the molecule, with a basic structure of CH_3–$(CH_2)_n$–COOH. Small amounts of shorter chain length fatty acids, C8–C10, are present in lamb fat.

About 40% of fatty acids are saturated (SFA), that is, each carbon has two hydrogen atoms attached, about 40% have one double bond (monounsaturated fatty acid, MUFA) where adjacent carbon atoms are attached to only one hydrogen atom each and a smaller proportion, about 2%–25% have more than one double bond (polyunsaturated fatty acid, PUFA). Fatty acids are commonly labeled according to carbon chain length and the number of double bonds, for example, linoleic acid is labeled as 18:2, being 18 carbons in length and containing two double bonds. Double bonds are either of the more common *cis*-type, in which the hydrogen atoms point in the same direction, or of the *trans*-type, in which they point in opposite directions resulting in a straighter molecular configuration (Figure 5.1). Oleic acid (18:1 *cis*-9) is the major fatty acid in all meats, contributing over 30% of total fatty acids.

The length, degree of unsaturation, and configuration of the fatty acid molecule influence physical properties such as melting point. The longer the chain length and the fewer the number of double bonds present in the molecule, the higher the melting point. Saturated and *trans* fatty acids have a higher melting point than unsaturated and *cis* fatty acids (Table 5.1). A high proportion of SFA therefore causes hard rather than soft fat tissue.

The fatty acids in ruminant tissues are more complex than those in nonruminants, containing higher proportions of *trans* fatty acids, fatty acids with an odd number of carbon atoms (arising from rumen-derived proprionic acid rather than acetate as a precursor for fatty acid synthesis, e.g., C15 and C17), fatty acids with branched chains (derived from the amino acids, leucine, valine, and isoleucine, i.e., 4-methyl octanoic acid, C8:0 and 4-methyl nonanoic acid, C9:0) and fatty acids with conjugated double bonds (i.e., the bonds are on adjacent carbon atoms rather than being separated by a CH_2 group). These variations are the result of the actions of enzymes present in microorganisms in the rumen that degrade plant structures and dietary fatty acids, producing a wide range of products, some of which are absorbed in the small intestine and incorporated into tissue lipids. An important group of fatty acids in ruminants are the conjugated linoleic acids (CLAs) with 18 carbons and 2 conjugated double bonds. These have been shown to have a range of physiological actions in the body of the animal and consumers of meat.

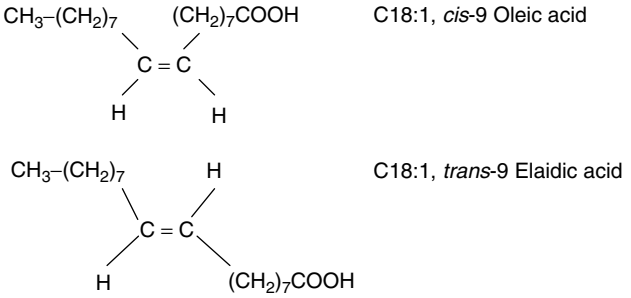

FIGURE 5.1 *Cis* and *trans* double bonds.

TABLE 5.1
Effect of Chain Length and Number and Configuration of Double Bonds on the Melting Point of Fatty Acids

Increasing Chain Length		Increasing Unsaturation	
Fatty Acid	**Melting Point (°C)**	**Fatty Acid**	**Melting Point (°C)**
Lauric acid, 12:0	44.2	Stearic, 18:0	69.6
Myristic acid, 14:0	54.4	Elaidic, 18:1 *trans*-9	43.7
Palmitic acid, 16:0	62.9	Oleic, 18:1 *cis*-9	13.4
Stearic acid, 18:0	69.6	Linoleic, 18:2	–5.0
Arachidic acid, 20:0	75.4	Linolenic, 18:3	–11.0

TABLE 5.2
Sources of Some Major Fatty Acids in Meat

Fatty Acid Type	Source
Saturated	Synthesis + diet + biohydrogenation
Monounsaturated-*cis*	Synthesis + diet
Monounsaturated-*trans*	Biohydrogenation
Polyunsaturated-18:2n-6	Diet (essential FA)
Polyunsaturated-18:3n-3	Diet (essential FA)
Conjugated linoleic acid	Synthesis + biohydrogenation
Polyunsaturated C*20*, C*22*	Synthesis + diet

The SFA in meat can be derived from the diet, produced in the rumen from unsaturated dietary fatty acids, or synthesized from glucose or acetate in liver or adipose tissue (Table 5.2). MUFA (e.g., 18:1*cis*-9) are mainly formed in adipose tissue from SFA by the action of desaturase enzymes, for example, delta-9-desaturase that forms oleic acid (18:1*cis*-9) from stearic acid (18:0) and palmitoleic acid (16:1*cis*-9) from palmitic acid (16:0). This same enzyme complex forms the main CLA isomer, *cis*-9, *trans*-11 CLA from 18:1 *trans*-11, which is produced in the rumen. Most CLA formation occurs in adipose tissue (the mammary gland in the case of lactating animals) but some occurs in the rumen.

PUFAs are of the n-6 or n-3 type that describes the position along the carbon chain from the methyl end where the first double bond is inserted. The n-6 fatty acid present in the largest amount is linoleic acid (18:2n-6), which is an essential fatty acid, that is, it is derived entirely from the diet (e.g., oilseeds and grains). The animal possesses desaturase and elongase enzymes that can convert 18:2 to longer-chain n-6 fatty acids such as arachidonic acid (20:4n-6). Similarly, the most common n-3 fatty acid is α-linolenic acid (18:3n-3), which is present in the leaves of plants and grasses. This fatty acid can be converted to long-chain n-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). There is competition between 18:2n-6 and 18:3n-3 for conversion to the long-chain PUFA because the enzymes are shared. Evidence suggests that 18:3n-3 is the preferred substrate but the presence of much more 18:2n-6 usually results in greater synthesis and deposition of long-chain PUFA derived from this fatty acid (Williams and Burdge, 2006). These long-chain n-6 and n-3 fatty acids have important physiological roles in the body through their conversion to eicosanoids, which among other actions, control thrombosis and tissue inflammation.

Ruminant and nonruminant species differ greatly in their proportions of PUFA in tissues and meat because, whereas these are hardly changed by digestion in pigs and poultry and are incorporated directly into adipose tissue, in ruminants they are extensively hydrogenated by microorganisms

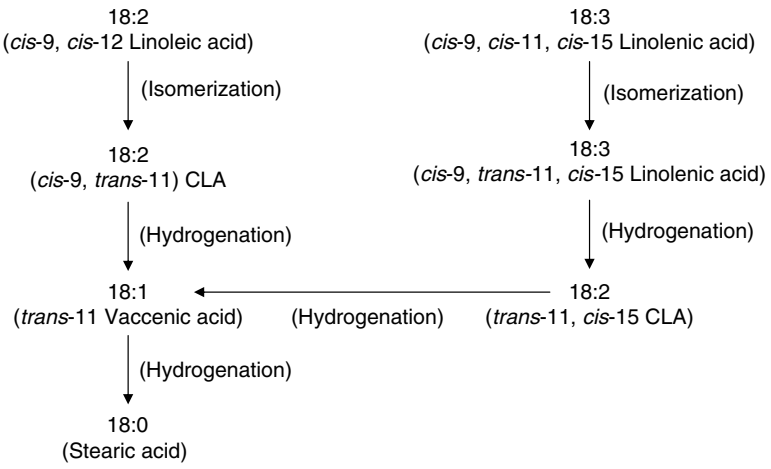

FIGURE 5.2 A simple representation of biohydrogenation pathways in the rumen.

in the rumen (Figure 5.2). This microbial action results in generally low levels (10% or less) of dietary PUFAs being available for absorption into body tissues after passing through the rumen and a wide range of modified fatty acids.

The fatty acids in meat are found in two main lipid classes, neutral triacylglycerol (storage role) and more polar glycerophospholipid (structural and metabolic role). The former is the main lipid component (>90%) of adipose tissue in mature animals (visible fat) and the latter, a constituent of cell membranes, contributes between 10% and 40% of the total fatty acids in muscle. Phospholipid has a much higher concentration of PUFA than triacylglycerol. For example, pig loin muscle neutral lipid (triacylglycerol) and phospholipid contained 12% and 34% 18:2n-6, respectively. in a pig study (Wood et al., 2004). Some average values for the fatty acid composition of adipose tissue triacylglycerol and muscle phospholipid are given in Table 5.3 and are derived from studies in which the different species were fed grain-based (concentrate) diets. As meat animals grow toward the point of slaughter they deposit increasing amounts of fat in the carcass including that within the muscle (marbling fat). This results in an increasing ratio of triacylglycerol to phospholipid, producing a lower concentration of PUFA in total lipids. A clear picture of the effects of production factors on fatty acid composition can therefore only be obtained by analyzing triacylglycerol and phospholipid separately.

III. MEASUREMENT OF FATTY ACID COMPOSITION

Analysis of the amount and type of fat present in meat can be carried out in a variety of ways depending on the requirements of the research project. Results should always be judged in relation to the procedures used.

The widely used Soxhlet method that involves continuous refluxing of ether or light petroleum spirit over a prepared ground and dried sample for several hours will readily extract neutral triacylglycerol but not the phospholipid component. If the amount of total fat (without subsequent fatty acid analysis) is required, then after the Soxhlet extraction, the sample can be hydrolyzed using 5 M hydrochloric acid, washed with distilled water and oven dried, and then reextracted with light petroleum spirit using the Soxhlet method once again.

In order to identify fatty acids present in the different lipid classes, the extraction mixture chloroform/methanol (2:1, v/v) proposed by Folch et al. (1957) is ideal for the initial total lipid extraction process since it will extract both the neutral triacylglycerol components as well as more polar phospholipid components of the tissue without damage to the fatty acids. An antioxidant such

TABLE 5.3
Major Fatty Acids of Adipose Tissue Triacylglycerol and *Longissimus* Muscle Phospholipid in Cattle, Sheep, and Pigs Given Concentrate-Based Diets (% of Total Fatty Acids)

	Triacylglycerol in Adipose Tissue			Phospholipid in Muscle		
Fatty Acids	**Cattle**	**Sheep**	**Pigs**	**Cattle**	**Sheep**	**Pigs**
14:0	3.8	3.0	1.4	0.4	0.5	0.7
16:0	26.1	21.3	24.8	14.9	15.4	17.5
16:1 *cis*[a]	6.2	2.9	3.2	1.5	1.6	1.8
18:0	10.9	9.2	12.0	10.6	10.3	13.7
18:1 *cis*-9	36.5	32.0	35.7	22.0	25.8	17.2
18:2n-6	1.9	1.9	13.3	18.4	12.3	25.3
18:3n-3	0.2	1.7	1.3	0.4	5.0	1.0
20:4n-6	ND	ND	0.2	10.4	5.6	9.1

ND: not detected.

[a]All isomers.

Source: Warren, H.E., et al. 2007a. *Meat Science*, in press; Demirel, G., et al. 2004. *British Journal of Nutrition*, **91**, 551–565; Riley, P.A., et al. 2000. *Animal Science*, **71**, 483–500.

as butylated hydroxy toluene (BHT) is added to prevent oxidation of the unsaturated fatty acids. The neutral and polar fractions can then be separated by applying this total lipid extract to silicic acid solid-phase extraction columns or by thin layer chromatography. Following this step, individual fatty acids can be isolated by saponification, extracted into organic solvents, prepared as fatty acid methyl esters, and then subsequently analyzed by gas–liquid chromatography (GLC).

The gas chromatograph (GC) is widely accepted as a valuable tool in the analysis of fatty acids, and with the introduction of 100-m silica narrow bore capillary columns, the development of liquid phases suitable for a variety of applications, and the appropriate use of temperature programming, separation of individual fatty acids from complex mixtures is possible. Identification is carried out by comparison with standards and, where necessary, by simultaneous mass spectrometry. Modern technology with automation and the use of sophisticated integration software means that routine measurement is possible.

For complex mixtures containing different isomers of particular fatty acids such as CLA, the use of multiple-column silver-ion high-performance liquid chromatography (HPLC) has proved very effective.

IV. MEAT FATTY ACIDS AND HUMAN HEALTH

Some SFA, that is, those with less than 18-carbon atoms chain length, raise blood levels of low-density lipoprotein (LDL) cholesterol, which increases the risk of atherosclerosis leading to CVD in man (Williamson et al., 2005). On the other hand, MUFA and PUFA lower blood levels of LDL-cholesterol. As a result of these findings, health bodies around the world have issued dietary guidelines for the fatty acid composition of the human diet. The World Health Organization (2003) has said that total fat should constitute not more than 15%–30% of total energy in the diet, SFA around 10%, n-6 PUFA around 5%–8%, and n-3 PUFA 1%–2%. The U.K. Department of Health (1994) recommended that ratios between these fatty acid groups should be >0.4 for PUFA:SFA and <4.0 for n-6:n-3 PUFA. Several studies agree that n-3 PUFA levels are too low in Western diets and that these fatty acids reduce the risk of CVD, are necessary for proper brain and visual development in the fetus, and have a role in reducing various cancers (Enser, 2001).

Trans fatty acids have potentially more potent effects on LDL-cholesterol than SFA, although *trans* fatty acids are generally low in meat and there is some evidence that the *trans* fatty acids in meat and milk are less damaging to human health than those in other processed fatty foods (Williamson et al., 2005). *Trans*-11 18:1 (*trans* vaccenic acid) is the precursor in tissues of the major CLA isomer, *cis*-9, *trans*-11 CLA, which is recognized to have several positive health benefits including inhibition of carcinogenesis and atherosclerosis and enhancement of the immune response.

V. SPECIES EFFECTS ON FATTY ACID COMPOSITION

The results in Table 5.3 show data for cattle, sheep, and pigs for specific lipid classes and dietary treatments. Results for the composition of the total fatty acids of muscle tissue from loin chops or steaks representing a wide range of production systems are shown in Table 5.4. The samples were purchased from four supermarkets in the town of Weston-Super-Mare, United Kingdom, in 1994 and therefore represent the meat on sale to the public.

The values for the weight of total fatty acids in muscle shown in Table 5.4 are high in comparison with other studies in which cores from the central part of the *longissimus* muscle have been examined. The results were intended to reflect muscle normally consumed so they include the epimysial connective tissue and some adhering subcutaneous and intermuscular fat that would be consumed by people separating fat from muscle on the plate.

The results in Table 5.4 show that beef and lamb have higher proportions of most SFA than pork. Conversely, pork is much higher than beef and lamb in the main PUFA, 18:2n-6. Values for 18:3n-3

TABLE 5.4
Fatty Acid Composition of *Longissimus* Muscle from Beef, Lamb, and Pork Loin Steaks Obtained at Retail[a]

	Percentage of Total Fatty Acids		
Fatty Acid	**Beef**	**Lamb**	**Pork**
12:0	0.08	0.31	0.12
14:0	2.66	3.30	1.33
16:0	25.0	22.2	23.2
16:1 *cis*[b]	4.54	2.20	2.71
18:0	13.4	18.1	12.2
18:1 *trans*[b]	2.75	4.67	ND
18:1 *cis*-9	36.1	32.5	32.8
18:1 *cis*-11	2.33	1.45	3.99
18:2n-6	2.42	2.70	14.2
18:3n-3	0.70	1.37	0.95
20:3n-6	0.21	0.05	0.34
20:4n-6	0.63	0.64	2.21
20:5n-3	0.28	0.45	0.31
22:4n-6	0.04	ND	0.23
22:5n-3	0.45	0.52	0.62
22:6n-3	0.05	0.15	0.39
Total fatty acids (g/100 g muscle)	3.8	4.9	2.2

ND: not detected.

[a] Results are means for 50 samples from each species.
[b] All isomers.

Source: Enser, M., et al. 1996. *Meat Science*, **42**, 443–456.

TABLE 5.5
Fatty Acid Ratios Related to Healthy Nutrition from the Retail Study of Enser et al. (1996)

Sample	P:S[a]	18:2n-6 / 18:3n-3	n-6 / n-3
Beef			
Muscle	0.11	3.42	2.11
Adipose tissue	0.05	2.30	2.30
Lamb			
Muscle	0.15	1.90	1.32
Adipose tissue	0.09	1.37	1.37
Pork			
Muscle	0.58	14.7	7.22
Adipose tissue	0.61	10.0	7.64

[a] P:S is 18:2n-6 + 18:3n-3/12:0 + 14:0 + 16:0.

are more similar between the species, reflecting its presence at a high level in grass and forages. Although a high proportion (about 90%) of 18:3n-3 in the diet of ruminants is normally hydrogenated in the rumen (Scollan et al., 2001b), some does escape to the duodenum, to be absorbed and deposited in tissues as in pigs. The proportions of long-chain PUFA are also similar between the species, apart from 20:4n-6 that is high in pigs because of the high concentration of its precursor, 18:2n-6.

The study of Enser et al. (1996) also included analysis of subcutaneous adipose tissue removed from the steak. Values for the proportions of the C12–C18 fatty acids were similar to those for muscle but whereas small proportions of the C20–C22 PUFA were present in pork, these were not detected in beef and lamb, reflecting the lack of incorporation of the long-chain PUFA into ruminant triacylglycerols (Enser et al., 1996).

Values for the nutritional indices of fatty acid composition in muscle and adipose tissue, with explanations of how they were calculated, are shown in Table 5.5. The P:S ratio was much lower than advocated for the whole diet (>0.4) in beef and lamb tissues but was beneficially high in pork. This ratio was higher in pork adipose tissue (subcutaneous fat) than in muscle, whereas this was not the case in beef and lamb. The ratios of n-6 to n-3 PUFA were within the range advocated for the whole diet for beef and lamb (<4.0) but well above this (less desirable) for pork. These results mainly reflect the high level of 18:2n-6 in pig diets, coming from grains and oil seeds.

In a comparison of different species, Rossell (1992) found that broiler chicken meat fatty acid composition was similar to that of pork, although the proportion of 18:2n-6 was much higher, that is, 18.9% against 9.5% in that study. Rule et al. (2002) also found a high value for chicken 18:2n-6 in a comparative study, 17.0% of total fatty acids in breast muscle. Among other characteristic species differences is the very high value for 18:3n-3 in horse muscle and adipose tissue (Rossell, 1992; Robb et al., 1972).

VI. DIET EFFECTS ON FATTY ACID COMPOSITION

Compared with other production factors, diet has the largest effect on fatty acid composition in all species, particularly in the monogastrics.

A. Pigs

Studies conducted in the United States in the 1920s showed clearly the effects of different oil sources in the diet on the fatty acid composition of pork (Ellis and Isbell, 1926). Different diets produced different commercial grades of subcutaneous fat tissue, from brewer's rice that produced hard fat to soybeans grazed in the field, which produced oily fat. These effects were due to the incorporation of relatively saturated fat from brewer's rice into body fat to a large amount of highly unsaturated fat as in the case of grazed soybeans. The fatty acid most affected by diet was 18:2n-6, which was 1.9% and 30.6% of total fatty acids in subcutaneous fat of pigs fed brewer's rice and grazed soybeans, respectively.

A review by Wood (1984) showed that 18:2n-6 from oil sources such as soybean meal is incorporated into muscle and adipose tissue in direct proportion to its concentration in the diet. Similar results were found in other PUFA sources having characteristic fatty acid compositions; for example, linseed, which contains a high proportion of 18:3n-3 (Enser et al., 2000) and fish oil, which contains the long-chain n-3 PUFA EPA and DHA (Irie and Sakimoto, 1992).

Feeding SFA does not raise their proportions in muscle and adipose tissue as much as feeding PUFA. This is because of a lower incorporation into lipid and elongation and desaturation of these fatty acids into other SFA and MUFA. Results in Table 5.6 contrast the fatty acid composition of the diet and of the muscle tissue from pigs fed palm kernel oil, palm oil, or soybean oil when these supplied 2.8% of the diet (Teye et al., 2006a). The proportion of 18:0 was three times higher in the palm kernel oil diet than in the soybean oil diet but values in muscle were similar. On the other hand, a doubling of the proportion of 18:2n-6 in the soybean oil diet compared with the palm kernel oil diet produced a 33% increase in the proportion of this fatty acid in muscle.

Comparison of papers published in the 1970s and 1980s with more recent ones shows that the level of 18:2n-6 in pig muscle and adipose tissue has greatly increased during this time. This is partly not only because the oil content of diets has increased to promote faster growth but also due to lower carcass fat levels. When carcass fat deposition is reduced for reasons of genetics or a lower feed intake this decreases the fatty acids synthesized from carbohydrates (SFA and MUFA); therefore, the relative contribution of dietary PUFA to body fat deposition increases. One consequence is softer fat tissues in modern lean pigs compared with the fatter pigs of former years.

B. Cattle

Despite the hydrogenating effect of the conditions of the rumen on dietary PUFA, small but significant amounts enter the duodenum to be absorbed into blood and delivered to tissues as

TABLE 5.6
Fatty Acid Composition (%) of Dietary Fat (D) and *Longissimus* Total Fatty Acids (M) in Pigs Fed *Ad Libitum* to 100 kg Live Weight

	Palm Kernel Oil		Palm Oil		Soybean Oil	
	D	M	D	M	D	M
12:0	14.70	0.23	0.07	0.09	ND	0.13
14:0	3.80	1.41	0.29	0.99	ND	1.09
16:0	7.40	22.86	15.80	22.10	8.20	22.74
18:0	4.20	11.10	1.22	10.27	1.40	10.95
18:2n-6	17.80	9.92	17.38	11.13	35.11	13.24

ND: not detected.

Source: Teye, G.A., et al. 2006a. *Meat Science*, **73**, 157–165.

TABLE 5.7
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Longissimus* Muscle of Steers Fed a Concentrate or Grass Silage Diet from 6 to 24 Months of Age

	Concentrate		Grass Silage	
	NL	PL	NL	PL
14:0	2.8	0.39	3.2	1.0
16:0	28.7	14.9	29.3	18.2
16:1 *cis*[a]	4.5	1.5	4.8	3.0
18:0	12.3	10.6	12.0	10.7
18:1 *trans*[a]	2.9	0.9	1.0	0.4
18:1 *cis*-9	38.4	19.5	39.4	28.0
18:1 *cis*-11	2.0	2.5	1.8	2.4
18:2n-6	1.7	18.4	0.6	5.8
CLA[b]	0.6	0.2	0.2	0.2
18:3n-3	0.1	0.4	0.4	3.3
20:3n-6	ND	2.7	ND	0.8
20:4n-6	ND	10.4	ND	3.7
20:5n-3	ND	0.4	ND	2.7
22:5n-3	ND	1.1	ND	3.9
22:6n-3	ND	0.1	ND	0.4
Total fatty acids (g/100 g muscle)	4.7	0.6	8.9	0.6

ND: not detected.

[a] All isomers.
[b] 18:2 *cis*-9, *trans*-11.

Source: Warren, H.E., et al. 2007a. *Meat Science*, in press.

in pigs. A recent study conducted by the University of Bristol and the Institute of Grassland and Environmental Research (IGER) has compared muscle fatty acid composition in cattle of two breeds (Holstein-Friesian and Aberdeen Angus cross) fed either a grain-based concentrate diet or a grass silage diet from 6 to 14, 19, or 24 months of age. Results for the Aberdeen Angus cross animals slaughtered at 24 months are shown in Table 5.7. The concentrate diet raised the proportion of 18:2n-6 and 20:4n-6, whereas the grass silage diet increased levels of the n-3 PUFA 18:3, 20:5, and 22:6. All these changes reflect the fatty acid composition of the diets. The increased concentration of 22:6n-3 in the grass silage group was significant since the provision of more 18:3n-3 does not always result in higher levels of 22:6n-3 in tissues (Scollan et al., 2003). The higher levels of SFA and MUFA in the grass silage groups can partly be explained by overall greater fatness resulting from de novo fatty acid synthesis in these cattle.

When cattle are fed concentrate alone, the rate of PUFA passage through the rumen is rapid, limiting the access to microorganism action, compared with a mixed forage/concentrate diet. This helps to explain the high values for muscle PUFA proportions seen in young bulls fed a "barley beef" diet (Enser et al., 1998), although a low carcass fat level in bulls will also be important, leading to a high proportion of phospholipid in total lipid.

The concentrate portion of beef cattle diets can be fortified with dietary oils with positive effects on muscle and adipose tissue fatty acid composition. Scollan et al. (2001a) fed linseed and fish oils to increase proportions of 18:3n-3 and the long-chain n-3 PUFA, respectively (Table 5.8). The concentrate portion of the diet contained 6% oil of which half was the test oil. These diets were fed for 120 days along with grass silage in a 60:40 forage/concentrate combination. The results

TABLE 5.8
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Longissimus* Muscle of Steers Fed Different Oil Sources in the Concentrate Portion of a 60:40 Forage/Concentrate Mixed Diet

	Control		Linseed		Fish Oil		Linseed/Fish	
	NL	PL	NL	PL	NL	PL	NL	PL
14:0	3.7	0.7	4.1	0.6	4.6	0.9	4.4	0.7
16:0	30.0	17.2	27.1	14.7	32.0	17.5	29.0	15.5
16:1 *cis*[a]	3.7	2.2	4.0	2.0	4.2	2.3	4.2	2.3
18:0	15.8	10.5	14.2	10.4	13.2	9.7	12.9	9.6
18:1 *trans*[a]	1.9	0.6	3.8	1.3	4.4	1.8	4.9	1.8
18:1 *cis*-9	36.0	23.4	35.7	21.6	30.1	17.4	33.2	20.7
18:1 *cis*-11	0.9	1.7	1.0	1.7	1.0	2.3	0.9	1.9
18:2n-6	0.9	11.4	0.8	10.1	0.6	8.4	0.7	9.2
18:3n-3	0.4	2.1	0.6	4.3	0.4	2.4	0.4	3.5
20:3n-6	ND	1.5	ND	1.2	ND	1.1	ND	1.0
20:4n-6	ND	5.1	ND	4.5	ND	3.0	ND	3.9
20:5n-3	ND	2.3	ND	3.5	ND	4.9	ND	3.6
22:5n-3	ND	4.3	ND	4.6	ND	4.8	ND	4.5
22:6n-3	ND	0.5	ND	0.6	ND	1.1	ND	1.2
Total fatty acids (g/100 g muscle)	2.9	0.45	3.2	0.44	3.9	0.50	2.9	0.45

ND: not detected.

[a]All isomers.

Source: Scollan, N.D., et al. 2001a. *British Journal of Nutrition*, **85**, 115–124.

show that there was a limited effect of oil source on neutral lipid fatty acid composition (e.g., 18:3n-3 increased from 0.4% in the control diet to 0.6% in linseed) but a bigger effect on phospholipid (18:3n-3 increased from 2.1% in controls to 4.3% in the linseed diet; 20:5n-3 increased from 2.3% in controls to 4.8% in the fish oil diet). These results again emphasize the importance of the phospholipid fraction in muscle PUFA proportions.

The effects of dietary oils on meat fatty acid composition are enhanced if they are protected from biohydrogenation in the rumen. Chemical protection can be achieved if protein in the diet is treated with formaldehyde, which results in a matrix structure within which dietary fatty acids are "encapsulated." In a study where soybean (0.7), linseed (0.2), and sunflower (0.1) seed oils were combined and protected in this way, %18:3n-3 in neutral lipid was raised from 0.4% to 1.7% and in phospholipid from 2.5% to 3.3% (Scollan et al., 2003). Interestingly, there were no increases in proportions of 20:5n-3 and 22:6n-3 in phospholipid despite this increase in 18:3n-3. These results contrast with those in Table 5.7, which show that providing 18:3n-3 in grass increased all the long-chain n-3 PUFA, including 22:6n-3.

Several studies have shown that there is a linear relationship in muscle and adipose tissue between the proportions of 18:1 *trans*-11 (*trans* vaccenic acid) and the main CLA isomer, *cis*-9, *trans*-11 CLA, reflecting the synthesis of CLA from its precursor. In the study of Warren et al. (2007) described in Table 5.7, a group of steers was reared on fresh grazed grass from 14 to 19 months in addition to those steers fed concentrate and grass silage to the same ages. The results for adipose tissue (Figure 5.3) confirm the linear relationship between 18:1 *trans*-11 and CLA and show that both fatty acids are at higher proportions following the consumption of fresh grass compared with

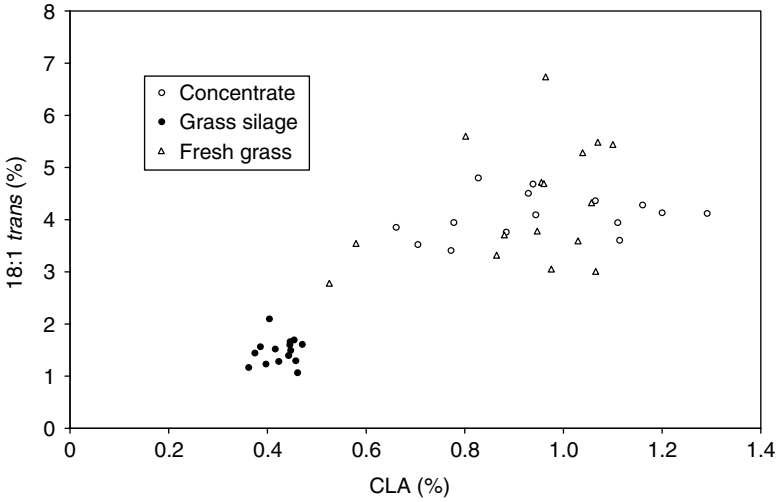

FIGURE 5.3 Relationship between proportions of 18:1 *trans*-11 and CLA in adipose tissue of steers given different diets. (Warren, H.E., et al. 2007a. *Meat Science*, in press.)

grass silage. Values for the group fed fresh grazed grass were similar to those fed concentrate, in contradiction to other findings showing higher values after consuming grass.

Wood et al. (2005) showed that there were three main CLA isomers in beef muscle and the levels were affected by dietary oil source. The *cis*-9, *trans*-11 isomer always exceeded 80% of the total, the other two isomers being *trans*-11, *cis*-13 and *trans*-11, *trans*-13.

The 18:3n-3 content of grass changes through the growing season in parallel with other nutrients such as nitrogen (Walker et al., 2004). Other factors that affect grass fatty acid composition and therefore meat fatty acid composition include the conservation process. Excessive drying of grass prior to producing hay or silage reduces PUFA proportions through the action of plant enzymes (Dewhurst et al., 2003) and fatty acid oxidation.

C. Sheep

The effects of grass-based diets compared with grain-based (concentrate) diets are similar in sheep to those in cattle. Results in Table 5.9 are from a study by Fisher et al. (2000) in which Suffolk cross sheep were grazed on lowland pasture or fed a standard concentrate diet for 3 months before slaughter. Values for the proportions of PUFA are high in comparison with the beef steers in Table 5.7 when those data are used to calculate proportions of total lipid. For example, 18:3n-3 was 0.6% of total lipid in the steers fed grass silage compared with 2.3% in the grazed lambs in Table 5.9. However, the much lower level of total muscle fatty acids is also a factor in these high values for lambs. As with the grass-fed steers, all the long-chain n-3 PUFA were higher in the grass-fed lambs than in those fed concentrate, including DHA.

Concentrate diets containing the same dietary oil sources as those used in the cattle study described in Table 5.8 were fed to three breeds of ram lambs in a study by Wachira et al. (2002). The diets contained 6% oil from different sources and were fed between 24 and 44 kg live weight. Results for the fatty acid composition of *longissimus* muscle total lipid (containing both neutral lipid and phospholipid) for Suffolk cross lambs are shown in Table 5.10. The proportion of 18:3n-3 in the group fed linseed was higher than for the grazed lambs in Table 5.9 and yet proportions of 20:5n-3, 22:5n-3, and 22:6n-3 were lower. These results confirm the effect of grass diets in raising levels of long-chain n-3 PUFA.

TABLE 5.9
Fatty Acid Composition (% of Total Fatty Acids) of Total Lipid in *Semimembranosus* Muscle of Suffolk Cross Ram Lambs Fed Concentrate or Fresh Grass (Grazing)

	Concentrate	Grazed Grass
14:0	2.3	3.2
16:0	19.4	18.6
18:0	12.3	14.3
18:1 *trans*[a]	4.3	4.3
18:1 *cis*-9	36.7	31.7
18:2n-6	9.7	6.8
18:3n-3	0.7	2.3
20:4n-6	3.3	2.6
20:5n-3	0.4	1.3
22:5n-3	0.8	1.5
22:6n-3	0.3	0.6
Total fatty acids (g/100 g muscle)	1.96	1.85

[a] All isomers.

Source: Fisher, A.V., et al. 2000. *Meat Science*, **55**, 141–147.

TABLE 5.10
Fatty Acid Composition (% of Total Fatty Acids) of Total Lipid in *Longissimus* Muscle of Ram Lambs Fed a Concentrate Diet Containing Different Oil Sources

	Control	Linseed	Fish Oil	Linseed/Fish
14:0	2.5	2.4	2.7	2.3
16:0	25.1	21.1	24.3	23.2
16:1 *cis*[a]	1.8	1.5	2.3	1.7
18:0	14.6	15.5	12.5	13.4
18:1 *trans*[a]	3.8	6.4	8.1	9.0
18:1 *cis*-9	34.1	31.3	24.4	27.3
18:1 *cis*-11	1.3	0.9	2.0	1.6
18:2n-6	4.7	3.6	3.1	3.3
18:3n-3	1.4	3.1	1.4	2.2
20:3n-6	0.1	0.1	0.2	0.1
20:4n-6	1.0	0.7	0.6	0.7
20:5n-3	0.8	0.8	2.4	1.5
22:5n-3	0.7	0.7	1.4	1.0
22:6n-3	0.3	0.3	1.0	0.7
Total fatty acids (g/100 g muscle)	3.15	3.09	3.66	3.03

[a] All isomers.

Source: Wachira, A.M., et al. 2002. *British Journal of Nutrition*, **88**, 697–709.

TABLE 5.11
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Longissimus* Muscle of Entire Male Pigs of Different Breeds Fed a Standard Concentrate Diet

	Berkshire		Duroc		Large White		Tamworth	
	NL	PL	NL	PL	NL	PL	NL	PL
14:0	1.7	0.3	1.6	0.3	1.4	0.2	1.7	0.2
16:0	26.8	16.5	23.8	16.6	23.1	16.3	25.9	16.7
16:1 *cis*[a]	4.4	1.1	2.6	0.8	3.3	0.8	4.0	0.8
18:0	12.8	11.6	15.6	12.1	11.7	11.8	12.6	10.9
18:1 *cis*-9	38.7	11.4	36.2	9.4	38.7	10.9	38.5	9.8
18:1 *cis*-11	4.3	3.3	3.4	2.6	4.0	3.0	4.3	3.5
18:2n-6	7.8	29.5	12.0	31.4	12.8	29.9	8.8	31.5
18:3n-3	0.6	0.7	1.0	0.6	0.9	0.6	0.7	0.8
20:3n-6	ND	1.1	ND	1.3	ND	1.2	ND	1.3
20:4n-6	0.2	10.3	0.2	10.5	0.4	11.1	0.3	9.8
20:5n-3	ND	0.7	ND	1.0	ND	0.8	ND	0.7
22:5n-3	ND	2.0	ND	2.0	ND	2.2	ND	1.9
22:6n-3	ND	0.8	ND	1.2	ND	0.9	ND	0.6
Total fatty acids (g/100 g muscle)	1.67	0.39	1.35	0.42	0.60	0.38	0.82	0.38

ND: not detected.

[a]All isomers.

Source: Wood, J.D., et al. 2004. *Meat Science*, **67**, 651–667.

VII. GENETIC EFFECTS ON MEAT FATTY ACID COMPOSITION

A. Pigs

Several authors have shown effects of breeds and genetic lines on muscle and adipose tissue fatty acid composition. In pigs, Wood et al. (2004) compared four pure breeds reared from 9 weeks of age for 12 weeks on two different concentrate diets. Results for the standard (control) diet are shown in Table 5.11. The two traditional breeds, Berkshire and Tamworth, were fatter than the two modern breeds, Duroc and Large White, at slaughter. Values for P_2 fat thickness (fat thickness at the last rib position, 6.5 cm from the midline) were 15.1, 9.1, 7.8, and 14.7 mm in Berkshire, Duroc, Large White, and Tamworth, respectively. In general, differences in fatty acid composition between the breeds were restricted to neutral lipid and were quite small, reflecting differences in carcass fatness. Thus the two modern lean breeds, Duroc and Large White, had higher proportions of 18:2n-6 (12.0% and 12.8%) than the two traditional breeds, Berkshire and Tamworth (7.8% and 8.8%). The main product of de novo synthesis (16:0) was higher in the two traditional breeds. Many of the other differences between the breeds were statistically significant but numerically small. The content of total muscle fatty acids (neutral lipid plus phospholipid) did not reflect the differences in carcass fat, Durocs having a higher value for muscle lipid than Large Whites but a similar P_2 fat thickness. Similarly, Tamworth pigs had a lower muscle fat value than Berkshires but similar subcutaneous fat thickness (Figure 5.4). These results show that partition of fat in the body is under genetic control.

B. Cattle

Results in Table 5.12 show the fatty acid composition of *longissimus* muscle in steers reared from 6 to 24 months of age on a grass silage diet. There were two breeds, a beef crossbreed (Aberdeen

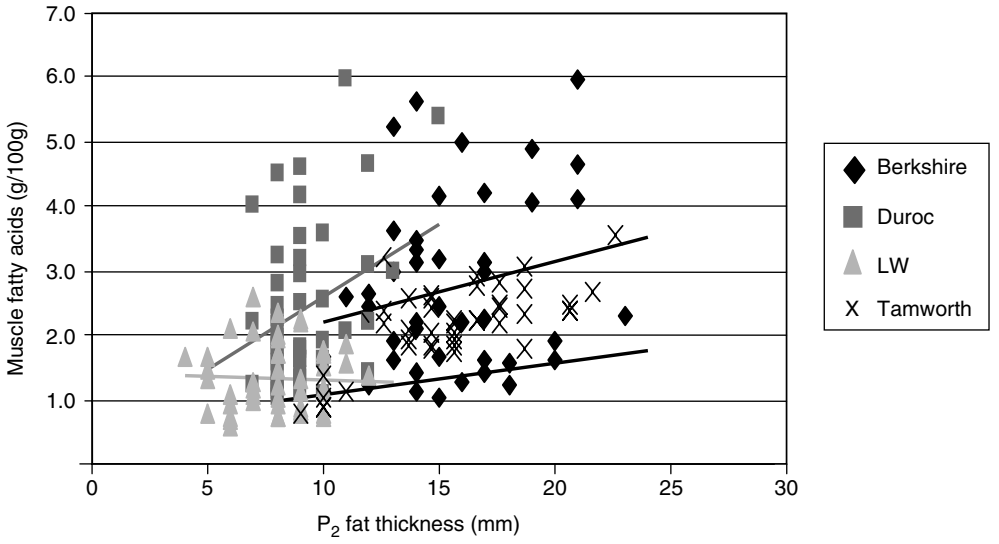

FIGURE 5.4 Relationship between total muscle fatty acids in *longissimus* muscle and thickness of subcutaneous fat (P_2, mm) in four pig breeds. (Wood, J.D., et al. 2004. *Meat Science*, **67**, 651–667.)

TABLE 5.12
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Longissimus* Muscle of Aberdeen Angus Cross and Holstein-Friesian Steers Fed a Grass Silage Diet to 24 Months of Age

	Aberdeen Angus		Holstein-Friesian	
	NL	PL	NL	PL
14:0	3.2	1.0	2.9	0.5
16:0	29.3	18.2	28.0	16.0
16:1 *cis*[a]	4.8	3.0	5.1	2.1
18:0	12.0	10.7	12.0	11.0
18:1 *trans*[a]	1.0	0.4	1.1	0.4
18:1 *cis*-9	39.4	28.0	40.0	23.8
18:1 *cis*-11	1.8	2.4	2.0	2.5
18:2n-6	0.6	5.8	0.7	8.1
18:3n-3	0.4	3.3	0.5	4.1
20:3n-6	ND	0.8	ND	1.0
20:4n-6	ND	3.7	ND	4.9
20:5n-3	ND	2.7	ND	4.2
22:5n-3	ND	3.9	ND	4.5
22:6n-3	ND	0.4	ND	1.0
Total fatty acids (g/100 g muscle)	8.92	0.65	4.34	0.71
Carcass fat score	108		66	

[a]All isomers.

Source: Warren, H.E., et al. 2007a. *Meat Science*, in press.

Angus × Holstein-Friesian) and a dairy breed (Holstein-Friesian). The Aberdeen Angus steers were considerably fatter, judged by the carcass fat score, which is a visual estimation of subcutaneous fat deposition. Values approximate to percentage subcutaneous fat in the carcass 10 times. Aberdeen Angus also had more neutral lipid and more phospholipid fatty acids in muscle. Values for the main fatty acid proportions follow a pattern similar to those for pig breeds differing in carcass fat content (Table 5.11). Thus, the fatter Aberdeen Angus steers had higher proportions of 16:0 in neutral lipid and lower proportions of PUFA. However, the differences in proportions of n-6 and n-3 PUFA in phospholipid, with Holstein-Friesians having much higher values than Aberdeen Angus, are an indication of a genetic effect that is independent of fatness. Differences are much bigger than seen in the pig study (Table 5.11) despite similarly large differences in fatness. We hypothesize that these differences are due to breed differences in the expression of enzymes forming long-chain n-3 PUFA from 18:3n-3.

Studies in Belgium have shown that the Belgian Blue breed has very high proportions of PUFA in muscle tissue linked directly to a low fat content. In a study by Raes et al. (2004), values for 18:2n-6 in *longissimus* muscle of Belgian Blue cattle fed different diets were above 10% of total lipid (almost as high as in pigs) and the P:S ratio was above 0.3. Contrast this with 0.11 in United Kingdom beef in Table 5.5. The authors concluded that a low concentration of muscle lipid (<1% of muscle weight) resulted in a high ratio of phospholipid to total lipid and this caused the high-PUFA proportions.

C. Sheep

Considerable variations in body size and shape exist between sheep breeds, explained by the wide range of environments inhabited by sheep and selection of breeding animals for different production characteristics. Wachira et al. (2002) compared Suffolk cross, Friesland cross, and Soay breeds representing extremes of breed types in the United Kingdom (i.e., a meat type, a milk type, and a primitive/traditional type). Ram lambs were fed a concentrate diet containing different oil sources to about 50% of the breed mature weight. Results for the groups fed megalac, a relatively saturated oil source, are shown in Table 5.13. Suffolks were slightly fatter than Soays and Frieslands, although the total fatty acid content of muscle (neutral lipid plus phospholipid) was higher in Soays. The differences in total lipid fatty acid composition were small when compared with the results for cattle (Table 5.12). Although the proportions of 18:2n-6 and 18:3n-3 were highest in the lean Soay breed, long-chain n-3 fatty acid proportions were similar. Fisher et al. (2000) found that PUFA proportions were higher in grass-fed Soays than in Welsh Mountain and Suffolk cross animals fed on grass.

Recent results from Bishop et al. (2006) show high heritabilities for fatty acid composition in sheep and evidences that genetic differences in fatty acid composition can be detected using genetic markers (QTLs).

VIII. FATTY ACID COMPOSITION OF DIFFERENT MUSCLES, TISSUES, AND MEAT PRODUCTS

Muscles in the carcass differ in fiber type, with muscles involved in rapid movement having predominantly white glycolytic type II fibers and those involved in posture retention having predominantly red oxidative type I fibers. All muscles contain a mixed population of fiber types, including intermediate types between these two extremes.

Red oxidative fibers contain more mitochondria and a higher proportion of phospholipid than white glycolytic fibers and as a result contain a higher proportion of PUFA. Results in Table 5.14 show the fatty acid composition of the predominantly "white" *longissimus* and the "red" *psoas* muscle in Duroc pigs fed the control diet in the study of Wood et al. (2004). The proportion of 18:2n-6

TABLE 5.13
Fatty Acid Composition (% of Total Fatty Acids) of Total Lipid in *Longissimus* Muscle of Ram Lambs from Three Breeds Fed a Concentrate Diet Containing the Saturated Oil Source Megalac

	Suffolk	Soay	Friesland
14:0	2.5	2.5	2.6
16:0	25.1	26.0	25.1
16:1 *cis*[a]	1.8	1.9	1.9
18:0	14.6	15.1	13.8
18:1 *trans*[a]	3.8	4.2	3.4
18:1 *cis*-9	34.1	32.1	36.1
18:1 *cis*-11	1.3	1.0	1.4
18:2n-6	4.7	5.6	4.2
18:3n-3	1.4	1.7	1.2
20:3n-6	0.1	0.1	0.1
20:4n-6	1.0	1.1	1.2
20:5n-3	0.8	0.6	0.6
22:5n-3	0.7	0.6	0.6
22:6n-3	0.3	0.3	0.2
Total fatty acids (g/100 g muscle)	3.15	3.42	3.13
Carcass fat (%)	19.0	16.5	17.2

[a]All isomers.

Source: Wachira, A.M., et al. 2002. *British Journal of Nutrition*, **88**, 697–709.

was higher in phospholipid from *psoas* but the main difference between the two muscles was the higher proportion of phospholipid in total lipid of *psoas* (41.5% vs. 23.7%).

Liver is also a metabolically active tissue with a high proportion of phospholipid in total lipid. Results in Table 5.15 for lambs show that total lipid was 2.4% and 5.4% of tissue weight in muscle and liver, respectively, phospholipid being 24.1% and 56.9% of total lipid. The phospholipid in liver was less unsaturated than that in muscle. For example, 18:2n-6 was 12.4% and 5.8% of phospholipid fatty acids in muscle and liver, respectively.

The fatty acid composition of various tissues in pigs fed a control diet is shown in Table 5.16. The range in values for 18:2n-6 was from 10.9% in muscle neutral lipid to 29.0% in muscle phospholipid. Values for 18:2n-6 and 20:4n-6 were higher in pig liver than lamb liver (Table 5.15), whereas 18:3n-3 and 20:5n-3 were higher in lamb liver. These differences again reflect the different diets consumed. The presence of long-chain PUFA in pig subcutaneous fat albeit at low levels, contributes to a high nutritional value in pigmeat.

IX. EFFECTS OF FATTY ACIDS ON MEAT QUALITY

The total amount of fat in meat and its fatty acid composition affect many aspects of meat quality and only a few examples will be given here. More information is in Wood et al. (2003).

In pigs, the fatty acid composition of subcutaneous fat affects its firmness, which is a factor in product quality. High concentrations of PUFA in fat tissue, caused by a high level in the diet, produce soft, oily fat with low visual and handling quality as shown in the early studies of pigs fed soybeans by Ellis and Isbell (1926). The two fatty acids having the biggest effect on firmness are 18:0 and 18:2n-6, and Whittington et al. (1986) found that the ratio between these provided the best prediction of firmness measured by using a penetrometer. The reduced "fat quality" of pigs with thin

TABLE 5.14
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Longissimus* and Psoas Muscles of Duroc Pigs Fed a Control Concentrate Diet

	Longissimus		Psoas	
	NL	PL	NL	PL
14:0	1.6	0.3	1.3	0.2
16:0	23.8	16.6	23.5	15.6
16:1 *cis*[a]	2.6	0.8	2.4	0.7
18:0	15.6	12.1	15.5	13.2
18:1 *cis*-9	36.2	9.4	31.0	7.9
18:1 *cis*-11	3.4	2.6	2.9	3.3
18:2n-6	12.0	31.4	15.9	35.1
18:3n-3	1.0	0.6	1.1	0.8
20:3n-6	ND	1.3	ND	1.0
20:4n-6	0.2	10.5	1.1	9.6
20:5n-3	ND	1.0	ND	0.9
22:5n-3	ND	1.9	ND	1.8
22:6n-3	ND	1.2	ND	0.9
Total fatty acids (g/100 g muscle)	1.35	0.42	0.76	0.54

ND: not detected.

[a]All isomers.

Source: Wood, J.D., et al. 2004. *Meat Science*, **67**, 651–667.

as opposed to thick subcutaneous fat, which includes separation between fat layers and a dull color as well as softness, was found by Wood et al. (1986, 1989) to be closely related to changes in the proportions of these two fatty acids as well as water and lipid. An increase in fat thickness indicates a more mature tissue with larger fat cells, a higher proportion of lipid, and a corresponding reduction in the proportion of water.

Fatty acid composition plays an indirect role in the color of meat in retail display. Vatansever et al. (2000) examined the beef produced using different dietary oil sources reported by Scollan et al. (2001a) (Table 5.8). The sources were megalac (relatively saturated), linseed (high in 18:3n-3), and fish oil (high in 20:5n-3 and 22:6n-3). Sirloin steaks were displayed under simulated retail display conditions and lipid oxidation and surface color were measured over 11 days. As the display period progressed, lipid oxidation increased, more so in the steaks produced using fish oil, which had the highest concentration of PUFA. These are relatively unstable, oxidation leading to production of many compounds, including aldehydes, ketones, and alcohols. There was a parallel decline in the color saturation of the steaks during the display period, this being a measure of the intensity of the red color. Saturation declined fastest in the steaks produced using fish oil and slowest in the megalac controls. The implication is that lipid oxidation products catalyze the oxidation of the muscle pigment myoglobin leading to accelerated production of the oxidized form, metmyoglobin.

A difference in the color of beef sirloin steaks develops during retail display in grass- and concentrate-fed cattle. In the study of Warren et al. (2007b), color saturation declined more quickly in the concentrate-fed steers than in those fed grass silage (Figure 5.5). This was associated with higher lipid oxidation in the steaks from animals fed concentrate. This was partly related to higher proportions of PUFA in these steers (Table 5.7) although a much lower concentration of vitamin E in muscle tissue compared with that from steers fed grass silage was probably a more important factor.

TABLE 5.15
Fatty Acid Composition (% of Total Fatty Acids) of Neutral Lipid (NL) and Phospholipid (PL) in *Semimembranosus* Muscle and Liver of Ram Lambs Fed a Concentrate Diet Containing Megalac as the Oil Source

	Muscle		Liver	
	NL	PL	NL	PL
14:0	3.0	0.4	1.1	0.4
16:0	25.6	15.0	17.9	12.9
16:1 *cis*[a]	2.2	1.5	2.8	1.7
18:0	13.6	10.4	10.4	23.7
18:1 *trans*[a]	2.0	0.5	2.4	1.6
18:1 *cis*-9	43.8	22.1	30.4	19.8
18:1 *cis*-11	1.2	3.6	2.4	1.8
18:2n-6	1.5	12.4	5.3	5.8
18:3n-3	1.2	4.6	3.8	2.9
20:3n-6	ND	0.6	ND	ND
20:4n-6	ND	5.9	2.1	4.8
20:5n-3	ND	4.1	2.7	4.6
22:5n-3	ND	3.8	4.1	5.8
22:6n-3	ND	1.2	2.0	4.1
Total fatty acids (g/100 g tissue)	1.86	0.59	2.33	3.08

ND: not detected.

[a]All isomers.

Source: Demirel, G., et al. 2004. *British Journal of Nutrition*, **91**, 551–565.

TABLE 5.16
Some Fatty Acids (% of Total Fatty Acids) in *Longissimus* Muscle Total Lipid, Neutral Lipid (NL), and Phospholipid (PL) and Total Lipid of Subcutaneous Fat, Sausages Made from Muscle and Fat Tissue from the Shoulder and Liver of Entire Male Pigs Fed a Standard Concentrate Diet

	18:2n-6	18:3n-3	20:4n-6	20:5n-3
Muscle total lipid	17.3	0.9	3.9	0.4
NL	10.9	0.9	0.7	0.04
PL	29.0	0.9	9.7	0.9
Subcutaneous fat	20.2	1.9	0.2	0.05
Sausages	18.9	1.9	0.5	0.09
Liver	19.2	1.0	14.0	0.75

Source: Enser, M., et al. 2000. *Meat Science*, **55**, 201–212.

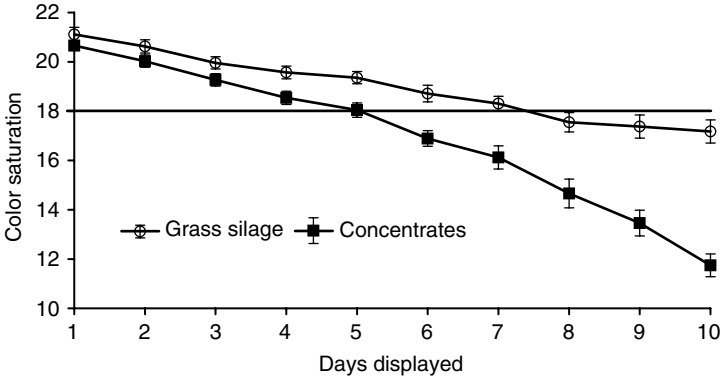

FIGURE 5.5 Color of beef sirloin steaks from steers fed concentrate or grass silage diets to 14 months of age. (Warren, H.E., et al. 2007b. *Meat Science*, in press.)

Vitamin E is present at high concentrations in leafy grass when n-3 PUFA proportions are also high, protecting the PUFA from oxidation, both in the grass and in the animal tissues.

Fatty acids contribute to the meat flavors generated during cooking, either directly through the lipid oxidation products, some of which are odorous, or indirectly by reactions between lipid oxidation products and proteins and carbohydrates present in the meat. High levels of 20:5n-3 and 22:6n-3 in lamb, created by feeding fish oils or marine algae, produced high taste panel scores for "rancid" and "fishy" and low scores for "overall liking" in recent research by Nute et al. (2007). On the other hand, an increased concentration of 18:3n-3 from linseed elicited the highest scores for "lamb flavor" and "overall liking." In a companion paper, Elmore et al. (2005) identified high concentrations of compounds generated from the thermal oxidation of n-3 PUFA in headspace volatiles from the samples produced from marine algae.

Campo et al. (2006) observed that beef sirloin steaks displayed in "modified atmosphere" packs containing a high concentration of oxygen showed increased lipid oxidation as the display period progressed. Samples with high concentrations of PUFA had the highest oxidation and they received the lowest taste panel scores for "beef flavor." They scored highest for "abnormal flavor" and "rancid" and there were strong negative correlations between TBARS (thiobarbituric acid reactive substances, the standard test for meat lipid oxidation) and "beef flavor" and between "beef flavor" and "rancid." These results show that fatty acid composition is a major factor in beef flavor.

X. CONCLUSIONS

This chapter shows that the fatty acid composition of meat in pigs, cattle, and sheep is affected by diet and breed, and differs between tissues. The levels of fat tissue in the carcass and meat are important underlying factors because fatty acid composition changes as fat is deposited. One important consequence of increasing fat deposition is that the proportion of phospholipid is reduced, leading to a decrease in the proportion of the major PUFA, 18:2n-6.

The fatty acid composition of pork can be readily modified by diet since fatty acids are deposited unchanged by digestion. In ruminants, the rumen is a barrier to the incorporation of PUFAs into meat although the effect of grass diets in increasing proportions of n-3 PUFA and possibly CLA is an interesting area of current research, leading to more desirable meat products for the consumer.

The fatty acid composition of meat is important for human health reasons and also has crucial effects on meat quality, for example, fat tissue firmness, color, shelf life, and flavor. Changes in production that affect fatty acid composition should therefore only be introduced after examining the impact on meat quality.

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6 Fatty Acids in Milk Fat

Donald L. Palmquist and Robert G. Jensen

CONTENTS

I. INTRODUCTION

The bulk of the lipids in milk, 97%–98%, are triacylglycerols (TG), with sterols (mostly cholesterol) and phospholipids next in quantity (Table 6.1). The di- and monacylglycerols (DG and MG, respectively) and nonesterified fatty acids (FA) in quantities greater than traces are the products of postharvest lipolysis. Freshly drawn milk that is promptly pasteurized or frozen and stored at –70°C contains little of these lipolytic compounds. However, increased milking frequency, a practice that is increasing in the dairy industry, increases lipolysis in fresh milk (Wiking et al., 2006). The FA of commercial milk do not vary greatly in proportion because of the averaging effect of pooling by milk processors; many FA that are found in milk in low or minute quantities may have important nonnutritive roles as nutriceuticals (Milner, 1999; Parodi, 2006).

Most of the lipids in milk and dairy products exist as globules 1–4 μm in diameter as oil in water emulsion (Jensen and Newburg, 1995; Huppertz and Kelly, 2006). The emulsion is stabilized by a bipolar membrane of phospholipids and proteins existing as a loose layer at the oil–water interface. In making butter, the membrane is removed, causing reversal of the emulsion to a water–oil type.

Numerous reviews on lipid composition of milk fat are available (Jensen and Newburg, 1995; Jensen, 2002; MacGibbon and Taylor, 2006). This chapter complements a thorough review on bovine milk lipids published by Jensen (2002). Readers who need more historical information are referred to the chapter in the first edition of this monograph (Jensen, 1992); and for those desiring detailed discussions of all milk lipids see the recent volume *Advanced Dairy Chemistry. Volume 2. Lipids, 3rd edition* (Fox and McSweeney, 2006).

TABLE 6.1
Lipids in Milk at Lactation Day 42[a]

Lipid Class	mg/g of Lipid
Phospholipid	11.1
Cholesterol	4.6
Triacylglycerol	958
1,2-Diacylglycerol	22.3
Fatty acid	2.8
Monoacyglycerol	0.8
Cholesteryl ester	0.2

[a] Based on a study of 12 cows; total fat, 3.25 g/dL of milk.

Source: Adapted from Bitman, J. and Wood, D.L. (1990). *J. Dairy Sci.* 73:1208–1216.

II. ANALYSIS OF MILK FATTY ACIDS

Extraction of milk fat in preparation for gas–liquid chromatography (GLC) analysis is discussed by Jensen et al. (1997). For GLC analysis, milk FA are usually transesterified to FA methyl esters (FAME) with sodium methoxide or acids (H_2SO_4, HCl, or BF_3) as catalysts. Kramer et al. (1997) evaluated the applicability of catalysts for converting milk FA to FAME. They recommended that sodium methoxide be used even though FA in sphingomyelin were not esterified. The amount of FA excluded (approximately 0.1% of total FA) is so low that there would be little or no effect on the amounts or proportions of FA measured. Acid catalysts were reported to cause isomerization of conjugated bonds, resulting in decreased recovery, whereas sodium methoxide did not lower the quantities of these. It is important to determine FA by appropriate methods; if referencing Kramer et al. (1997), one must take care to specify which procedure was used, as these authors describe numerous combinations of methods for FAME preparation and analysis. If lipolysis has occurred (not common in commercial samples), then acid-catalyzed transesterification should be employed so that the Ca-FA salts (soaps) will be cleaved, allowing complete derivative formation. Modern technologies employ both mild acid and alkaline transesterification for milk fat samples (Kramer et al., 1997). See the discussion on acid transesterification by Kramer et al. (1997) and Carrapiso and García (2000), and on using internal standards for quantifying FA (Palmquist and Jenkins, 2003). Up-to-date literature and discussion of advances in lipid analysis can be found at http://www.lipidlibrary.co.uk.

Butyric acid (4:0) as the methyl ester is difficult to measure because of its extreme volatility and water solubility; repeated extraction of methyl butyrate from the aqueous reaction mixture with organic solvent is required to achieve complete recovery and to prevent biased estimates of FA proportions. If the measured content of 4:0 is less than 3% by weight, probability is that some of the butyrate has been lost. Molkentin and Precht (1997) reported 3.42% ± 0.144 (range 3.07–3.75) in 136 samples. Butyl (Iverson and Sheppard, 1986; Ulberth et al., 1999) and isopropyl (Wolff and Fabien, 1989) esters, rather than methyl esters, have been utilized to minimize loss of 4:0–10:0. Butyl and isopropyl esters can be prepared by substituting butanol or isopropanol for methanol in the esterification reaction. Temperature programming is applied to separate the esters of the short-chain FA from the solvent peak and from each other.

Proper identification of isomers is critical; this is particularly difficult in the 16:1 to 18:2 region of GLC chromatograms, where the numerous C18 monoenoic isomers elute. Comparison of retention

times with authentic standards is a minimum requirement, though not conclusive. Use of authentic standards, aided by comparison with published profiles such as those by Kramer et al. (1997) or Precht and Molkentin (1996, 1997b,c) may have greater reliability, whereas greatest certainty for the identification of isomers is by combined gas chromatography–mass spectrometry (GC–MS). Use of a certified butter oil reference standard, such as CRM 164 (Commission of the European Community, Bureau of References, Brussels, Belgium; Precht et al., 1998), is encouraged.

FA compositions are usually reported as relative weight percentages based on the peak areas from the GLC chart, though for some investigations, calculation of mole percentages is more informative. For fluid products, weights of FA/dL should be reported, so that the actual yields (in the case of production studies) or intake (for nutrition studies) of FA can be calculated. The amounts of FA can be determined by use of an internal standard with GLC analysis (Carpenter et al., 1993; Palmquist and Jenkins, 2003).

In the United States, the Food and Drug Administration and the Department of Agriculture (USDA) require that fats and FA contents be reported on food labels as the sum of TG equivalents (Carpenter et al., 1993). For example, if the weight of 16:0 taken from the GLC chart is 1 g then it is reported as 1.044946 g of tripalmitin, that is, the molecular weight (MW) of tripalmitin divided by the 3 × MW of palmitic acid. The factors for milk FA range from 1.074 for 10:0 to 1.037 for 22:0; most are close to 1.05, which may be utilized. Although not stated by Carpenter et al. (1993), the glyceride fragments of CH_3O and CH_2O and the methyl portion CH_3O esterified to FA have nearly equal MWs: 30 and 31; thus, the equivalent weights of FAME and TG FA are nearly identical, and the FAME units summed equals the sample TG weight. Although the weights of FA calculated from the areas on a GLC chart in relation to a FFA internal standard will not be equal to the weight of the extracted lipid, because the glyceride fragment is not included, the FA weight can be converted to TG by using the conversion factors, usually 1.05, as described above. The calculations described here allow one to report FA analysis as TG, according to package labeling requirements for commercial products. However, for computing energy values, it is more appropriate to consider FA and glycerol separately, because glycerol is metabolized and has the energy value of carbohydrate.

III. MILK FATTY ACIDS

Hilditch and Williams (1964) described early efforts to analyze milk FA when separations were attained by distillation of FAME, which were then characterized by determination of fat constants. Some of their data are presented in Table 6.2 and compared with results from GLC analyses of methyl esters from Posati et al. (1975) and Precht and Molkentin (1997a). The results described by Hilditch and Williams (1964) compare well with modern GLC analyses, remarkable when the differences in time, location, and methods are considered. Differences could be attributed to changes in feeding practices alone (Palmquist et al., 1993). Note that Precht and Molkentin (1997a) resolved 18:1c from 18:1t, a separation not possible with most packed GLC columns. Nevertheless, unless isomers of FAME coeluted, as with 18:1c and 18:1t, the weight percentages reported before use of capillary GLC columns were precise. However, the older procedures for measuring the areas of peaks on the GLC charts lacked sensitivity and thus are less reliable and report fewer FA than current analyses that utilize long capillary columns. Vaccenic acid is the predominant *trans* FA in milk fat (Wolff, 1995), and may comprise more than half of the 18:1 isomers in milk under some feeding conditions. Vaccenic acid was first described in milk fat by Bertram (1928); the name is derived from the latin word for cow and is the trivial name for the naturally occurring t11 18:1. Use of the term *trans* to describe this isomer is redundant and unnecessary.

The improved sensitivity and greater resolving power of capillary GLC have increased the number of FA detected and identified in milk. For example, 11 FA were listed in a 1929 publication (Hilditch and Williams, 1964). The number increased from 11 in 1929 to 167 in 1967 (Jensen et al., 1967) and

TABLE 6.2
Comparison of Milk Fatty Acid Contents (g/100 g) Obtained by Distillation or Gas–Liquid Chromatography of Methyl Esters[a]

Fatty Acid	English Market Samples,[b] 1929	1960–1974 Data[c]	German Milk, 1997[d]		
4:0	3.2	3.2	4.1	18:1t	3.8
6:0	1.8	1.9	2.4	18:3	0.7
8:0	0.8	1.1	1.3	20:1	0.2
10:0	2.1	2.4	2.9	20:4	0.1
12:0	3.8	2.8	3.6	22:0	0.1
14:0	10.4	10.1	11.1	22:1	Tr
16:0	28.0	26.3	28.6	24:0	0.1
18:0	10.0	12.1	9.5		
20:0	0.7	2.2	0.2		
18:1	34.1	25.1	22.6		
18:2	4.5	2.3	1.2		

[a] Minor components omitted.
[b] Hilditch, T.P. and Williams, P.N. (1964). The *Chemical Constitution of Natural Fats*, 4th ed., Wiley, New York, p. 146. Analysis by distillation of methyl esters. Identities of 20:0 and 18:2 not established.
[c] Posati, J.P., et al. (1975). *J. Am. Diet. Assoc*. 66:482–488. Analyses by gas–liquid chromatography of methyl esters, average of literature data. Identity of 20:0 given as other.
[d] Precht, D. and Molkentin, J. (1997a). *Kieler Milchwirtsch. Forsch. Ber*. 49:17–34. Analyses by gas–liquid chromatography of methyl esters with a capillary column, 100 m, n = 100.

406 in 1995 (Jensen and Newburg, 1995). Jensen (2002) lists 416 FA and explains corrections made to some earlier accountings of the number of identified milk FA. Many of these FA were not identified by GLC alone. Other techniques were utilized to obtain fractions enriched in certain types of FA, such as separation of classes of unsaturated FA with $AgNO_3$-thin-layer chromatography (TLC) before GLC analysis. Identifications have been confirmed with GLC–MS. A typical milk FA profile with modern analysis is in Table 6.3.

The variety of FA found in milk fat is accounted for mainly by the ruminal biohydrogenation (BH) process (see below). Under most feeding conditions, 18:0 is the predominant FA absorbed from the intestine of ruminants. This is desaturated variably to c9-18:1 by Δ-9 desaturase (stearoyl-CoA desaturase; SCD; EC 1.14.99.5); activity is minimal in the intestinal tissue and most active in mammary gland and adipose tissue (Palmquist et al., 2005). Because absorbed FA in ruminants are highly saturated, unique adaptations have evolved to accommodate the requirement that milk fat must be liquid at body temperature. In addition to Δ-9 desaturase activity, ruminant milk fat is unique in its content of short chain and branched FA; these, together with oleic acid, primarily are responsible for maintaining milk fat fluidity. Among bovine breeds, the contents of oleic acid and short chain FA tend to be reciprocal; also, breeds that secrete milk of higher fat content tend to have lower c9-18:1 and higher content of the short chain de novo-synthesized FA.

The TG structure of milk fat contributes to its unique rheological properties. If the 400 + FA of milk fat were randomly distributed in the TG, the total number of TG species would exceed 64×10^6. Clearly, arrangement is not random, but very few specific TG species exceed 1 mol. % of the total. The *sn*-1 position is dominated by 16:0 and 18:1 (34 and 30 mol. %, respectively), *sn*-2 by 14:0, 16:0, and 18:1 (18, 32, and 19 mol. %, respectively), and *sn*-3 by 4:0 and 18:1 (35 and 23 mol. %, respectively). All of the 4:0 and most of the 6:0 occur at *sn*-3, where they are readily released by lipases, thus contributing importantly to the characteristic flavor of milk fat.

Ruminant milk fat contains glycerides with TG number (the total number of FA carbon atoms in the glyceride) ranging from 24 to 54, with modes at 36–40 and 48–50. Feeding high-fat diets to

TABLE 6.3
Fatty Acids in Bovine Milk Fat

Fatty Acid	Milk[a] (g/100 g)	Butter[b] (g/100 g ± SD)	Fatty Acid	Milk (g/100 g)	Butter (g/100 g ± SD)
		Saturated fatty acids			
4:0	4.5	5.31 ± 0.30	16:0i	—	02.9 ± 0.01
6:0	2.3	2.81 ± 0.09	16:0	28.2	28.13 ± .37
8:0	1.3	1.56 ± 0.08	17:0i	0.7	0.52 ± 0.01
10:0	2.7	3.14 ± 0.06	17:0ai	—	0.50 ± 0.01
11:0	0.3	—			
12:0	3.0	3.39 ± 0.06	17:0	0.6	0.57 ± 0.01
13:0i[c]	—	0.13 ± 0.01	18:0i	—	0.090 ± 0.11
13:0	0.2	0.11 ± 0.00	18:0	12.6	10.62 ± 0.11
14:0i	0.1	0.15 ± 0.00	19:0	—	0.14 ± 0.03
14:0	10.6	10.78 ± 0.17	20.0	—	0.20 ± 0.03
15:0i	0.7	0.30 ± 0.01			
15:0ai	—	0.49 ± 0.01			
15:0	1.0	1.03 ± 0.01			
		Monounsaturated fatty acids			
10:1	—	0.31 ± 0.01	18:1t	1.7	—
12:1	—	0.09 ± 0.01	18:1n9	21.4	20.84 ± 0.79
14:1n5	0.9	0.90 ± 0.02	18:1n7	—	0.15
15:1	0.3				
16:1t	—	0.27 ± 0.02	20:1n9	0.6	0.29 ± 0.06
16:1n7	1.8	1.38 ± 0.03	22:1	—	0.09 ± 0.05
17:1	0.4	0.28 ± 0.04			
		Polyunsaturated fatty acids			
18:2t	0.4	0.47 ± 0.04	20:2n6	—	0.03 ± 0.02
18:2n7	—	0.15 ± 0.02	20:3	—	0.10 ± 0.01
18:2n6	2.9	2.01 ± 0.14	20:4n6	0.2	0.14 ± 0.01
18:2n4	—	0.09 ± 0.03	20:4n3	—	0.11 ± 0.05
18:3n6	—	0.08 ± 0.02	20:5n3	—	0.08 ± 0.04
18:3n3	0.3	0.48 ± 0.05	22:6n3	—	0.09 ± 0.05
18:4n3	—	0.27 ± 0.04	Unknown	—	0.23 ± 0.26

[a] J. Sampugna, Personal communication, University of Maryland (1993). Analyses of the butyl esters of 4:0–14:0 and methyl esters of >14:0 with temperature programing.

[b] J. lverson and R.G. Ackman, Personal communication, Technical University of Nova Scotia (1993). Analyses of butyl esters and temperature programing. Both previously published (Jensen, R.G. and Newburg, D.S. (1995). Bovine milk lipids, in *The Handbook of Milk Composition* (R.G. Jensen, ed.), Academic Press, San Diego, CA, pp. 543–575. With permission).

[c] i, iso; ai, anteiso.

cows shifts the modes to higher TG numbers. The plasticity of milk fat most notable in butter is caused by suspension of the mostly liquid unsaturated TG in a crystalline matrix of a small amount of long-chain fully saturated TG (German et al., 1997).

IV. VARIABILITY OF MILK FAT COMPOSITION

The greatest sources of variation in bovine milk fat composition are attributable to feeding management of the cow (especially the proportion of forage and the amount and type of fat in the ration), stage of lactation and breed. Numerous reviews on factors affecting the FA contents of milk

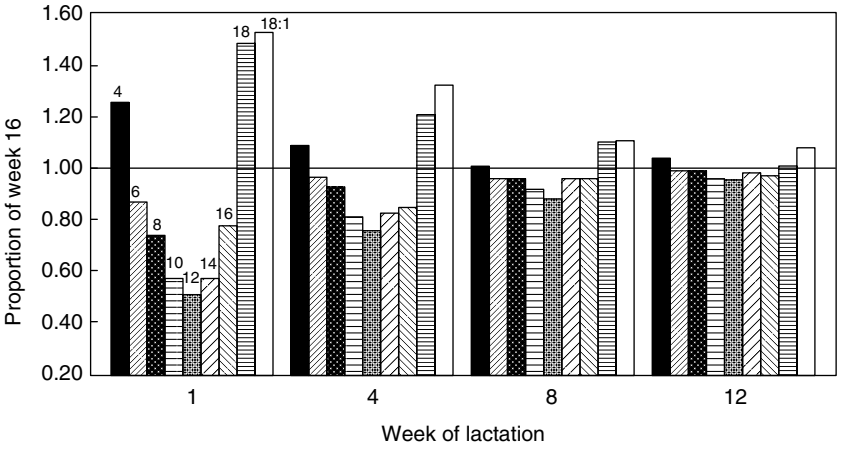

FIGURE 6.1 Concentrations of individual milk fatty acids at weeks 1, 4, 8, and 12 of lactation as proportions of their concentrations at week 16. (From Palmquist, D.L., et al. (1993). *J. Dairy Sci.* 76:1753–1771. With permission.)

are available; for example, see Grummer (1991), Palmquist et al. (1993), Mansbridge and Blake (1997), Bauman and Griinari (2001, 2003), and Chilliard and Ferlay (2004).

A. Animal Effects on Milk Fat Composition

Differences in milk fat composition occur among animals due to physiological effects (stage of lactation, disease) and to genetics (species, breed, individual animal). Breed differences among bovines tend to be less than the individual differences. In early lactation, relatively higher proportions of long-chain FAs with lower proportions of the short chain, de novo-synthesized FA are observed (Palmquist et al., 1993; Figure 6.1), caused primarily by mobilization of long-chain FA from adipose tissue and their subsequent deposition in milk fat. Differences among breeds in the activity of SCD are evidenced by differences in ratios of desaturase pairs (14:1/14:0; 16:1/16:0; *c*-9 18:1/18:0; *c*-9, *t*-11-18:2/*t*-11-18:1) and in relative abundance of rumenic acid (DePeters et al., 1995; Kelsey et al., 2003). Proportions of short-chain de novo-synthesized FA in milk fat are higher in breeds with higher milk fat percent, and the proportions of de novo-synthesized FA and long-chain unsaturated FA tend to be reciprocal (Karijord et al., 1982).

B. Seasonal (Feed) Influence on Fatty Acid Composition

Seasons as such do not influence milk fat composition; however, in traditional dairy systems the feeding program changes with changing seasons, and in some management systems, calving (the lactation cycle) is seasonal; both of these factors will influence the composition of the milk fat in the market. Jahreis et al. (1999) reviewed species, seasonal, dietary, and management f actors influencing CLA in milk, concluding that the strong seasonal effects are attributable to the relatively high content of unsaturated FA in fresh forages from cows grazing pasture. Lock and Garnsworthy (2003) measured changes in CLA concentration of milk during a full year in which cows were fed in a winter barn feeding/summer pasture regimen. During the winter months, a total mixed ration was fed, whereas through the summer fresh grass was fed with increasing levels of rumen buffer to maintain optimal ruminal pH as the summer progressed. The CLA content of milk in May, June, and July was higher than all other months, averaging 1.50 g CLA/100 g FAME compared with a mean of 0.77 g/100 g for other months. Milk fat produced during the summer contained greater amounts of short chain FA at the expense of medium chain FA, indicating that fresh grass may alter the pattern of FA synthesis

TABLE 6.4
Seasonal Variation of Major Fatty Acids (g/100 g of Fatty Acids) in the United States Milk Supply[a]

	4:0	6:0	8.0	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2
February												
Average	3.47	2.44	1.25	2.96	3.52	11.64	2.58	29.92	3.33	9.65	26.48	2.76
Maximum	3.61	2.51	1.29	3.06	3.84	12.00	2.68	30.50	3.51	10.36	27.86	3.41
Minimum	3.38	2.36	1.15	2.76	3.31	10.73	2.30	29.13	3.05	9.30	25.78	2.48
May												
Average	3.41	2.34	1.20	2.83	3.39	11.25	2.59	28.55	3.38	10.08	27.93	3.02
Maximum	3.57	2.42	1.25	2.92	3.47	11.67	2.69	29.58	3.54	10.64	29.58	3.67
Minimum	3.24	2.25	1.11	2.66	3.18	10.68	2.45	27.36	3.23	9.61	26.92	2.63
August												
Average	3.06	2.28	1.13	2.56	3.10	10.94	2.67	28.85	3.42	10.24	28.90	2.84
Maximum	3.27	2.38	1.19	2.77	3.33	11.30	2.79	30.41	3.57	10.71	30.70	3.38
Minimum	2.79	2.16	1.00	2.31	2.92	10.46	2.53	17.38	3.30	9.76	26.75	2.40
November												
Average	3.32	2.30	1.20	2.89	3.53	11.80	2.69	30.79	3.37	9.39	26.23	2.51
Maximum	3.48	2.45	1.28	3.00	3.68	12.13	2.77	31.54	3.48	10.26	27.80	3.42
Minimum	3.07	2.19	1.13	2.66	3.22	10.83	2.49	29.49	3.22	9.03	25.54	2.13

[a] Packed GLC columns. Samples from 50 cheese plants in 10 areas of the United States, 1984.

Source: Adapted from Palmquist, D.L., et al. (1993). *J. Dairy Sci*. 76:1753–1771.

in the mammary gland. Fat composition of milk produced from "ecological" or "organic" systems have higher concentrations of n-3 FA (Ellis et al., 2006). Couvreur et al. (2006) reported a linear relationship between the proportion of fresh grass in the diet and unsaturated FA of milk fat; these changes were, in turn, responsible for linear decreases in final melting temperature and solid fat content of butter and the sensation of butter firmness in the mouth. See Dewhurst et al. (2006) for more discussion on the effects of forages on milk FA unsaturation. Only a relatively small proportion of the U.S. milk supply is from cows grazing pasture, so that seasonal effects on milk composition in the United States is minor, as shown in Table 6.4 (Palmquist et al., 1993). These samples were collected from 50 cheese plants in 10 areas in the United States and were analyzed by packed GLC columns. Though the contents of 18:1 and 18:2 were higher during May and August than in the period of November through February, differences were not attributed to any specific feeding systems.

C. Effects of Feeding Fat to the Cow on Milk Fat Composition

The ruminant diet typically provides less than 3% of feed dry matter as FA; however, these are highly unsaturated, being predominantly 18:3n-3 derived from forages and 18:2n-6 from cereals. Feeding management of dairy cows has changed as genetic progress has increased yield potential; it is now common to feed supplemental fat to increase energy intake by the animal. Supplemental fat increases the proportion of long-chain (mainly 18-carbon) FA in milk fat, thus decreasing the relative contribution of short-chain de novo-synthesized FA. Rumen microbial BH of dietary unsaturated fat causes very little dietary unsaturated fat to appear in milk. Only a brief summary of BH will be presented here; for more in-depth treatment, refer to Harfoot and Hazlewood (1997) and Palmquist et al. (2005).

Optimal feeding (adequate fiber intake from forages) of dairy cattle causes the rumen pH to remain near or above 6.0; in this environment unsaturated FA are BH predominantly to 18:0. Intermediate steps involve isomerization of the c9, c12 double bonds to c9, *t*11conjugated dienes or trienes, followed by hydrogenation of the c9 and c15 bonds to yield t11-18:1 (vaccenic acid), which is then hydrogenated to 18:0. More detailed discussion of the BH of 18:3 and the formation of its various isomers is presented by Destaillats et al. (2005), who have proposed the trivial names of *rumelenic acid* for c9, t11, c15-18:3 and *isorumelenic acid* for c9, t13, c15-18:3. High amounts of unsaturated FA in the diet inhibit the final BH step, causing the accumulation of vaccenic acid. The long-chain unsaturated FA of fish oil seem to be especially potent in this regard. When feeding conditions (decreased forage, highly fermentable carbohydrates) allow rumen pH to remain below 6.0 for extended periods of the day, the microbial population is altered, accompanied by the accumulation of numerous *trans* monoenoic isomers, especially t10-18:1. Some of these isomers have been implicated as causal in the inhibition of milk fat synthesis (Bauman and Griinari, 2003). The diversity of *cis* and *trans* monoenes and dienes formed during BH contribute to the large number of different FA identified in milk fat.

The conjugated diene, c9, t11-18:2 (trivial name, *rumenic acid*), formed transiently during the BH of linoleic acid, accounts for only a part of this isomer found in milk fat; 60%–90% of rumenic acid in milk fat arises from Δ-9 desaturation of vaccenic acid in the mammary gland by SCD (Griinari et al., 2000; Palmquist et al., 2005). This discovery has stimulated research to develop feeding systems that maximize ruminal synthesis of vaccenic acid as a means to increase concentration of rumenic acid in milk fat. Generally, supplementing free oils increases vaccenic acid and other unsaturated isomers in the rumen and milk FA, whereas feeding whole oil seeds is less effective. Slow release of FA from the matrix of whole oilseeds minimizes the accumulation of free unsaturated FA in ruminal content, allowing more complete BH of unsaturated FA to 18:0 (Morales et al., 2000).

A very detailed analysis of the isomers of milk fat as influenced by type and amount of forage in the diet and a sunflower/fish oil supplement is in Tables 6.5 through 6.7 (Shingfield et al., 2003, 2005). To achieve this analysis, the milk fat FAME were separated with a high-performance liquid chromatography system, using dual heated columns impregnated with $AgNO_3$. Separation of *cis*/*trans* isomers was improved by use of a manually operated switching valve that allowed fractions to be recycled between columns.

In the studies reported in Tables 6.5 through 6.7, the control diet dry matter (DM) was grass silage/concentrate (60:40), containing 377 g/day of FA, of which 60% was polyunsaturated FA. The control milk 18:0 content (Table 6.5), 19.54%, is unusually high, about double the usual concentration. This has been reported recently to be caused by the addition of transition metals as digesta flow markers to the diet. Inhibition of SCD activity by transition metals, especially Cu, has been documented; although identification of the specific inhibitor of SCD was not determined by Shingfield et al. (2006), Co was implicated. Addition of oil (30 g/kg of diet DM) as a mixture of fish oil and sunflower oil (2:3) reduced 18:0 content to only 5%–8% of the milk FA. The reduction was greater when corn silage was fed than when grass silage was fed, indicating differential effects of the forage on the ability of fish oil to inhibit ruminal BH. Note the low accumulation of the eicosanoic FA in milk fat when fish oil was fed. Transfer of these to milk fat varies and is invariably low, ranging from 1% for 22:6n-3 to 11% for 22:5n-3 (Palmquist and Griinari, 2006). The fish oil/sunflower oil mixture modified BH end products dramatically, as seen in the diversity of monoenoic (Table 6.6) and dienoic (Table 6.7) isomers measured in the milk fat. During recycling of the *trans* unsaturated region of the chromatograms by Shingfield et al. (2005), an unidentified isomer was found to elute immediately after the *c*9, *t*11-18:2 peak. Extensive purification of this isomer and comparison with chemically synthesized compounds confirmed the identity as t9, c11-18:2 (“reverse CLA”), previously unidentified in milk fat.

TABLE 6.5
Effect of Forage Type and Concentrate Level on Milk Fatty Acid Composition (g/100 g Total Fatty Acids)

		Treatment[b] Maize Silage		Grass Silage	
Fatty Acid	**Control[a]**	**L**	**H**	**L**	**H**
4:0	4.58	3.35	2.69	3.49	3.17
6:0	2.23	1.62	1.47	1.82	1.61
8:0	1.11	0.85	0.77	0.88	0.82
10:0	2.22	2.03	1.83	1.78	1.82
12:0	2.40	2.56	2.39	1.95	2.26
12:1 c11	ND	0.06	0.06	0.05	0.05
13:0 iso	ND	0.04	0.04	0.03	0.05
13:0 anteiso	ND	0.07	0.07	0.05	0.06
14:0	10.2	9.68	10.13	8.75	9.00
14:0 iso	0.10	0.06	0.05	0.08	0.06
14:1 c9	0.56	1.34	1.55	0.97	1.22
15:0	0.83	0.78	0.88	1.22	1.12
15:0 iso	0.22	0.18	0.16	0.21	0.19
15:0 anteiso	0.47	0.37	0.36	0.39	0.47
16:0	24.7	23.6	25.6	24.2	23.8
16:0 iso	0.24	0.23	0.15	0.21	0.21
16:1 c9	1.29	2.53	2.88	1.89	2.44
16:1[c]	ND	0.39	0.49	0.17	0.33
17:0	0.52	0.54	0.48	0.63	0.60
17:0 iso	0.41	0.91	0.67	0.74	0.80
18:0	19.54	4.68	5.49	8.77	7.41
18:0 iso	ND	0.01	0.01	0.03	0.01
18:0 anteiso	ND	0.20	0.19	0.22	0.22
18:1 c	19.0	12.1	11.8	17.0	14.7
18:1 t	4.5	21.2	20.8	12.0	17.3
18:1 total	23.5	33.3	32.9	30.4	32.0
18:2[d]	1.41	3.29	3.66	3.21	3.91
CLA	0.56	3.43	1.91	3.15	2.43
18:3n-3	0.42	0.24	0.20	0.40	0.32
18:4n-3	0.00	0.03	0.03	0.02	0.03
20:0	0.90	0.17	0.12	0.31	0.18
20:1 c11	0.23	0.59	0.49	0.78	0.64
20:2n-3	0.00	0.05	0.07	0.04	0.07
20:2n-6	0.00	0.02	0.01	0.02	0.03
20:3n-3	ND	0.08	0.12	0.05	0.01
20:3n-6	0.05	0.08	0.07	0.05	0.06
20:4n-3	0.05	0.17	0.14	0.11	0.13
20:4n-6	0.00	0.10	0.10	0.09	0.09
20:5n-3	0.05	0.09	0.09	0.07	0.08
22:0	0.04	0.09	0.06	0.12	0.08
22:1 c13	0.00	0.06	0.06	0.07	0.07
22:2n-3	ND	0.02	0.02	0.08	0.06
22:3n-3	ND	0.02	0.01	0.01	0.02
22:4n-3	0.00	0.09	0.12	0.03	0.10
22:4n-6	0.00	0.02	0.01	0.01	0.01
22:5n-3	0.00	0.19	0.16	0.11	0.14
22:5n-6	0.00	0.19	0.09	0.07	0.10

Continued

TABLE 6.5
(Continued)

		Treatment[b] Maize Silage		Grass Silage	
Fatty Acid	**Control[a]**	**L**	**H**	**L**	**H**
22:6n-3	0.00	0.07	0.06	0.04	0.06
24:0	0.04	0.07	0.05	0.07	0.07
24:1 c15	0.00	0.03	0.02	0.04	0.03

CLA: conjugated linoleic acid; ND: not determined.

[a] Shingfield, K.J., et al. (2003). *Anim. Sci*. 77:165–179. Control diet was grass silage, 600 g/kg DM plus a protein–concentrate mixture, no oil supplement.
[b] Shingfield, K.J., et al. (2005). *Anim. Sci.* 80:225–238. Treatments consisted of total mixed rations based on maize or grass silage containing 650 (L) or 350 (H) g forage per kg dry matter (DM) plus a protein–energy concentrate and supplemented (30 g/kg DM) with a mixture (2:3, w/w) of fish oil and sunflower oil.
[c] Sum of three geometric and positional isomers of $C_{16:1}$ of unknown double bond position.
[d] Sum of $C_{18:2}$ fatty acids excluding isomers of conjugated linoleic acid.

TABLE 6.6
Effect of Forage Type and Concentrate Level on Milk $C_{18:1}$ Content (g/100 g Total Fatty Acids)

		Treatment[b] Maize Silage		Grass Silage	
Fatty Acid	**Control[a]**	**L**	**H**	**L**	**H**
18:1 c9	18.11	10.70	10.30	15.90	13.41
18:1 c11	0.59	1.04	1.10	0.56	0.86
18:1 c12	0.18	0.20	0.32	0.20	0.31
18:1 c13	0.076	0.15	0.10	0.15	0.12
18:1 c15	ND	0.08	0.05	0.07	0.06
18:1 c16	ND	0.13	0.12	0.16	0.13
18:1 t4	0.019	0.04	0.02	0.03	0.02
18:1 t5	0.014	0.05	0.02	0.03	0.02
18:1 t-6, 7, and 8	0.26	0.80	0.96	0.68	0.78
18:1 t9	0.26	0.97	0.96	0.78	0.84
18:1 t10	0.21	7.84	12.73	0.39	7.81
18:1 t11	1.80	8.04	3.48	6.26	4.48
18:1 t12	0.34	1.29	1.01	1.18	1.11
18:1 t13 and 14	0.63	1.36	0.93	1.34	1.24
18:1 t15	0.50	0.49	0.39	0.63	0.58
18:1 t16	0.46	0.33	0.21	0.63	0.44

[a] Shingfield, K.J., et al. (2003). *Anim. Sci*. 77:165–179. Control diet was grass silage, 600 g/kg DM plus a protein–concentrate mixture, no oil supplement.
[b] Shingfield, K.J., et al. (2005). *Anim. Sci*. 80:225–238. Treatments consisted of total mixed rations based on maize or grass silage containing 650 (L) or 350 (H) g forage per kg dry matter (DM) plus a protein–energy concentrate and supplemented (30 g/kg DM) with a mixture (2:3, w/w) of fish oil and sunflower oil.

TABLE 6.7
Effect of Forage Type and Concentrate Level on Milk $C_{18:2}$ Content (mg/100 g Total Fatty Acids)

		Treatment[b] Maize Silage		Grass Silage	
Fatty Acid	**Control[a]**	**L**	**H**	**L**	**H**
18:2 c9, c12	902	1537	1923	1202	1615
18:2 t8, c13	54	72.7	82.2	61.7	96.7
18:2 c9, t12	88	133	119	160	131
18:2 c9, t13	46	395	324	533	461
18:2 t9, c12	21	150	113	235	188
18:2 t11, c15	189	420	440	418	589
18:2 t9, t12	9	66.4	109	33.2	117
18:2 t, tb	104	450	489	551	677
18:2 c, t/t, c total	398	1132	1090	1261	1410
CLA c9, t11	394	3003	1412	2835	1969
CLA c11, t13	ND	0.4	0.7	0.8	0.7
CLA c12, t14	4	2.0	1.1	4.9	1.4
CLA t7, c9	30	168	220	176	180
CLA t8, c10	8	38.8	16.1	34.9	20.9
CLA t9, c11	ND	115	165	26	125
CLA t10, c12	2.0	17.2	26.2	3.1	15.1
CLA t11, c13	38	6.4	7.2	14.5	7.7
CLA t7, t9	13	14.7	12.1	22.1	24.1
CLA t8, t10	2	12.9	15.1	13.5	18.8
CLA t9, t11	11	22.3	20.4	27.6	31.0
CLA t10, t12 CLA	3	9.4	8.9	4.5	9.8
CLA t11, t13	23	3.0	3.6	6.0	5.9
CLA t12, t14	15	4.9	7.4	9.4	9.2
CLA c, t/t, c total	477	3350	1830	3049	2320
CLA t, t total	66	67.2	66.7	83.2	98.7

[a] Shingfield, K.J., et al. (2003). *Anim. Sci.* 77:165–179. Control diet was grass silage, 600 g/kg DM, no oil supplement.
[b] Shingfield, K.J., et al. (2005). *Anim. Sci*. 80:225–238. Treatments consisted of total mixed rations based on maize or grass silage containing 650 (L) or 350 (H) g forage per kg dry matter (DM) plus a protein–concentrate mixture and supplemented (30 g/kg DM) with a mixture (2:3, w/w) of fish oil and sunflower oil.

D. Fat Supplements

Because free oils or unsaturated FA in ruminant diets tend to decrease diet fiber digestion and milk fat percent (Palmquist and Jenkins, 1980; Bauman and Griinari, 2003), the dairy feed industry has developed alternate fat sources as energy supplements for high-milk producing cows. Although tallow and whole oilseeds are widely used as fat supplements, feeding saturated FA and calcium salts of FA has become standard practice. Whereas oilseeds contain high amounts of 18-carbon FA and increase the proportion of these in milk fat, most commercial fat supplements are based mainly on palm oil by-products, containing about 50% of 16:0. These increase the proportion of 16:0 in milk fat; thus, the FA profile of the commercial milk supply is influenced by the sources of fat used as energy supplements. The extent to which feeding supplemental oils or oilseeds modify contents of c9-18:1 and 18:2n-6 in milk FA was summarized by Jenkins and McGuire (2006).

E. Protected Lipids

Since 1970, there has been concerted effort by many research groups to develop fat supplements that are not susceptible to ruminal BH (i.e., they are "protected"). The first of these, drawing much research attention, was a process developed by CSIRO in Australia whereby vegetable oils were incorporated into a protein matrix, followed by treatment with formaldehyde and heat. Under ideal conditions, the fat was not subjected to BH; the low pH of the ruminant stomach and intestine then broke the formaldehyde linkages, releasing the fat for absorption. Depending on the extent of treatment, variable protection and availability of the FA occurred. Using this process, milk fat containing as much as 35% 18:2 was produced (McDonald and Scott, 1977). However, such milks, even at 10%–20% 18:2, are readily susceptible to the development of oxidized flavor (McDonald and Scott, 1977; Timmons et al., 2001). For this and other reasons, the process has not gained wide acceptance. Later applications by CSIRO (Ashes et al., 1992) have shown that significant decreases in 14:0 and 16:0 of milk fat can be achieved by feeding protected canola oil, with only modest increases in 18:2 content. A recent development (Carroll et al., 2006) has shown significant protection of unsaturated FA from BH by using a processed whey complex that avoids the negative aspects of using products with added formaldehyde. Because BH requires the presence of a free carboxyl group in the substrate, providing dietary unsaturated FA as amides is also effective to protect FA from BH; *c*9-18:1 content greater than 40% of total milk FA has been achieved by feeding amide derivatives of canola oil (Jenkins, 1999). Although Ca salts of FA dissociate in ruminal content with subsequent BH of the unsaturated FA, BH of 18:1 and 18:2 in the Ca salts of palm oil is less than complete, being usually about one-half the extent of BH observed with FA in oils and oilseeds. Increased unsaturation improves FA digestibility by the animal, but has minimal effects on milk FA composition (Wu et al., 1991).

F. Fatty Acid Composition of Other Dairy Products

FA composition of dairy products has long been available as USDA Agriculture Handbook 8-1, but modern summaries are no longer available in the USDA database. However, FA composition of dairy products reflects closely the composition of the parent milk fat. Although changes in feeding practice and newer analytical methods lead to changes in reported milk FA profiles, very few manufacturing practices cause the FA profiles of products to differ from those of the raw source. Because of this and widespread availability of product data in the literature, these are not duplicated in this chapter.

V. HEALTH CONCERNS

Some FA in milk, chiefly lauric (12:0), myristic (14:0), and palmitic (16:0), may be atherogenic (Jensen, 1992); therefore, the FA composition of milk is of interest. Newer research suggests that palmitic acid is not atherogenic if consumed with adequate linoleic acid intake (Clandinin, 2000); Mensink et al. (2003), in a meta analysis of 60 controlled studies, showed that lauric acid increases the HDL cholesterol/LDL cholesterol ratio, whereas myristic and palmitic acids had little effect on this ratio in plasma. Although widely regarded as a high-cholesterol food, whole milk contains only about 15 mg of cholesterol/dL and cannot be considered a major contributor to dietary cholesterol levels. However, the fat and cholesterol contents of dairy products increase roughly in parallel, so that butter, for example, with minimum 80% fat, contains about 200 mg of cholesterol per 100 g. Cardiovascular and other public health problems are discussed elsewhere in this volume and by Miller et al. (1995); Parodi (1999, 2006) has addressed the nutritional significance of milk lipids, focusing on anticarcinogenic properties. Readers are referred to Patton (2004) for a comprehensive overview of the qualities of milk as a food.

The major health concern has been that the 12:0, 14:0, and 16:0 and cholesterol in milk fat contribute to the risk of coronary heart disease (CHD) by increasing plasma cholesterol and low-density lipoprotein (LDL). These FA comprise 45%–50% of milk fat; thus, the often-stated "milk fat is 70% saturated fat" is inappropriate because the 4:0–10:0 and 18:0 are neutral with respect to

atherogenic properties. The *trans* FA content of milk (1.9%–6.3% of milk fat or 63–208 mg/dL of milk containing 3.3% fat) is low compared with some food fats, and is below 0.5 g/serving, the level required for declaration by food labeling laws. The role of *trans* FA in nutrition, metabolism, and health has been a subject of research for many years (Gurr, 1996). Research became more focused with publication of studies by Mensink and coworkers (Katan et al., 1995) that linked *trans* FA in the diet to increased lipoprotein Lp(a), LDL-cholesterol and decreased HDL cholesterol; higher Lp(a) and an increase in the ratio of LDL/HDL-cholesterol are believed to increase the risk of atherosclerosis and cardiovascular disease (CHD). Interesting effects of *trans* FA were reported by Willett et al. (1993), summarizing data from the Nurses' Health Study that included dietary and health questionnaires over an 8-year period from 1970 to 1980 for over 69,000 female registered nurses. They found that persons in the highest quintile of *trans* FA intake (mean *trans* FA intake as percentage of energy ranged from 1% to 3.5%) had a 67% greater risk of CHD than those in the lowest quintile of intake ($p < .002$). When *trans* isomers from only vegetable fats (60% of total *trans* FA intake) were considered, the highest quintile risk was 78% higher than the lowest ($p < .009$). However, when *trans* isomers from only animal fat (40% of *trans* FA intake, mainly vaccenic acid) were considered, there was no significant relationship ($p = .23$), and the mean risk actually tended to decrease as *trans* FA intake increased. Updated analysis of data from a 14-year period of the study (Hu et al., 1997) was consistent with the conclusion of no effect of *trans* FA from animal fats on relative risk of CHD. These studies show that all *trans* FA are not the same in their biological effects; it is presumed that the differences are attributable to vaccenic acid that constitutes >80% of the *trans* FA in ruminant fats (Lock et al., 2004).

A. Conjugated Linoleic Acid and Other FA with Biological Effects

Because conjugated linoleic acid (CLA) is a product of ruminal BH, ruminant foods are the most important source of this highly biologically active FA (Pariza, 1999). Rumenic acid, the predominant CLA isomer found in ruminant foods, is reported in Table 6.8 (Lin et al., 1995), and is presented in detail elsewhere by Parodi (2003). Concentration of CLA in milk fat from countries where grazing is a major component of dairy management (e.g., Ireland, New Zealand) is about double that where intensive feedlot dairy production is practiced (Parodi, 2003). Estimates of intake of CLA in the human diet range from 80–120 mg/day in the United States to 300–450 mg/day in Germany (McGuire et al., 1999). CLA is synthesized in the body by desaturation of vaccenic acid (Turpeinen et al., 2002), essentially doubling the dietary supply of CLA to the tissues (Parodi, 2006).

In addition to CLA, the branched chain FA of milk fat have been shown to have cell regulatory properties. Phytanic and pristanic acids, metabolites of chlorophyll metabolism, and 13-methyl

TABLE 6.8
Amounts of Rumenic Acid in Dairy Products[a]

Sample	c9, t11-18:2 mg/100 g Sample	mg/g Fat
Sharp cheddar cheese	161.20	4.59
Cottage cheese	19.60	4.80
Mozzarella cheese	91.40	4.31
American processed cheese	91.10	3.64
Homogenized milk	14.20	4.49
Yogurt	7.40	3.82

[a] Data from Lin, H., et al. (1995). *J. Dairy Sci*. 78:2358–2365.

tetradecanoic acid, a product of rumen microbial metabolism, or their CoA esters, strongly bind the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), inducing β-oxidation of FA (Hostetler et al., 2006), which may result in decreased blood lipids (Bremer, 2001). Concentration of the branched chain FA of milk is less variable and less subject to manipulation than is CLA.

It is becoming an increasing practice to report "atherogenic index" (AI) values in studies on milk fat composition, to suggest that certain modified fats are more "healthy." This is an inaccurate and misleading exercise; inaccurate because milk fat provides only a part of the daily intake of fat and misleading because it reinforces a concept that milk fat is an "unhealthy" food. Only diets, not individual foods, are unhealthy; similarly, if AI has any merit as to measuring healthfulness, it applies only to diets, not individual foods.

VI. CONCLUSION

There continues to be too few publications that take care to report in detail the minor milk FA. As has been found with CLA isomers and the branched chain FA, many of these may have important biological activities. More analyses are needed to report the minor FA. We have published pleas to take more care in reporting the minor FA (Jensen, 1999), and continue to do so in this monograph. More than 400 FA have been characterized in milk fat; a few have been found to have powerful biological effects. How many others with biological activity remain to be discovered?

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This chapter is dedicated to the memory of R.G. 'Bob' Jensen, a pioneer in analysis and composition of milk fat. Dr Jensen died 9 July 2006.

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7 Fatty Acids in Poultry and Egg Products

Austin H. Cantor, Eric A. Decker, and Victoria P. Collins

CONTENTS

I. FATTY ACID COMPOSITION OF POULTRY MEATS

This section deals with the differences in fatty acid composition of several species of poultry meats including chicken, turkey, duck, goose, and game birds. The fatty acid composition of variety meats, mechanically deboned meat, and separable fat and the effect of cooking methods and other processing techniques are also discussed.

A. Fatty Acid Composition of Chicken Muscle

The total lipid concentration of raw skinless chicken (broiler) light muscle is approximately 1.7% (Table 7.1). Light muscle lipids are composed of 36.6% saturated, 32.5% monounsaturated, and 30.8% polyunsaturated fatty acids. The primary saturated fatty acids are palmitic acid followed by stearic acid. The major monounsaturated fatty acids are oleic and palmitoleic acids; linoleic acid, arachidonic acid (AA), and docosahexaenoic acid (DHA) are the primary polyunsaturated acids (PUFAs). All other fatty acids account for less than 1% of the total fatty acid concentration.

TABLE 7.1
Fatty Acid Composition of Raw Chicken (Broiler) Meat[a]

	Light Meat		Dark Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	—	0.01 (0.1)	0.02 (0.6)	0.02 (0.1)
14:0	0.01 (0.8)	0.09 (0.9)	0.03 (0.9)	0.15 (0.9)
16:0	0.28 (23.3)	2.33 (23.3)	0.74 (21.1)	3.82 (22.6)
18:0	0.13 (10.8)	0.63 (6.3)	0.29 (8.3)	1.07 (6.3)
Total saturated	0.44 (36.6)	3.16 (31.5)	1.10 (31.3)	5.26 (31.2)
16:1	0.04 (3.3)	0.60 (6.0)	0.20 (5.7)	1.02 (6.0)
18:1	0.34 (28.3)	3.74 (37.3)	1.11 (31.6)	6.34 (37.6)
20:1	0.01 (0.8)	0.12 (1.2)	0.01 (0.3)	0.18 (1.1)
22:1	—	—	—	—
Total monounsaturated	0.39 (32.5)	4.52 (45.1)	1.34 (38.2)	7.65 (45.3)
18:2	0.22 (18.3)	2.07 (20.6)	0.82 (23.4)	3.55 (21.0)
18:3	0.01 (0.8)	0.10 (1.0)	0.04 (1.1)	0.17 (1.0)
20:4	0.06 (5.0)	0.06 (0.6)	0.10 (2.8)	0.09 (0.5)
20:5	0.01 (0.8)	0.01 (0.1)	0.01 (0.3)	0.01 (0.1)
22:5	0.01 (0.8)	0.01 (0.1)	0.02 (0.6)	0.02 (0.1)
22:6	0.02 (1.7)	0.02 (0.2)	0.04 (1.1)	0.03 (0.2)
Total polyunsaturated	0.37 (30.8)	2.34 (23.4)	1.07 (30.5)	3.96 (23.5)
Total fat (g/100 g edible portion)	1.65	11.07	4.31	18.34

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

In raw chicken (broiler) light muscle with skin, the concentrations of saturated and polyunsaturated fatty acids are 14% and 24%, respectively, less and the concentration of monounsaturated fatty acids is 39% more than in skinless light muscle (see Table 7.1). The predominant fatty acids in each of these classifications are the same as in light muscle without skin; however, light muscle with skin contains a higher proportion of palmitoleic, oleic, gondoic (20:1), and linoleic acids and a lower proportion of palmitic, stearic, arachidonic, and docosahexaenoic acids. This increase in monounsaturated fatty acids and the decrease in saturated and polyunsaturated fatty acids are due to the presence of triacylglycerols associated with the skin (see Table 7.13, separable chicken fat). Whereas the percentages of fatty acids are similar in light muscle with and without skin, the fat content is 6.7-fold as higher in muscle with skin (11.07 g/100 g of muscle). Therefore, the amounts of individual fatty acids in muscle with skin are higher than in muscle without skin for the same serving size.

The fatty acid composition of raw chicken (broiler) dark muscle without skin (4.3 g fat/100 g muscle) is 31.3% saturated, 38.3% monounsaturated, and 30.5% polyunsaturated fatty acids (see Table 7.1). The fatty acid profile is similar to that of light muscle, with the predominant fatty acids being palmitic, stearic, palmitoleic, oleic, linoleic, linolenic, arachidonic, and docosahexaenoic acids. Raw chicken dark muscle with skin contains 4.3-fold more total fat and 18% more monounsaturated, 23% less polyunsaturated, and equal proportions of saturated fatty acids compared with skinless dark muscle. Chicken dark muscle with skin has a fatty acid profile similar to that of skinless dark muscle except that it contains a higher proportion of oleic and gondoic acids and lower percentages of linoleic acid, AA, and DHA owing to an increase in the triacylglycerol content from the skin.

TABLE 7.2
Fatty Acid Composition of Raw Turkey (All Classes) Meat[a]

	Light Meat		Dark Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.01 (0.8)	0.01 (0.2)	0.01 (0.3)	0.01 (0.1)
14:0	0.01 (0.8)	0.05 (0.8)	0.03 (0.8)	0.06 (0.8)
16:0	0.23 (19.3)	1.33 (20.3)	0.77 (20.4)	1.61 (20.5)
18:0	0.15 (12.6)	0.43 (6.6)	0.44 (11.7)	0.63 (8.0)
Total saturated	0.5 (42.0)	2.00 (30.6)	1.47 (39.0)	2.58 (32.8)
16:1	0.04 (3.4)	0.48 (6.5)	0.15 (4.0)	0.50 (6.4)
18:1	0.23 (19.3)	2.27 (34.7)	0.82 (21.8)	2.43 (30.9)
20:1	0.01 (0.8)	0.01 (0.2)	0.02 (0.5)	0.01 (0.1)
22:1	0.01 (0.8)	0.01 (0.2)	0.01 (0.3)	0.01 (0.1)
Total monounsaturated	0.27 (22.7)	2.81 (43.0)	0.99 (26.3)	3.00 (38.2)
18:2	0.27 (22.7)	1.48 (22.6)	1.06 (28.1)	1.95 (24.8)
18:3	0.01 (0.8)	0.10 (1.5)	0.04 (1.1)	0.11 (1.4)
20:4	0.08 (6.7)	0.09 (1.4)	0.16 (4.2)	0.15 (1.9)
20:5	—		—	—
22:5	0.01 (0.8)	0.01 (0.2)	0.02 (0.5)	0.02 (0.25)
22:6	0.02 (1.7)	0.01 (0.2)	0.03 (0.8)	0.03 (0.4)
Total polyunsaturated	0.42 (35.3)	1.73 (26.5)	1.31 (34.7)	2.28 (29.0)
Total fat (g/100 g edible portion)	1.56	7.36	4.38	8.80

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

B. Fatty Acid Composition of Turkey Muscle

The lipid content of raw turkey (all classes) light muscle with and without skin is 7.36% and 1.56%, respectively (Table 7.2). Lipids from skinless turkey light muscle are composed of 42.0% saturated, 22.7% monounsaturated, and 35.3% polyunsaturated fatty acids. The fatty acid profile is similar to that of chicken light muscle, with the major fatty acids being palmitic, stearic, oleic, palmitoleic, linoleic, and arachidonic acids. Turkey light muscle with skin contains less saturated (30.6%) and polyunsaturated (26.5%) fatty acids and more monounsaturated (43.0%) fatty acids than skinless light muscle (Table 7.2). Turkey light muscle with skin contains lower proportions of stearic acid, AA, and DHA but higher proportions of palmitoleic and oleic acids.

Turkey dark raw muscle (all classes) with and without skin contains 8.8% and 4.38% fat, respectively (see Table 7.2). Dark muscle without skin is composed of 39.0% saturated, 26.3% monounsaturated, and 34.7% polyunsaturated fatty acids. The predominant fatty acids within this classification are the same as in skinless light muscle except that they are found in higher concentrations, because dark muscle contains 2.8 times more lipid. As in turkey light muscle, turkey dark muscle with skin contains a lower proportion of saturated (32.8%) and polyunsaturated (29.0%) fatty acids but a higher percentage of monounsaturated fatty acids (38.2%) than skinless muscle, which is primarily due to an increase in palmitic and oleic acids (see Table 7.2).

C. Fatty Acid Composition of Duck, Goose, and Other Game Birds

Duck and goose muscle are predominantly dark muscle throughout the carcass. Duck muscle contains 5.95% lipid without skin and 39.34% with skin (Table 7.3). Muscle alone contains 50.3%

TABLE 7.3
Fatty Acid Composition of Raw and Roasted Duck Meat[a]

	Raw Meat		Roasted Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.03 (0.7)	0.05 (0.1)	0.04 (0.4)	0.04 (0.2)
14:0	0.02 (0.4)	0.25 (0.7)	0.05 (0.5)	0.17 (0.6)
16:0	1.22 (26.5)	9.58 (26.0)	2.44 (26.2)	6.80 (25.9)
18:0	0.77 (16.7)	3.20 (8.7)	1.27 (13.7)	2.43 (9.3)
Total saturated	2.32 (50.3)	13.22 (35.7)	4.17 (44.8)	9.67 (36.9)
16:1	0.22 (4.8)	1.54 (4.2)	0.43 (4.6)	1.11 (4.2)
18:1	1.32 (28.7)	16.73 (45.2)	3.24 (34.8)	11.52 (43.9)
20:1	—	0.40 (1.1)	0.04 (0.4)	0.26 (1.0)
22:1	—	—	—	—
Total monounsaturated	1.54 (33.4)	18.69 (50.5)	3.70 (39.8)	12.90 (49.2)
18:2	0.67 (14.5)	4.69 (12.7)	1.29 (13.9)	3.36 (12.8)
18:3	0.08 (1.7)	0.39 (1.1)	0.14 (1.5)	0.29 (1.1)
20:4	—	—	—	—
20:5	—	—	—	—
22:5	—	—	—	—
22:6	—	—	—	—
Total polyunsaturated	0.75 (16.3)	5.08 (13.7)	1.43 (15.4)	3.65 (12.8)
Total fat (g/100 g edible portion)	5.95	39.34	11.20	28.35

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

saturated, 33.4% monounsaturated, and 16.3% polyunsaturated fatty acids, whereas duck with skin contains 35.7% saturates, 50.5% monounsaturates, and 13.7% polyunsaturates. As with chicken and turkey, the addition of the skin increases the proportion of monounsaturated fatty acids in the lipid from duck. The major fatty acids in duck fat are similar to those in chicken and turkey except for the absence of long-chain PUFAs and a higher proportion of linolenic acid.

Goose contains 7.13% fat in the muscle and 33.6% fat in muscle with skin (Table 7.4). Skinless goose muscle lipids are composed of 50.3% saturated, 33.4% monounsaturated, and 16.3% polyunsaturated fatty acids, whereas goose muscle with skin contains less saturated (31.2%) and polyunsaturated (12.0%) fatty acids and more monounsaturated fatty acids (52.7%). The fatty acid profiles of goose and duck muscle are very similar.

The fatty acid content of pheasant muscle (3.64% fat) is 40.9% saturated, 38.6% monounsaturated, and 20.5% polyunsaturated fatty acids; pleasant muscle with skin (9.29% fat) is 32.9%, 52.7%, and 14.4% saturated, monounsaturated, and polyunsaturated fatty acids, respectively (Table 7.5). The fatty acid profile of pheasant muscle is similar to those of duck and goose muscle; however, pheasant muscle generally contains less saturated and more monounsaturated fatty acids.

Quail meat without skin contains 3.7% fat, and the fatty acids are 35.0% saturated, 34.0% monounsaturated, and 31.0% polyunsaturated (see Table 7.5). Quail with skin contains 2.8-fold more total fat, 8% less saturated, 17% more monounsaturated, and 9% less polyunsaturated fatty acids than skinless quail muscle. The fatty acid profile of quail muscle is very similar to that of chicken light muscle except for the absence of DHA and the presence of a higher proportion of linoleic acid.

TABLE 7.4
Fatty Acid Composition of Raw and Roasted Goose Meat[a]

	Raw Meat		Roasted Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.03 (0.5)	0.03 (0.1)	0.04 (0.4)	0.04 (0.2)
14:0	0.03 (0.5)	0.17 (0.5)	0.05 (0.5)	0.11 (0.6)
16:0	1.47 (26.5)	6.95 (22.2)	2.61 (25.0)	4.53 (23.1)
18:0	0.92 (16.6)	2.33 (7.4)	1.40 (13.4)	1.81 (9.2)
Total saturated	2.79 (50.4)	9.78 (31.2)	4.56 (43.7)	6.87 (35.0)
16:1	0.27 (4.9)	0.98 (3.1)	0.44 (4.2)	0.68 (3.5)
18:1	1.58 (28.5)	16.68 (53.3)	3.88 (37.2)	9.52 (48.5)
20:1	—	0.04 (0.1)	0.01 (0.1)	0.02 (0.1)
22:1	—	—	—	—
Total monounsaturated	1.85 (33.4)	17.77 (56.8)	4.34 (41.6)	10.25 (52.2)
18:2	0.80 (14.4)	3.34 (10.7)	1.37 (13.1)	2.24 (11.4)
18:3	0.10 (1.8)	0.21 (0.7)	0.15 (1.4)	0.18 (0.9)
20:4	—	—	—	—
20:5	—	—	—	—
22:5	—	—	—	—
22:6	—	—	—	—
Total polyunsaturated	0.9 (16.2)	3.76 (12.0)	1.54 (14.8)	2.52 (12.8)
Total fat (g/100 g edible portion)	7.13	33.62	12.67	21.92

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

D. Fatty Acid Composition of Cooked Poultry Muscle

The total fat composition of cooked muscle foods is affected by the cooking method. In general, cooking will cause a loss of water from skinless poultry meat, resulting in an increase in total lipid concentration when 100 g of cooked muscle is compared with 100 g of raw muscle. Dry cooking methods such as roasting will cause more moisture loss from skinless muscle and result in a greater increase in total fat content (compared with raw muscle) than moist cooking methods such as stewing. Cooking meat with skin decreases the amount of water lost and causes subcutaneous fat associated with the skin to be removed from the meat. Therefore, cooking meat with skin causes an overall decrease in the lipid concentration when expressed on a 100-g portion. Roasting meat with the skin on results in smaller fat losses than stewing. The greater loss of fat in stewed meat could be due to longer cooking times and/or the direct contact of the cooking medium with the meat, which could facilitate the removal of adipose fat.

Total fat content of skinless light muscle (broiler) increases from 1.65 to 4.51 g/100 g when the meat is roasted (Table 7.6) and 3.99 g/100 g when it is stewed (Table 7.7). The fatty acid compositions of roasted and stewed light muscle without skin are similar; however, when compared with raw muscle, the saturated and polyunsaturated fatty acid concentrations decrease and the monounsaturated fatty acid concentration increases. The increase in monounsaturated fatty acids is mainly due to increases in palmitoleic and oleic acids. Decreases in PUFAs are due to the loss of 20- and 22-carbon PUFAs, which could reflect a change in membrane lipids during cooking.

The total fat content of chicken (broiler) light muscle with skin will decrease from 11.07 g/100 g meat to 10.85 g/100 g meat when the meat is roasted (see Table 7.6) and 9.97 g/100 g meat when

TABLE 7.5
Fatty Acid Composition of Raw Pheasant and Quail Meat[a]

	Pheasant		Quail	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.05 (1.7)	0.05 (0.6)	0.03 (0.8)	0.04 (0.4)
14:0	0.03 (1.0)	0.09 (1.1)	0.03 (0.8)	0.10 (1.0)
16:0	0.75 (24.8)	2.21 (27.0)	0.78 (20.9)	2.19 (20.8)
18:0	0.36 (11.9)	0.65 (7.9)	0.46 (12.2)	0.88 (8.3)
Total saturated	1.24 (40.9)	2.70 (32.9)	1.32 (35.0)	3.38 (32.1)
16:1	0.20 (6.6)	0.81 (9.9)	0.24 (6.4)	0.62 (5.9)
18:1	0.96 (31.7)	3.08 (37.6)	1.02 (26.5)	3.52 (33.3)
20:1	—	0.01 (0.1)	0.01 (0.3)	0.01 (0.1)
22:1	—	—	—	—
Total monounsaturated	1.17 (38.6)	4.32 (52.7)	1.28 (34.0)	4.18 (39.7)
18:2	0.54 (17.8)	0.81 (9.9)	0.93 (24.7)	2.30 (21.8)
18:3	0.08 (2.6)	0.10 (1.2)	0.02 (0.5)	0.42 (4.0)
20:4	—	—	0.12 (3.2)	0.14 (1.3)
20:5	—	—	0.01 (0.3)	0.01 (0.1)
22:5	—	—	0.02 (0.5)	0.03 (0.3)
22:6	—	—	—	—
Total polyunsaturated	0.62 (20.5)	1.18 (14.4)	1.17 (29.2)	2.98 (28.3)
Total fat (g/100 g edible portion)	3.64	9.29	4.53	12.05

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

it is stewed (see Table 7.7). The fatty acid profiles of roasted and stewed light muscle with skin are essentially identical, and the fatty acid composition changes only slightly compared with raw muscle because of a slight decrease in monounsaturated fatty acids and an increase in PUFAs. This change is most likely due to a loss of triacylglycerols from the subcutaneous fat.

Dark chicken muscle without skin contains 4.3%, 9.7%, and 9.0% fat in raw (see Table 7.1), roasted (see Table 7.6), and stewed meat (see Table 7.7), respectively. The fatty acid profiles are similar for roasted and stewed skinless dark muscle but lower in monounsaturated fatty acids and higher in PUFAs than for raw skinless dark muscle. Changes in total fat content in cooked chicken (broiler) dark muscle with skin decrease in a trend similar to that of light muscle. Dark muscle with skin contains 18.3% (see Table 7.1), 15.8% (see Table 7.6), and 14.7% (see Table 7.7) fat when raw, roasted, and stewed, respectively. Roasted and stewed dark muscle with skin have similar fatty acid compositions; however, they differ from that of raw muscle in that they contain slightly less monounsaturated fatty acids and more PUFAs.

Turkey increases in fat content when roasted with or without skin (Table 7.8). This increase in fat content is greater in turkey without skin (2.1-fold for light and 1.6-fold for dark muscle) than with skin (1.1-fold for light and 1.3-fold for dark muscle). The increase in fat content in roasted turkey is primarily due to the loss of water. Roasting turkey has little effect on the fatty acid profile of skinless meat; however, in both light and dark roasted turkey muscle with skin, the percentages of saturated and polyunsaturated fatty acids increase, whereas monounsaturated fatty acids decrease owing to loss of triacylglycerol. These changes are due to increases in stearic and linoleic acids and decreases in palmitoleic and oleic acids.

Duck (see Table 7.3) and goose (see Table 7.4) lose fat when roasted with skin (35% and 52% decrease for goose and duck, respectively), but the fat content increases when roasted without skin

TABLE 7.6
Fatty Acid Composition of Roasted Chicken (Broiler) Meat[a]

	Light Meat		Dark Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.01 (0.3)	0.01 (0.1)	0.03 (0.4)	0.03 (0.2)
14:0	0.04 (1.1)	0.09 (0.9)	0.07 (0.8)	0.12 (0.90)
16:0	0.87 (23.0)	2.25 (23.1)	1.84 (21.7)	3.19 (22.7)
18:0	0.32 (8.4)	0.62 (6.4)	0.63 (7.4)	0.90 (6.4)
Total saturated	1.27 (33.5)	3.05 (31.7)	2.66 (31.4)	4.37 (31.1)
16:1	0.18 (4.7)	0.57 (5.9)	0.49 (5.8)	0.86 (6.1)
18:1	1.30 (34.3)	3.51 (36.4)	2.97 (35.0)	5.11 (36.4)
20:1	0.03 (0.8)	0.12 (1.2)	0.05 (0.7)	0.15 (1.1)
22:1	—	—	—	—
Total monounsaturated	1.54 (40.6)	4.26 (44.3)	3.56 (42.0)	6.19 (44.1)
18:2	0.74 (19.5)	1.98 (20.6)	1.87 (22.1)	3.04 (21.6)
18:3	0.04 (1.1)	0.09 (0.9)	0.09 (1.1)	0.14 (1.0)
18:4	—	—	—	—
20:4	0.08 (2.1)	0.09 (0.9)	0.14 (1.7)	0.14 (1.0)
20:5	0.01 (0.3)	0.01 (0.1)	0.01 (0.1)	0.02 (0.1)
22:5	0.02 (0.5)	0.02 (0.2)	0.03 (0.4)	0.03 (0.2)
22:6	0.03 (0.8)	0.03 (0.3)	0.05 (0.6)	0.05 (0.4)
Total polyunsaturated	0.98 (25.9)	2.31 (24.0)	2.26 (26.7)	3.49 (24.8)
Total fat (g/100 g edible portion)	4.51	10.85	9.73	15.78

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

(78% and 88% increase for goose and duck, respectively). Loss of fat in roasted waterfowl with skin is due to the loss of subcutaneous fat associated with the skin. Increases in fat content for birds roasted without skin is due to loss of water from the muscle. Fatty acid compositional changes in roasted goose and duck with skin are due to increases in saturated and polyunsaturated fatty acids and decreases in monounsaturated fatty acids, with the proportions of palmitic (goose only), stearic, and linoleic acids increasing and that of oleic acid decreasing. Roasting goose and duck without skin has the opposite effect, causing an overall decrease in saturated and polyunsaturated fatty acids and an increase in monounsaturated fatty acids. The effects of roasting without skin on fatty acid content include decreases in palmitic, stearic, palmitoleic, and linoleic acids, and increases in oleic acid.

E. Poultry Variety Meats

The fat content of raw liver is similar for chicken (3.86%), turkey (4.64%), duck (4.64%), and goose (4.28%). The content of specific fatty acids in raw liver is quite variable among species; however, liver generally contains more saturated and less polyunsaturated fatty acids than muscle (Tables 7.9 and 7.10). The major fatty acids in raw liver from poultry species are palmitic, stearic, oleic, and linoleic acids. Cooking (simmering) chicken and turkey livers has little effect on their fatty acid composition but does increase the total fat content (Table 7.9).

Raw chicken and turkey heart muscle contain 9.33% and 6.99% fat, respectively (Table 7.11). The major fatty acids in heart muscle are similar for turkey and chicken, with palmitic, stearic, oleic, linoleic, and arachidonic acids predominating. Simmering the heart decreases the fat content by 15% for chicken and 13% for turkey but has little effect on the fatty acid profile (Table 7.11). Raw and

TABLE 7.7
Fatty Acid Composition of Stewed Chicken (Broiler) Meat[a]

	Light Meat		Dark Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.01 (0.3)	0.01 (0.1)	0.03 (0.4)	0.03 (0.2)
14:0	0.03 (0.9)	0.08 (0.9)	0.06 (0.8)	0.11 (0.8)
16:0	0.77 (23.1)	2.07 (23.4)	1.69 (21.7)	2.97 (22.8)
18:0	0.28 (8.7)	0.56 (6.3)	0.58 (7.4)	0.83 (6.4)
Total saturated	1.12 (33.5)	2.8 (31.7)	2.45 (31.4)	4.06 (31.1)
16:1	0.16 (4.8)	0.53 (6.0)	0.45 (5.8)	0.80 (6.1)
18:1	1.14 (34.1)	3.23 (36.5)	2.71 (34.7)	4.74 (36.3)
20:1	0.03 (0.9)	0.11 (1.2)	0.05 (0.6)	0.14 (1.1)
22:1	—	—	—	—
Total monounsaturated	1.35 (40.4)	3.92 (44.3)	3.26 (41.8)	5.75 (44.1)
18:2	0.65 (19.5)	1.83 (20.7)	1.73 (22.2)	2.82 (21.6)
18:3	0.03 (0.9)	0.08 (0.9)	0.08 (1.0)	0.13 (1.0)
20:4	0.07 (2.1)	0.08 (0.9)	0.13 (1.7)	0.13 (1.0)
20:5	0.01 (0.3)	0.01 (0.1)	0.01 (0.1)	0.01 (0.1)
22:5	0.02 (0.6)	0.02 (0.2)	0.03 (0.40)	0.02 (0.2)
22:6	0.03 (0.9)	0.03 (0.3)	0.05 (0.6)	0.05 (0.4)
Total polyunsaturated	0.87 (26.0)	2.12 (24.0)	2.09 (26.8)	3.24 (24.8)
Total fat (g/100 g edible portion)	3.99	9.97	8.98	14.66

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

cooked chicken gizzards contain 2.06% and 2.68% fat, respectively, while the respective values for raw and cooked turkey gizzards are 4.58% and 3.87% (Table 7.12). Cooking has little effect on the fatty acid composition of gizzards, and the predominating fatty acids are the same as for heart muscle.

F. Separable Fat and Mechanically Deboned Muscle

Poultry fat and mechanically deboned muscle are commonly used in processed poultry products. Chicken fat contains primarily triacylglycerols and therefore would have less PUFAs than muscle, which contains more membrane lipids. Overall, the predominant fatty acids in separable fat are similar to those in chicken muscle, with palmitic, oleic, and linoleic acids predominating; however, separable fat has an absence or lower proportion of 20-carbon PUFAs (Table 7.13) compared with muscle lipids. The amount of monounsaturated fatty acids is higher in separable fat than in muscle fat (46.8% vs. 32.5% for light and 38.2% for dark muscle without skin). This increase is due to increases in palmitoleic and oleic acids.

Mechanical deboning processes are used to increase the utilization of poultry meat by removing meat from necks, backs, or whole carcasses. This process involves crushing the bones and separating the meat from the bones by extruding it through a sieve. Mechanically deboned meat has a high amount of triacylglycerols, some of which are derived from adipose fat and bone marrow (Dawson and Gartner, 1983). Therefore, the total fat content (15.48%–24.73% for chicken backs and necks with and without skin; Table 7.14) is higher than in raw muscle (4.31% and 18.34% for dark muscle with and without skin; see Table 7.1). The fatty acid composition of mechanically deboned turkey frames (Table 7.14) is similar to that of turkey light and dark muscle, but, as in mechanically

TABLE 7.8
Fatty Acid Composition of Roasted Turkey (All Classes) Meat[a]

	Light Meat		Dark Meat	
Fatty Acid	**No Skin**	**With Skin**	**No Skin**	**With Skin**
12:0	0.01 (0.4)	0.01 (0.1)	0.02 (0.3)	0.01 (0.1)
14:0	0.02 (0.8)	0.06 (0.8)	0.05 (0.8)	0.08 (0.8)
16:0	0.47 (19.2)	1.45 (20.2)	1.28 (20.6)	2.1 (20.5)
18:0	0.31 (12.7)	0.55 (7.6)	0.72 (11.6)	0.9 (8.8)
Total saturated	1.03 (42.0)	2.34 (32.5)	2.42 (38.9)	3.49 (34.1)
16:1	0.07 (2.9)	0.47 (6.5)	0.24 (3.9)	0.59 (5.8)
18:1	0.46 (18.8)	2.29 (31.8)	1.35 (21.7)	2.97 (29.0)
20:1	0.01 (0.4)	0.01 (0.1)	0.03 (0.5)	0.02 (0.2)
22:1	0.01 (0.4)	0.01 (0.1)	0.02 (0.3)	0.02 (0.2)
Total monounsaturated	0.56 (22.9)	2.84 (39.5)	1.64 (26.4)	3.65 (35.7)
18:2	0.57 (23.3)	1.64 (22.8)	1.75 (28.1)	2.61 (25.5)
18:3	0.01 (0.4)	0.10 (1.4)	0.07 (1.1)	0.14 (1.4)
20:4	0.17 (6.9)	0.16 (2.2)	0.26 (4.2)	0.24 (2.3)
20:5	—	—	—	—
22:5	0.03 (1.2)	0.03 (0.4)	0.04 (6.4)	0.03 (0.29)
22:6	0.03 (1.2)	0.03 (0.4)	0.06 (9.6)	0.05 (0.5)
Total polyunsaturated	0.86 (35.1)	2.01 (28.0)	2.16 (34.7)	3.09 (30.2)
Total fat (g/100 g edible portion)	3.22	8.33	7.22	11.54

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

deboned chicken muscle, the proportion of monounsaturated fatty acids (especially oleic acid) is higher than in hand-deboned chicken muscle. The increase in oleic acid is most likely due to the increase in triacylglycerol concentration.

G. Processed Poultry Products

In recent years, an increasing number of processed poultry products have become available to consumers. The purpose of this section is not to cover all processed poultry products but to give examples of products that represent the processing techniques used on a variety of poultry meats.

Canned poultry meats are cooked at high temperature in broth. Therefore, both total fat concentration and fatty acid composition (see Table 7.1) are similar to those of stewed meats. Canned chicken is slightly higher in monounsaturated fatty acids than stewed chicken owing to the retention of triacylglycerols in the broth of these products. Poultry meat is also marketed as boneless rolls that are produced by restructuring processes that use a combination of sodium chloride and phosphate salts to bind together whole or coarsely cutup meat pieces (Addis, 1986). These products are often marketed as ready-to-cook roasts and luncheon meats. The total fat concentration of chicken and turkey rolls made from light meat is higher than in cooked muscle without skin but lower than in cooked muscle with skin (Table 7.15). This is because skin or separable fat is included in these products and/or less fat is lost during the cooking process. The fatty acid compositions of chicken and turkey rolls are similar to those of cooked chicken and turkey light muscle with skin.

Numerous cured poultry products are available to consumers, including frankfurters and luncheon meats. The fat and fatty acid contents of these products are dependent on the type of meat used in the formulation. Chicken frankfurters contain 19.48% fat and have a fatty acid profile that contains more monounsaturated and less saturated and polyunsaturated fatty acids than either chicken light or dark muscle (Table 7.16). The increase in the proportion of monounsaturated fatty

TABLE 7.9
Fatty Acid Composition of Raw and Cooked (Simmered) Chicken and Turkey Livers[a]

	Chicken		Turkey	
Fatty Acid	**Raw**	**Cooked**	**Raw**	**Cooked**
12:0	—	—	—	—
14:0	0.01 (0.3)	0.01 (0.2)	0.09 (0.6)	0.11 (0.6)
16:0	0.88 (24.3)	1.11 (20.2)	3.95 (27.1)	4.82 (26.7)
18:0	0.65 (18.0)	0.92 (16.8)	1.47 (10.1)	2.02 (11.2)
Total saturated	1.56 (43.1)	2.06 (37.5)	5.51 (37.8)	6.94 (38.4)
16:1	0.11 (3.0)	0.12 (2.2)	1.02 (7.0)	1.07 (5.9)
18:1	0.13 (3.6)	1.27 (23.1)	6.30 (43.3)	7.96 (44.1)
20:1	0.01 (0.3)	0.02 (0.4)	0.04 (2.5)	0.04 (0.2)
22:1	—	—	—	—
Total monounsaturated	1.24 (34.3)	1.42 (25.9)	7.40 (50.8)	9.11 (50.4)
18:2	0.48 (13.1)	0.72 (13.1)	1.28 (8.8)	1.55 (8.6)
18:3	0.01 (0.3)	0.02 (0.4)	0.00	0.02 (0.1)
20:4	0.33 (9.0)	0.51 (9.3)	0.33 (2.3)	0.40 (2.2)
20:5	0.00	0.00	—	—
22:5	0.00	0.00	—	—
22:6	0.00	0.00	—	—
Total polyunsaturated	0.82 (22.7)	2.01 (36.6)	1.65 (11.3)	2.02 (11.2)
Total fat (g/100 g edible portion)	4.83	6.51	16.36	20.54

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

acids in frankfurters is due to the presence of mechanically deboned chicken and/or separable fat, which are high in monounsaturated fatty acids (Addis, 1986). Turkey ham is another example of a cured poultry product available to consumers. Turkey ham is made primarily from dark muscle (Addis, 1986), and, as one would expect, its fatty acid composition (see Table 7.16) is similar to that of cooked turkey dark muscle without skin.

H. Influence of the Fatty Acid Composition of Feeds on the Fatty Acid Composition of Chicken

Like that of most monogastrics, the fatty acid composition of chicken is influenced by the type of fat in the diet (Miller et al., 1967; Edwards et al., 1973; Phetteplace and Watkins, 1989, 1990). Changes in fatty acid composition due to diet are seen in all tissues, including muscle, liver, heart, and adipose fat (Phetteplace and Watkins, 1990). Edwards and coworkers (1973) reported that the adipose lipids of 8- to 9-week-old broilers had different fatty acid compositions depending on the source of fat in the diet (Table 7.17). The adipose fat of broilers fed a basal diet with no added fat contained 30.7% saturated, 34.7% monounsaturated, and 34.5% polyunsaturated fatty acids. Adipose fat from chickens fed the basal diet plus cottonseed oil contained 29% less monounsaturated, 29% more polyunsaturated, and about the same proportion of saturated fatty acids as chickens fed the basal diet. The fatty acid changes were due to a decrease in the amount of palmitoleic and oleic acids and an increase in linoleic acid. Feeding beef tallow in addition to the basal diet both increased monounsaturated and decreased polyunsaturated fatty acids 25% while having little influence on saturated fatty acid content compared with the basal diet. The change in the fatty acid profile of adipose fat

TABLE 7.10
Fatty Acid Composition of Raw Duck and Goose Livers[a]

Fatty Acid	Duck	Goose
12:0	—	—
14:0	0.01 (0.4)	0.01 (0.4)
16:0	0.80 (28.8)	0.80 (30.0)
18:0	0.63 (22.7)	0.76 (28.6)
Total saturated	1.44 (51.8)	1.59 (59.8)
16:1	0.05 (1.8)	0.06 (2.3)
18:1	0.65 (23.4)	0.74 (27.8)
20:1	—	0.01 (0.4)
22:1	—	—
Total monounsaturated	0.71 (25.5)	0.81 (30.5)
18:2	0.37 (13.3)	0.18 (6.8)
18:3	—	0.01 (0.4)
20:4	0.26 (9.4)	0.07 (2.6)
20:5	—	—
22:5	—	—
22:6	—	—
Total polyunsaturated	0.63 (22.7)	0.26 (9.8)
Total fat (g/100 g edible portion)	4.64	4.28

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

from chickens fed beef tallow was due to increases in oleic acid and decreases in linoleic acid. These changes in the chicken fat directly reflected the fatty acid composition of the beef tallow used in the feeding studies.

Although n-3 fatty acid levels in poultry meat can be increased by inclusion of fish oil or fish meal in the diet, there is relatively little use of fish products in poultry diets in the United States due to the cost. In contrast, in other geographic areas (e.g., South America), there is considerable use of fish meal. Valenzuela (1999) indicated that pork, poultry meat, and eggs were important sources of n-3 fatty acids for people in Chile due to the use of fish products in animal diets.

Phetteplace and Watkins (1990) fed chickens fish (menhaden) oil to increase the proportion of n-3 fatty acids in chicken. Abdominal fat from chickens fed menhaden oil contained 14% more saturated, 47% more polyunsaturated, and 32% less monounsaturated fatty acids than chickens fed chicken fat (Table 7.18). The increase in n-3 fatty acids in chicken fed menhaden oil was more than eightfold that of chickens fed chicken fat. The increase in the n-3 fatty acid content was primarily due to increases in eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids.

In recent years, there has been increasing concern regarding consumption of *trans* fatty acids in the human diet. Intake of *trans* fatty acids has been associated with negative effects on lipid profiles (increased low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol). However, there is evidence that suggests the intake of certain *trans* fatty acids (*trans* 18:1) may have beneficial health effects (Lemaitre et al., 2006). One major source of *trans* fatty acids is hydrogenated vegetable oil (shortening), which is extensively used in baking and other food manufacturing. Another source is animal products derived from ruminants, as a consequence of biohydrogenation in the rumen by microorganisms. As discussed previously, the fatty acid profile in poultry meat can be altered by the type of fat used in the animals' diet. Very little *trans* fatty acids would be expected

TABLE 7.11
Fatty Acid Composition of Raw and Cooked (Simmered) Chicken and Turkey Hearts[a]

	Chicken		Turkey	
Fatty Acid	**Raw**	**Cooked**	**Raw**	**Cooked**
12:0	—	—	—	—
14:0	0.06 (0.8)	0.05 (0.8)	0.02 (0.5)	0.02 (0.6)
16:0	1.45 (18.7)	1.23 (18.7)	0.91 (22.9)	0.83 (22.9)
18:0	0.78 (10.1)	0.66 (10.0)	0.38 (9.5)	0.43 (11.9)
Total saturated	2.66 (34.4)	2.26 (34.4)	1.32 (33.2)	1.29 (35.6)
16:1	0.39 (5.0)	0.33 (5.0)	0.21 (5.3)	0.15 (4.1)
18:1	1.98 (25.6)	1.68 (25.6)	1.17 (29.4)	0.98 (27.1)
20:1	—	—	0.01 (0.25)	0.01 (0.3)
22:1	—	—	—	—
Total monounsaturated	2.37 (30.6)	2.01 (30.6)	1.39 (34.9)	1.14 (31.5)
18:2	1.91 (24.7)	1.62 (24.7)	0.93 (23.4)	0.84 (23.2)
18:3	0.07 (0.9)	0.06 (0.9)	0.01 (0.25)	0.02 (0.6)
20:4	0.72 (9.3)	0.61 (9.3)	0.30 (7.5)	0.32 (8.8)
20:5	—	—	—	—
22:5	—	—	—	—
22:6	—	—	—	—
Total polyunsaturated	2.71 (35.0)	2.30 (35.0)	1.27 (31.9)	1.19 (32.9)
Total fat (g/100 g edible portion)	9.33	7.92	4.79	4.64

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

to contained in the major grain and protein sources in the United States (corn and soybean meal, respectively). However, some *trans* fatty acids could enter the bird's diet by the inclusion of ingredients such as animal fats (derived from ruminants), restaurant grease, or bakery by-product. A major decline in the content of *trans* fatty acids in the latter two ingredients would be expected as a result of the recent efforts of the baking and restaurant industries to minimize *trans* fatty acid levels in their products. Thus, a consequent reduction in levels of *trans* fatty acids in poultry meat products would also be expected.

Conjugated linoleic acids (CLA) is another group of fatty acids receiving considerable attention due to potential health benefits. A number of studies (e.g., Crespo and Esteve-Garcia, 2001; Badinga et al., 2003; Sirri et al., 2003) have shown that content of CLA in broiler meat and fat can be increased by including CLA in the chickens' diet. Most of these studies have also shown that dietary CLA changes fatty acid levels in tissues by increasing saturated fatty acids and decreasing monounsaturated fatty acids, while usually not altering PUFAs.

I. Summary

The skin of poultry meat is associated with triacylglycerols, which have a higher proportion of monounsaturated fatty acid than muscle lipids. Therefore, poultry meats with skin not only contain more total fat but also have a greater proportion of monounsaturated fatty acids (primarily oleic acid) and lower proportions of saturated and polyunsaturated fatty acids than muscle alone. Poultry light muscle without skin contains more saturated and less monounsaturated fatty acids but about the same amount of PUFAs as dark muscle.

TABLE 7.12
Fatty Acid Composition of Raw and Cooked (Simmered) Chicken and Turkey Gizzards[a]

	Chicken		Turkey	
Fatty Acid	**Raw**	**Cooked**	**Raw**	**Cooked**
12:0	—	—	—	—
14:0	0.01 (0.7)	0.00	0.03 (0.8)	0.03 (1.0)
16:0	0.35 (25.0)	0.41 (26.3)	0.96 (26.1)	0.85 (29.5)
18:0	0.17 (12.1)	0.24 (15.3)	0.31 (8.5)	0.27 (9.5)
Total saturated	0.53 (37.9)	0.67 (43.2)	1.31 (35.7)	1.16 (40.4)
16:1	0.07 (5.0)	0.07 (4.8)	0.24 (6.5)	0.22 (7.8)
18:1	0.43 (30.7)	0.44 (28.6)	1.33 (36.1)	0.97 (33.7)
20:1	0.01 (0.7)	0.01 (0.07)	0.01 (0.4)	0.01 (0.3)
22:1	—	—	—	—
Total monounsaturated	0.51 (36.4)	0.53 (34.0)	1.59 (43.2)	1.21 (42.1)
18:2	0.25 (17.9)	0.22 (14.4)	0.64 (17.4)	0.41 (14.3)
18:3	0.003 (0.2)	0.01 (0.5)	0.00 (0)	0.02 (0.6)
20:4	0.09 (6.4)	0.10 (6.7)	0.10 (2.7)	0.07 (2.3)
20:5	—	—	—	—
22:5	—	—	—	—
22:6	—	—	—	—
Total polyunsaturated	0.36 (25.7)	0.35 (22.8)	0.78 (21.1)	0.50 (17.4)
Total fat (g/100 g edible portion)	2.06	2.68	4.58	3.87

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

Chicken light and dark muscle generally contain less saturated and polyunsaturated fatty acids but more monounsaturated fatty acids than turkey light and dark muscle. Skinless duck and goose muscle contain the highest proportion of saturated fatty acids and lowest proportion of PUFAs of all the poultry species. Duck and goose also contain the greatest amount of total fat in muscle with and without skin. Pheasant meat contains the same fatty acids as duck and goose; however, it contains a smaller proportion of saturated fatty acids, a greater proportion of monounsaturated fatty acids, and less total fat. The total fat and fatty acid content of quail are similar to that of chicken and turkey muscle.

The total fat content and the fatty acid profile of poultry variety meats are similar among species; however, they differ from organ to organ. Heart muscle contains the greatest amount of fat of all the organ meats. Hearts and gizzards contain a higher proportion of PUFAs (primarily AA) than liver or muscle. Liver contains the greatest amount of saturated fatty acids of all the organ meats.

Separable chicken fat and mechanically deboned poultry contain a high proportion of monounsaturated fatty acids compared with muscle lipids owing to the presence of high concentrations of triacylglycerols. Therefore, any processed poultry products that contain mechanically deboned meat or separable fat, such as frankfurters, would contain a higher proportion of monounsaturated fats than muscle. Processed poultry products that do not contain mechanically deboned muscle or separable fat can be expected to have a fatty acid composition similar to that of the muscle from which they are produced.

Poultry represents a muscle food in which diet can be used to increase the concentration of bioactive fatty acids in the final product. Increasing n-3 fatty acids in poultry products by dietary supplementation of marine lipids is possible; however, such practices will change the physical and chemical properties of the skeletal muscle. Muscle containing high concentrations of PUFAs will

TABLE 7.13
Fatty Acid Composition of Separable Chicken Fat[a]

Fatty Acid	Concentration
12:0	0.04 (0.1)
14:0	0.60 (0.9)
16:0	14.65 (22.6)
18:0	4.08 (6.3)
Total saturated	20.25 (31.3)
16:1	3.86 (6.0)
18:1	25.29 (39.1)
20:1	0.73 (1.1)
22:1	—
Total monounsaturated	30.30 (46.8)
18:2	13.26 (20.5)
18:3	0.70 (1.1)
20:4	0.04 (0.1)
20:5	—
22:5	—
22:6	—
Total polyunsaturated	14.20 (21.9)
Total fat (g/100 g edible portion)	67.95

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

have lipids with lower melting points leading to muscle with soft and even liquid fat. Reduction of fat hardness will cause problems during the processing of poultry products due to fat smearing and can lead to consumer rejection if fat softness is severe. In addition, high concentrations of unsaturated fatty acids will decrease the oxidative stability of poultry products leading to consumer rejection due to the development of rancidity and associated fishy odors. Reduction of oxidative stability in poultry products containing high concentrations of unsaturated fatty acids can be partially overcome by dietary supplementation of α-tocopherol and by vacuum or modified-atmosphere packaging technologies.

II. FATTY ACID COMPOSITION OF EGGS AND EGG PRODUCTS

The fatty acid composition of eggs and various cooked and uncooked egg products varies greatly owing to a number of factors. This section will discuss the variations in the fatty acid composition of raw and cooked chicken eggs, egg yolks, dried egg products, and egg substitutes. Factors affecting the fatty acid composition of eggs will also be discussed. Unless otherwise noted, mention of eggs and egg products will refer to chicken eggs. The composition of eggs and egg products has been reviewed by several authors (Cotterill et al., 1977; Naber, 1979; Cook and Briggs, 1986; Burley and Vadehra, 1989; Stadelman and Pratt, 1989).

A. Fatty Acid Composition of Chicken Eggs

The fatty acid composition of the edible portion of whole eggs, egg yolks, cooked eggs, egg products, and egg substitutes is shown in Tables 7.19–7.25. Two chicken eggs constitute a typical serving, with

TABLE 7.14
Fatty Acid Composition of Mechanically Deboned Chicken and Turkey[a]

	Chicken Backs and Necks		Dark Muscle	
Fatty Acid	**No Skin**	**With Skin**	**Chicken, Mature Hens**	**Turkey Frames**
12:0	—	—	—	0.13 (0.9)
14:0	0.12 (0.8)	0.26 (1.1)	0.15 (0.8)	0.27 (1.8)
16:0	3.57 (24.8)	5.27 (23.1)	3.53 (19.0)	3.26 (22.0)
18:0	1.02 (7.1)	1.82 (8.0)	0.98 (5.3)	1.65 (11.1)
Total saturated	4.71 (32.7)	7.45 (32.6)	4.73 (25.5)	5.31 (35.8)
16:1	0.71 (4.9)	0.55 (2.4)	0.86 (4.6)	0.25 (1.7)
18:1	6.70 (46.5)	9.87 (43.2)	8.36 (45.0)	4.77 (32.1)
20:1	—	—	—	—
Total monounsaturated	7.41 (51.5)	10.44 (45.7)	9.30 (50.1)	5.02 (33.8)
18:2	2.07 (14.4)	4.48 (19.6)	4.11 (22.1)	4.18 (28.2)
18:3	0.21 (1.5)	0.34 (1.5)	0.24 (1.3)	0.33 (2.2)
20:4	—	0.10 (0.4)	0.10 (0.5)	—
20:5	—	0.02 (0.1)	—	—
22:5	—	—	—	—
22:6	—	0.02 (0.1)	—	—
Total polyunsaturated	2.28 (15.8)	4.96 (21.7)	4.55 (24.5)	4.51 (30.4)
Total fat (g/100 g edible portion)	15.48	24.73	19.98	15.96

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TABLE 7.15
Fatty Acid Composition of Processed Chicken and Turkey Meat[a]

Fatty Acid	**Chicken Canned**	**Chicken Roll**	**Turkey Canned**	**Turkey Roll**
12:0	0.01 (0.1)	0.01 (0.2)	0.01 (0.2)	0.01 (0.2)
14:0	0.06 (0.8)	0.06 (0.9)	0.05 (0.8)	0.05 (0.8)
16:0	1.71 (22.8)	1.51 (23.5)	1.22 (20.3)	1.26 (20.1)
18:0	0.46 (6.1)	0.39 (6.1)	0.49 (8.2)	0.47 (7.5)
Total saturated	2.25 (30.0)	1.97 (30.7)	2.00 (33.3)	2.02 (32.3)
16:1	0.46 (6.1)	0.41 (6.4)	0.37 (6.2)	0.41 (6.5)
18:1	2.73 (36.4)	2.43 (37.9)	1.83 (30.4)	2.02 (32.3)
20:1	0.08 (1.1)	0.08 (1.2)	0.01 (0.2)	0.01 (0.2)
22:1	—	—	0.01 (0.2)	0.01 (0.2)
Total monounsaturated	3.27 (43.7)	2.92 (45.5)	2.26 (37.6)	2.50 (39.9)
18:2	1.59 (21.2)	1.36 (21.7)	1.46 (24.3)	1.42 (22.7)
18:3	0.07 (0.9)	0.06 (0.9)	0.08 (1.3)	0.09 (1.4)
20:4	0.07 (0.9)	0.08 (1.2)	0.14 (2.3)	0.14 (2.2)
20:5	0.01 (0.1)	—	—	—
22:5	0.01 (0.1)	0.01 (0.2)	0.02 (0.3)	0.02 (0.3)
22:6	0.21 (2.8)	0.02 (0.3)	0.03 (0.5)	0.02 (0.3)
Total polyunsaturated	1.97 (26.3)	1.53 (23.8)	1.75 (29.1)	1.74 (27.8)
Total fat (g/100 g edible portion)	8.10	7.38	6.86	7.22

[a]Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TA4BLE 7.16
Fatty Acid Composition of Cured Poultry Products[a]

Fatty Acid	Chicken Frankfurters	Turkey Ham
12:0	0.05 (0.3)	0.00
14:0	0.18 (1.0)	0.04 (1.4)
16:0	4.12 (22.8)	0.68 (23.4)
18:0	1.10 (6.0)	0.34 (11.7)
Total saturated	5.54 (30.7)	1.06 (36.6)
16:1	1.20 (6.6)	0.10 (3.4)
18:1	7.14 (39.5)	1.04 (35.9)
20:1	—	—
22:1	—	—
Total monounsaturated	8.48 (47.0)	1.14 (39.3)
18:2	3.74 (20.7)	0.67 (23.1)
18:3	0.15 (0.8)	0.03 (1.0)
20:4	—	—
20:5	—	—
22:5	—	—
22:6	—	—
Total polyunsaturated	4.04 (22.4)	0.70 (24.1)
Total fat (g/100 g edible portion)	19.48	4.10

[a] Fatty acid concentrations expressed as g/100 g edible portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TABLE 7.17
Fatty Acid Composition of Fat from Chickens Fed Various Fat Sources[a]

Fatty Acid	Basal Diet	Cottonseed	Beef Tallow
12:0	—	—	—
14:0	1.0	0.8	1.9
16:0	20.6	20.5	18.6
18:0	9.1	9.8	11.4
Total saturated	30.7	30.4	31.9
16:1	6.7	2.0	6.3
18:1	28.0	22.7	36.9
20:1	—	—	—
22:1	—	—	—
Total monounsaturated	34.7	24.7	43.2
18:2	32.5	42.8	23.0
18:3	2.0	1.4	1.8
20:4	—	—	—
20:5	—	—	—
22:5	—	—	—
22:6	—	—	—
Total polyunsaturated	34.5	44.2	24.8

[a] Fatty acid concentrations are expressed as the percentage of total fatty acid content.

Source: Adapted from Edwards, H.M., Jr., et al. (1973). *Poultry Sci. 52*: 934.

TABLE 7.18
Fatty Acid Composition of Chicken Fat from Broilers Fed Chicken Fat and Menhaden Oil[a]

Fatty Acid	Chicken Fat	Menhaden Oil
12:0	—	—
14:0	—	—
16:0	24.4	25.9
17:0	0.2	1.1
18:0	5.3	7.0
Total saturated	29.9	34.0
16:1	8.5	10.3
18:1	40.4	24.2
20:1	0.5	1.0
22:1	—	—
Total monounsaturated	49.4	35.5
18:2	19.1	15.2
18:3	1.4	2.1
20:4	0.3	0.6
20:5	—	7.3
22:5	—	2.7
22:6	—	2.7
Total polyunsaturated	20.8	30.5

[a] Fatty acid concentrations are expressed as the percentage of total fatty acid content.

Source: Adapted from Phetteplace, H.W., and Watkins, B.A. (1990). *J. Agric. Food Chem. 38*: 1848.

TABLE 7.19
Fatty Acid Composition of Chicken Eggs[a]

Fatty Acid	Whole Egg, Edible Portion[b]
12:0	0.03 (0.4)
16:0	2.26 (27.3)
18:0	0.78 (9.4)
Total saturated	3.10 (37.5)
16:1	0.30 (3.6)
18:1	3.47 (42.0)
Total monounsaturated	3.81 (46.0)
18:2	1.15 (13.9)
18:3	0.03 (0.4)
20:4	0.14 (1.7)
Total polyunsaturated	1.36 (16.4)
Total fat	9.94

[a] Fatty acid concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of total fatty acid content.
[b] Fresh and frozen, raw. Very similar values apply to whole eggs hard-cooked in shell.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TABLE 7.20
Reported Values for Saturated, Monounsaturated, and Polyunsaturated Fatty Acid Content of Whole Eggs[a]

Fatty Acids	Cotterill et al. (1977)	USDA (1979)	USDA (1988)	Cook and Briggs (1986)
Saturated	4.50	3.35	3.4	3.67
Monounsaturated	5.36	4.46	4.4	4.60
Polyunsaturated	1.69	1.45	1.4	1.32

Fatty acid composition of egg yolks.

[a] Expressed as g/100 g edible portion.

TABLE 7.21
Fatty Acid Composition of Chicken Egg Yolks[a]

Fatty Acid	Fresh Yolk[b]	Frozen Raw Yolk[c]	Frozen Raw Sugared Yolk	Dried Yolk
14:0	0.10 (0.4)	0.08	0.07	0.18
16:0	6.86 (26.9)	5.68	5.07	12.56
18:0	2.42 (9.5)	2.02	1.80	4.33
Total saturated	9.55 (37.5)	7.82	6.97	17.15
16:1	0.92 (3.6)	0.64	0.55	1.37
18:1	10.70 (42.0)	9.01	7.98	19.54
Total monounsaturated	11.74 (46.1)	9.75	8.61	21.13
18:2	3.54 (13.9)	2.94	2.60	6.42
18:3	0.10 (0.4)	0.06	0.06	0.13
20:4	0.44 (1.7)	0.43	0.36	0.84
Total polyunsaturated	4.20 (16.5)	3.63	3.24	7.90
Total fat	26.54	25.60	22.75	55.80

[a] Fatty acid concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of total fatty acid content.
[b] Includes a small portion of albumin.
[c] Includes approximately 17% albumin.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

each egg providing roughly 50 g of edible material. Thus, the values in tables showing the fatty acid concentration in g/100g edible portion can be used to approximate the fatty acid content of a serving of two chicken eggs. The values can be halved to obtain the amounts of fatty acids from a serving of one egg.

The fatty acid composition of chicken eggs is shown in Table 7.19. These values apply to raw eggs, fresh or frozen, as well as to whole eggs hard cooked in the shell. A 100 g edible portion of whole egg contains 3.35 g saturated fatty acids, 4.46 g monounsaturated fatty acids, and 1.45 g PUFAs. The predominant saturated fatty acid is palmitic acid (16:0), whereas oleic acid (18:1) and linoleic acid (18:2) are the major monounsaturated and polyunsaturated fatty acids, respectively. Almost one-half of the total fatty acids are monounsaturated, whereas a little more than one-third are saturated.

The reported values for the fatty acid content of whole eggs taken from several reports in the literature are shown in Table 7.20. In general, there is fair agreement on the amounts of saturated and unsaturated fatty acids in whole eggs. However, there is some variation among the various reports. The values of Cotterill et al. (1977) are higher than others shown in Table 7.20. The values reported

by the U.S. Department of Agriculture (1988) are approximately the same as those reported earlier by that organization (USDA, 1979).

The fatty acid composition of fresh chicken egg yolk and several other yolk products is shown in Table 7.21. The values for fresh yolk are for the yolk with a small amount of adhering albumen. The albumen is essentially fat free. Therefore, the effect of its inclusion is to dilute slightly the fatty acid concentration in the yolk. The frozen raw yolk listed in Table 7.21 is a product with approximately 17% albumin. Thus, the fatty acid content is appreciably diluted compared with fresh yolk. In the case of dried yolk, the moisture content is reduced and, therefore, the levels of fatty acids are considerably higher than the other yolk products. The relative amounts of fatty acids (% of total fatty acids) in all of the products shown in Table 7.21 are similar. The proportions of the various fatty acids for the fresh yolk reported in Table 7.21 are similar to the ratios for the whole egg. The levels of fatty acids for the yolk are higher than those of the whole egg, as all of the lipid in the yolk fraction. The values in Table 7.21 are expressed as g/100 g of edible portion. The yolk of an average large egg (60 g) weighs approximately 17 g. Thus, the amount of various fatty acids provided per yolk can be readily calculated. However, it should be noted that the percentage of yolk is not constant for eggs of various weight classes (Washburn, 1979). Smaller eggs tend to have a larger ratio of yolk to albumin, and they would therefore have relatively higher fatty acid levels.

B. Fatty Acid Composition of Cooked Eggs

Values for the fatty acid composition of whole eggs cooked in several ways are shown in Table 7.22. The amounts of fatty acids in whole eggs hard cooked in the shell are essentially the same as those for the whole fresh egg. In addition, similar values are noted for poached eggs. On the other hand, the values for fried and scrambled eggs and for omelets are different from those of fresh eggs owing to the use of butter and milk in the preparation. Fried eggs prepared with butter contain approximately 1.9 g more saturated fatty acids and 0.8 g more monounsaturated fatty acids per 100 g than raw, hard-cooked, and poached eggs. In addition to higher concentrations of myristic, palmitic, stearic, and oleic acids, fried eggs also contain small amounts of the shorter chain saturated fatty acids. These values would be higher in PUFAs and lower in saturated fatty acids if margarine and vegetable oil are used in place of butter. Omelets and scrambled eggs prepared with milk and butter have 1.19 more saturated fatty acids and 0.4 g less monounsaturated fatty acids per 100 g than raw eggs. However, owing to the dilution of the egg in omelets and scrambled eggs with milk, the total fat concentration is approximately the same as that of raw, poached, and hard-cooked eggs. Nevertheless, it should be recognized that a serving of scrambled eggs made from two eggs would contain more fat than two hard-cooked eggs.

C. Fatty Acid Composition of Dried Whole Eggs

The fatty acid composition of dried whole eggs is shown in Table 7.23. The proportions of the fatty acids, expressed as percentages of the total fatty acids, are the same as those of raw fresh whole eggs. However, the fatty acid concentrations are almost fourfold that of the fresh whole egg owing to the removal of water from the product. The amount of total fat per 100 g of dried eggs is approximately 42–44 g, whereas the respective value for fresh whole egg is 11 g. The concentration of total fat in dried eggs is also higher than that of fresh yolk (33 g/100 g) and lower than that of dried yolk (61 g/100 g) (see Table 7.21). The concentrations of fatty acids in stabilized, dried whole egg are slightly higher than those of dried whole egg due to differences in the moisture content of the products.

D. Fatty Acid Composition of Raw Whole Eggs of Several Avian Species

Most eggs used for human consumption are obtained from chickens. However, eggs from a number of other avian species are also consumed. The fatty acid composition of eggs from ducks, geese,

TABLE 7.22
Fatty Acid Composition of Cooked Eggs[a,b]

Fatty Acid	Fried[c]	Omelet or Scrambled[d]	Poached
4:0	0.00	0.00	
6:0	0.00	0.00	
8:0	0.003 (0.02)	0.003 (0.03)	0.003 (0.04)
10:0	0.003 (0.02)	0.003 (0.03)	0.003 (0.04)
12:0	0.003 (0.02)	0.003 (0.03)	0.003 (0.04)
14:0	0.048 (0.36)	0.038 (0.36)	0.030 (0.36)
16:0	2.949 (22.17)	2.308 (22.09)	2.217 (26.90)
18:0	1.238 (9.31)	0.973 (9.31)	0.781 (9.48)
Total saturated	4.294 (32.29)	3.360 (32.16)	3.087 (37.46)
16:1	0.333 (2.50)	0.260 (2.49)	0.297 (3.60)
18:1	5.971 (44.89)	4.698 (44.97)	3.459 (41.97)
Total monunsaturated	6.346 (47.71)	4.990 (47.76)	3.795 (46.05)
18:2	2.340 (17.59)	1.847 (17.68)	1.140 (13.83)
18:3	0.121 (0.91)	0.096 (0.92)	0.030 (0.36)
20:4	0.153 (11.50)	0.119 (1.14)	0.141 (1.71)
Total polyunsaturated	2.660 (20.00)	2.097 (20.07)	1.359 (16.49)
Total fat	15.31	12.02	9.90

[a] See Table 7.19 for fatty acid composition of whole eggs hard cooked in shell.
[b] Fatty acid concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of total fatty acid content.
[c] Fried whole egg prepared using butter as table fat.
[d] Product is cooked using whole egg, butter as table fat, and milk.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TABLE 7.23
Fatty Acid Composition of Dried Whole Eggs[a]

Fatty Acid	Dried Whole Egg	Dried Whole Egg Stabilized[b]
14:0	0.14 (0.4)	0.12 (0.3)
16:0	9.23 (27.3)	9.70 (26.6)
18:0	3.29 (9.7)	3.37 (9.2)
Total saturated	12.72 (37.6)	13.20 (36.2)
16:1	1.01 (3.0)	1.46 (4.0)
18:1	14.16 (41.8)	16.10 (44.1)
Total monounsaturated	15.34 (45.3)	17.56 (48.1)
18:2	4.61 (13.6)	4.88 (13.4)
18:3	0.11 (0.3)	0.12 (0.4)
20:4	0.57 (1.7)	0.37 (1.0)
Total polyunsaturated	5.80 (17.1)	5.71 (15.7)
Total fats	40.95	43.95

[a] Fatty concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of the total fatty acid content.
[b] Glucose reduced.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

TABLE 7.24
Fatty Acid Composition of Raw Whole Eggs of Several Avian Species[a]

Fatty Acid	Duck Eggs	Goose Eggs	Quail Eggs	Turkey Eggs
14:0	0.05 (0.4)	0.05 (0.5)	0.05 (0.5)	0.04 (0.4)
16:0	2.99 (26.2)	2.85 (25.9)	2.67 (29.0)	2.72 (27.6)
18:0	0.63 (5.5)	0.70 (6.4)	0.84 (9.1)	0.88 (8.9)
Total saturated	3.68 (32.2)	3.60 (32.7)	3.56 (38.7)	3.63 (36.8)
16:1	0.44 (3.9)	0.39 (3.5)	0.47 (5.1)	0.67 (6.8)
18:1	6.08 (53.3)	5.35 (48.5)	3.85 (41.8)	3.90 (39.6)
Total monounsaturated	6.52 (57.2)	5.75 (52.2)	4.32 (47.0)	4.57 (46.3)
18:2	0.56 (4.9)	0.68 (6.2)	0.94 (10.2)	1.17 (11.9)
18:3	0.10 (0.9)	0.55 (5.0)	0.04 (0.4)	0.08 (0.8)
20:4	0.32 (2.8)	0.28 (2.5)	0.12 (1.3)	0.13 (1.3)
22:5	Trace			
Total polyunsaturated	1.22 (10.7)	1.67 (15.2)	1.32 (14.3)	1.66 (16.8)
Total fat	13.77	13.27	11.09	11.88

[a] Fatty acid concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of total fatty acid content.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

quail, and turkeys is shown in Table 7.24. Total fat concentrations are higher in duck eggs and goose eggs than in quail eggs and turkey eggs. The fat concentration of the latter two types of eggs is similar to that of chicken eggs (see Table 7.19). Compared with goose eggs, duck eggs have a similar percentage of total saturated fatty acids, a higher percentage of total monounsaturated fatty acids (mainly due to a higher level of oleic acid), and a lower percentage of PUFAs. Quail and turkey eggs have a higher percentage of saturated fatty acids and a lower percentage of monounsaturated fatty acids compared with duck and goose eggs. These percentages of the three classes of fatty acids in quail and turkey eggs are similar to those of chicken eggs.

Another avian species that is used for egg production is the guinea fowl. Oguntona and Hughes (1988) observed that guinea fowl eggs contain approximately 12% total fat; that is, about 1% more fat than chicken eggs. These authors found that the guinea fowl eggs contain 49% saturated, 30% monounsaturated, and 21% polyunsaturated fatty acids (expressed as a percentage of the total fatty acid content). The respective values for chicken eggs are 36%, 48%, and 16% (see Table 7.19). Thus, the guinea fowl eggs are higher in saturated fatty acids, lower in monounsaturated fatty acids, and higher in PUFAs.

The composition of eggs from a number of wild birds were compared with that of several domesticated species by Bitman and Wood (1980). These authors observed that egg yolks of several aquatic carnivorous birds have much lower total fat levels than those of chickens. In addition, the aquatic birds had appreciable levels of 20:5, 22:5, and 22:6 fatty acids.

E. Fatty Acid Composition of Egg Substitutes

The fatty acid composition of several egg substitutes is shown in Table 7.25. Two of these products, the liquid and the frozen products, are made with vegetable oils and without egg yolk. The percentage of total saturated and monounsaturated fatty acids is low and the value for PUFAs is high compared with respective values for chicken eggs. Although the frozen product shown in Table 7.25 has a total fat value similar to that of fresh whole eggs, the total fat content of the liquid product is quite low. The powdered product illustrated in Table 7.25 contains whole egg solids. It contains a blend of

TABLE 7.25
Fatty Acid Composition of Egg Substitutes[a]

Fatty Acid	Liquid[b]	Powder[c]	Frozen[d]
12:0	<0.01 (0.1)	0.00	0.00
14:0	0.01 (0.2)	0.06 (0.6)	0.01 (0.1)
16:0	0.35 (11.1)	2.69 (24.9)	1.50 (14.2)
18:0	0.31 (9.8)	0.99 (9.2)	0.44 (4.2)
Total saturated	0.66 (20.9)	3.77 (34.9)	1.93 (18.2)
16:1	<0.01 (0.1)	0.50 (4.6)	
18:1	0.89 (28.2)	4.84 (44.9)	2.44 (23.0)
Total monounsaturated	0.90 (28.5)	5.34 (49.5)	2.43 (23.0)
18:2	1.41 (44.6)	1.41 (13.1)	6.18 (58.3)
18:3	0.18 (5.7)	0.09 (0.8)	0.06 (0.6)
Total polyunsaturated	1.60 (50.6)	1.68 (15.6)	6.24 (58.9)
Total fat	3.31	13.00	11.11

[a] Fatty acid concentrations expressed as g/100 g portion. Numbers in parentheses are the percentage of the total fatty acid content.
[b] Product contains egg white, hydrogenated soybean oil, and soy protein.
[c] Product contains egg white solids, whole egg solids, sweet whey solids, nonfat dry milk solids, and soy protein.
[d] Product contains egg white, corn oil, and nonfat dry milk.

Source: Adapted from USDA Nutrient Database for Standard Reference, Release 19 (Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata).

saturated, monounsaturated, and polyunsaturated fatty acids similar to that of fresh whole eggs and has a higher total fat concentration.

F. Factors Affecting the Fatty Acid Composition of Eggs

There are a number factors that can influence the fatty acid composition of eggs. The differences among various species of birds were discussed earlier. There are indications that variations can exist within a species. Washburn (1979) reviewed a number of studies that showed a difference in the relative proportions of yolk and albumen among various genetic strains of chickens. Increasing the proportion of yolk increases the total amount of fatty acids per egg. Washburn (1979) suggested that the variation in the proportion of yolk may be a secondary effect of changes in egg size. Studies in our laboratory (A.H.C., unpublished data) and elsewhere have indicated that although the yolk weight increases with the total egg weight, it is not proportional to the total egg weight. Smaller eggs tend to have a larger percentage of yolk. Consequently, the concentration of fatty acids would tend to be higher in smaller eggs. Another factor reviewed by Washburn (1979) is the genetic variation in the percentage egg solids. Variations in egg dry matter would alter the concentration of fatty acids without affecting the relative proportions of different fatty acids. The nature of the hen's diet is another important factor affecting the fatty acid composition of egg yolks. This topic is discussed in the next section.

Another possible source of differences in values reported for fatty acid composition of eggs and egg products is the analytical procedure that is employed. Fletcher et al. (1984) compared several methods for determining total yolk lipid content. They observed values ranging from 30.3% to 35.2% using three extraction procedures. This suggests that incomplete extractions can affect fatty acid determinations and calculated values derived from these assays.

G. Influence of the Hen's Diet on Fatty Acid Composition of Egg Yolks

The composition of the diet fed to hens has been shown to alter the fatty acid composition of eggs. Although most studies have dealt with the influence of altering the fatty acid composition of the diet, there are other dietary factors that may affect the composition of yolk lipids. For example, Shadad et al. (1985) studied the effect of administering oxytetracycline to hens upon egg lipid composition. These authors observed a decrease in the concentration of palmitic acid (as a percentage of total fatty acids in egg yolk) and increases in the concentrations of stearic, oleic, and linoleic acids.

As early as 1934, Cruikshank demonstrated that the fatty acid composition of eggs could be altered by the hen's diet. Linoleic and linolenic acid levels in the yolk were markedly increased, whereas oleic acid decreased upon feeding high levels of unsaturated fat. Many studies have confirmed this observation over the decades. The earlier studies focused on the use of oilseeds and animal fat sources (Cruikshank, 1941; Sell et al., 1968; Naber, 1979). Sim and Bragg (1978) observed that the substitution of 8% safflower oil for 8% hydrogenated coconut oil in the diet increased the concentrations of palmitic and linoleic acids and decreased the concentrations of palmitoleic and oleic acids without affecting the total lipid concentration of the yolk. Sell et al. (1987) observed increased yolk weights with no change in albumin weight following the addition of animal–vegetable fat to the diet of laying hens.

In recent years, especially following recommendations for the increased intake of n-3 fatty acids in human diets (Health and Welfare Canada, 1989), there has been considerable interest in providing consumers with eggs containing elevated levels of n-3 fatty acids. Most studies have shown that the egg composition could be altered within 2 weeks of dietary changes. Because much of the development of the egg yolk occurs within the 10 days before ovulation (Austic and Nesheim, 1990), a change in yolk fatty acid composition within a few weeks would be expected.

In some studies (Cruikshank, 1941; Navarro et al., 1972), fish oils were fed to laying hens, and the long-chain n-3 fatty acids in these oils appeared in eggs. In a typical study, 5% menhaden oil increased the egg DHA content from 2.9% to 11.8% of total yolk fatty acids (Couch and Saloma, 1973). Huang et al. (1990) found long-chain n-3 fatty acids increased from 0.78% to 4.42% of total fatty acids when 4% menhaden oil was fed. Adams et al. (1989) increased long-chain n-3 from 56 to 622 mg/100 g yolk by substituting menhaden oil for corn oil at 3% of the diet. Upon feeding 3% menhaden oil, Hargis et al. (1991) observed that the long-chain n-3 fatty acid concentration increased from 35 to 210 mg/100 g yolk, whereas the concentration of n-6 fatty acids, especially AA, decreased from about 100 to approximately 30 mg/100 g yolk. High levels of long-chain n-3 fatty acids might have suppressed the hepatic production of AA from dietary linolenic acid. Maurice (1994) added 4% menhaden oil to layer feed and increased eicosapentaenoic acid plus DHA from 1.67 to 18 mg/g. Most studies showed roughly a fivefold increase in the long-chain n-3 percentage when fish oils were fed, but Adams et al. (1989) and Maurice (1994) found approximately 11-fold increases.

Several researchers have addressed the possibility that feeding fish oils might produce fishy off-flavors in the eggs or tissue, and several observers reported significantly poorer organoleptic evaluations when fish oils were fed at levels as low as 1.5% of diet (Miller et al., 1967; Koehler and Bearse, 1975; Van Elswyk et al., 1992). Others have reported minimal flavor problems. Holdas and May (1966) found no effect on organoleptic scores when good-quality fish oil was fed at 2.5% of the diet. Huang et al. (1990) found an acceptable egg flavor when 3% fish oil, stabilized with ethoxyquin, was fed. Maurice (1994) reported that panelists were unable to detect off-flavors in eggs from hens fed deodorized menhaden oil at 8% of the diet. Minimizing oxidation of dietary fats by proper storage and antioxidant addition seems to be beneficial in preventing flavor problems and maximizing incorporation of long-chain n-3 into eggs (Huang et al., 1990; Hargis et al., 1991).

Herber and Van Elswyk (1996) reported a novel source of long-chain n-3 PUFAs: a marine alga that has approximately the same percentage (11%) of long-chain n-3 PUFAs as menhaden oil.

When fed at 2.4% of the diet, marine alga did not affect egg production or weight when compared with corn, although feeding 4.8% of marine alga depressed both egg production and weight. Feeding 2.4% alga and 1.5% menhaden oil gave very similar egg fatty acid compositions. In studies reported by Abril et al. (1999), use of a commercial marine algae product at 4.8% of the diet (providing 825 mg DHA per day) did not depress egg production, but decreased egg weight slightly. In addition to the high content of n-3 fatty acids, the algal products are also rich in β-carotene and canthaxanthin, which can enhance the pigmentation of the egg yolk.

Flax seed is another feed ingredient that has been reported to increase n-3 fatty acid levels in eggs. Freshly ground whole flax seed, rather than flax (linseed) oil, is used to minimize oxidation problems. Linolenic acid represents approximately 55% of total fatty acids in flax seed. Naber (1979) reviewed several studies showing large increases in the linolenic acid concentration in eggs due to high dietary levels of linseed oil and large increases in linoleic acid concentrations due to feeding vegetable oils. Aymond et al. (1994) increased egg linolenic acid from 0.95 to 16.08 mg/g yolk and long-chain n-3 PUFAs from 4 to 8.3 mg/g by feeding 15% flax seed. Using six different strains of laying hens, Ahn et al. (1995) found egg linolenic acid increased from about 0.5% to about 5% and long-chain n-3 PUFAs increased from about 1% to 3% of total fatty acids when a proprietary blend of "high linolenic acid ingredients" was fed at 17% of the diet. Cherian and Sim (1991) and Ajuyah et al. (1992) reported similar results—a ninefold increase in egg or tissue linolenic acid and only a twofold increase in long-chain n-3 PUFAs—when they fed 16% flax seed. Caston and Leeson (1990) reported that feeding graded levels of ground flax seed (up to 30% of the diet) increased the linolenic acid content from 0.4% to 11.5% of yolk fatty acids. The content of DHA was elevated from 0.04% to 0.22% by feeding 20% flax seed in the diet. Feeding a higher level of flax seed did not lead to a further increase in the concentration of DHA. This suggests that the hen's capacity for elongation of linolenic acid is limited.

Canola, a form of rapeseed which is low in erucic acid ($C_{22:1n9}$) and glucosinolates, has a fairly high level of linolenic acid, typically 9%–10% of total fatty acids, and a low level of saturated fatty acids (<10%). Nwokolo and Sim (1989) fed full-fat canola seed to layers at 10% of diet and increased egg linolenic acid by 50% and DHA by 26% compared with control treatment. By feeding 16% canola seed to laying hens, Cherian and Sim (1991) increased egg linolenic acid from 0.6% to 2.4% of total fatty acids and long-chain n-3 PUFAs from 1% to 1.7%. Increases in the n-3 PUFA percentage were accompanied by corresponding decreases in the saturated fatty acid content.

Studies by Collins et al. (1997) examined the effect of substituting pearl millet (*Pennisetum americanum*) for corn in laying hen diets on egg yolk fatty acids. Typical concentrations of linoleic and linolenic acids in these grains are 45% and 4% for pearl millet and 57% and 0.9% for corn, respectively. Replacing all of the corn in the diet with millet significantly decreased the linoleic acid content and increased the content of linolenic acid and DHA (Table 7.26). In addition, the ratio of n-6:n-3 fatty acids decreased from 13.1 to 8.3. A subsequent study by Collins (1997) examined egg fatty acid composition due to feeding diets that included corn, canola oil, pearl millet, menhaden fish oil, or flax seed. Substituting millet for corn or adding canola oil, menhaden oil, or flax seed to the diets significantly decreased the concentrations of linoleic and arachidonic acids and significantly increased concentrations of linolenic acids and long-chain n-3 fatty acids (Table 7.27). Feeding canola oil, pearl millet, or pearl millet plus canola oil greatly reduced the ratio of n-6:n-3 fatty acids compared with feeding corn. Greater reductions were observed when menhaden oil or flax seed were fed.

Farrell (1999) also examined the effects of several dietary several sources of n-3 fatty acids on egg fatty acid profiles. Cod liver oil was much more effective in elevating long-chain PUFAs in the yolk, compared with canola oil or flaxseed oil. Because the long-chain PUFAs are the ones associated with health benefits, Farrell indicated that all sources of n-3 fatty acids for enriching eggs should not be considered interchangeable with respect to expected health benefits.

As noted previously, there is increasing concern about levels of *trans* fatty acids in the human diet. Use of feed ingredients, such as restaurant grease and bakery products, two potential sources

TABLE 7.26
Effect of Grain Source in Diets of Hens on Egg Fatty Acid Content[a]

	Diet	
Fatty Acids	**Corn**	**Pearl Millet**
Saturated	119	122
Monounsaturated	156	170[6]
Linoleic acid, $C_{18:2n-6}$	56.2	40.4[b]
Arachidonic acid, $C_{20:2n-6}$	7.2	7.1[b]
Linolenic acid, $C_{18:3n-3}$	1.0	1.3[b]
Docosohesenoic acid, $C_{22:6n-3}$	1.8	2.7[b]
Total n-3	5.1	5.7[b]
n-6:n-3 ratio	13.1	8.3[b]

[a] Expressed as mg/g yolk; samples taken after 6 weeks of treatment.
[b] Significantly different from corn diet ($p < 0.05$).

Source: Adapted from Collins, V.C. et al. (1997). *Poultry Sci. 76*: 326.

TABLE 7.27
Effect of Changes in Diets of Laying Hens on Egg Fatty Acid Composition, Expressed as Percentage of Total Fatty Acids[a]

Diet								
Grain	**Addition**	**Saturated**	**Mono Unsaturated**	**Linoleic**	**Linolenic**	**Arachidonic**	**Long-Chain n-3[b]**	**n-6/n-3 Ratio**
Corn		33[d]	44[d,e]	16[c]	0.3[e]	2.4[c]	0.8[g]	19[c]
Corn	3% Canola oil	31[d]	49[c]	15[d,e]	1.1[c,d]	2.1[e]	1.6[e]	6[d]
Pearl millet		33[d]	46[d]	13[f,g]	0.6[d]	2.3[d]	1.3[f]	7[d]
Millet	3% Canola oil	32[d]	46[d]	15[d]	1.3[c,d]	1.9[f]	1.6[e]	6[d]
Corn	3% Menhaden oil	37[c]	42[e]	13[g]	0.6[d]	0.8[h]	5.3[c]	2[e]
Corn	8% Flaxseed	32[d]	46[d]	14[ef]	4.1[c]	1.4[g]	2.4[d]	3[e]
Pooled	SEM	0.5	1.7	0.2	0.2	0.1	0.3	0.9

[a] Data shown are means of three pooled eggs per replicate, eight replicates per treatment, sampled after 7 weeks of treatment.
[b] Long-chain n-3 represents the sum of $C_{20:5n3}$ and $C_{22:6n3}$.
[c–h] Values in a column not sharing a superscript differ significantly ($p < 0.05$).

Source: Adapted from Collins, V.C. *et al*. (1997). *Poultry Sci. 76*: 326.

of *trans* fatty acids, is typically much lower in diets for laying hens than for broilers or turkeys. Thus, very low levels of *trans* fatty acids would be expected in eggs. Levels of *trans* fat have been estimated to be 0.11 g per 100 g of whole liquid egg and 0.28 g per 100 g of liquid egg yolk (Egg Nutrition Center).

Because of potential health benefits of CLA in human nutrition, there have been a number of studies aimed at enriching egg yolk with CLA. Watkins and coworkers (1999) showed that supplementing hens with a human grade dietary supplement of CLA could effectively increase yolk CLA, without negative effects on production. Du et al. (2000) also noted that egg yolks could be enriched with CLA by adding CLA to the laying hen diet. They observed that using dietary CLA also resulted

in decreased monounsaturated fatty acids and increased PUFAs in both egg yolks and liver. Similar results on egg yolk fatty acid profiles due to CLA supplementation of the laying hen diet were also noted by Cherian et al. (2002). In these studies levels of PUFAs were not altered by CLA. In a study in which graded levels of CLA was substituted for soybean oil in the diet, Suksombat et al. (2006) observed increasing levels of saturated fatty acids and decreasing levels of both monounsaturated and polyunsaturated fatty acids in egg yolk.

Typical feeds used for laying hens in the United States contain corn and soybean meal as the major ingredients with very little added fat. Some of the alternative ingredients for laying hen diets mentioned above are used in various geographical areas owing to their lower costs and availability. In other cases, some of the ingredients discussed previously are specifically included in the diet for the purpose of producing nutritionally enhanced or "designer" eggs. Thus, the eggs that consumers eat may vary considerably in fatty composition from the values published for "normal" eggs (e.g., see Table 7.19).

III. SUMMARY

Raw chicken eggs contain a blend of fatty acids consisting of 36% saturated, 48% monounsaturated, and 16% polyunsaturated fatty acids. The fat in the edible portion of eggs is entirely in the yolk. Therefore, egg yolk and yolk products contain similar proportions of the three classes of fatty acids but have higher concentrations. This is also true for dried egg products. The fatty acid composition of cooked eggs is influenced by the ingredients used in the particular method of preparation. There are differences in fatty acid composition of eggs from different avian species. Eggs from quail and turkeys are similar in composition to chicken eggs. The composition of egg substitutes will vary with the ingredients used in their preparation. The use of vegetable oils in these products results in higher levels of PUFAs compared with chicken eggs.

There are a number of factors that influence the fatty acid composition of the chicken egg. These include genetic strain, egg size, and the nutrient composition of the hen's diet. Feeding high levels of vegetable oils increases the levels of unsaturated fatty acids in the egg. Inclusion of fish oil, canola oil, flax seed, marine alga, and pearl millet are some of the ingredients that have been used to enhance the content of n-3 fatty acids in egg yolks.

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8 Fatty Acids in Fish and Shellfish

Robert G. Ackman

CONTENTS

I. INTRODUCTION

"Fish is a brain food" is an old admonition given to children who decline to eat fish. It originated in the roughly similar appearance of white fish muscle lipids and those extracted from the human brain, and even the chemists of the nineteenth century found similarities in the phosphorus content, not realizing that this indicated that both tissues were rich in phospholipids. The fatty acids of fish were even more mysterious and the polyunsaturated ones were easily destroyed in the inexact analyses of the time. Fish was usually sold just as fish, a food to be eaten because it was Friday in Catholic countries, or because it was cheap. At about the turn of the century, a few organic chemists began to sort out the structures of two highly unsaturated fatty acids, known as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). By the 1950–1960 era, much patient work in Germany, Japan, Britain, and the United States had identified most of the fish fatty acids and Stansby (1967) could write an excellent book on fish oils, including early work on their hypercholesterolemic effects. However, the nutritional and biochemical aspects of these two particular fatty acids had to wait nearly two more decades for proper documentation of the role of "omega-3" fatty acids (Holman, 1998).

II. WHY THERE ARE SO MANY FISH FATTY ACID COMPOSITIONS

The author realised rather late in his career that he had developed one table of fatty acids of fish and fish oils and just kept changing the numbers (Ackman et al., 1988). This was almost independent of whether the analysis was of edible muscle, or the whole fish or bodies of molluscs, or whether of freshwater or marine species. The qualitative uniformity of the tables was a consequence of the

continuous recycling of fatty acids though different food webs in the ocean. The composition tables were also independent of whether the data were prepared for nutritionists and food scientists in g/100 g (Tables 8.1 and 8.2) or in weight % fatty acids (Table 8.3), but total lipid was also frequently requested (Table 8.4). Subtle differences among different diets or with animal life stage, or in the proportions of triacylglycerols and phospholipids of some species, made up most of the differences tabulated.

A. Coldwater Fish

Much of the work on fish lipids and fatty acids was, for decades, focused on North Atlantic and North Pacific species. Even the first tabulation from New Zealand included many products imported from Great Britain. Table 8.1 is an excellent presentation of marine fish fatty acids with the weights given in g/100 g. These units appeal to dieticians, who agreed on 100 g (or 3½ oz) as a "serving." The number of fish sampled is stated, with their size and proximate composition data. The scientific names are given in Table 8.2, because this is a regional survey and common names of fish can change from place to place.

There are 16 fatty acids listed (excluding 15:0). This is two more than are really needed to describe the fatty acids of fish, as 18:1n-7 (*cis*-vaccenic acid) is usually included with 18:1n-9 (oleic acid). These two isomeric octadecenoic acids illustrate the shorthand nomenclature system now in use, which facilitates the understanding of fatty acid interconversions. By computing *n* carbons from the terminal methyl group, it is easy to see that 16:1n-7 plus one acetate unit becomes 18:1n-7 (the hyphen is often omitted for brevity). Both 16:1n-7 and 18:1n-9 arise by action of one Δ9,10 desaturase on the corresponding saturated acid. Similarly, the two polyunsaturated fatty acids (PUFAs; 20:5n-3 and 22:6n-3) most often identified with fish oils and lipids are related to 18:3n-3 (α-linolenic acid), as shown in Figure 8.1. The 22:5n-3 is also related to 20:5n-3, and the very minor 22:5n-6 is clearly associated by the nomenclature system to 20:4n-6 (arachidonic acid) and ultimately to the common 18:2n-6 (linoleic acid) of plant origin (Figure 8.2).

North Atlantic fish depot fats are set apart from many fish fats by the presence of 5% or more of 22:1n-11 (cetoleic acid). This is usually accompanied by minor proportions of 22:1n-9 (Ackman et al., 1980). The latter isomer is erucic acid, the source of much concern in the past in health and nutrition circles but now recognized as probably harmless in humans (Sauer and Kramer, 1983; Sauer et al., 1989). Table 8.3 illustrates several points of interests with respect to marine fish lipids and fatty acids. The first is that phospholipids have high proportions of 22:6n-3, less 20:5n-3, and very little 22:1. Triglycerides have more 20:5n-3 than 22:6n-3. In some older analyses on highly polar gas chromatography columns, 20:4n-6 and 22:1 coincided (Ackman, 1987). The other fat details include the extraordinary distribution of fat possible in some species, the nape tissue of Canadian redfish being very fatty indeed, although the fillet of the same fish has only 1.5% fat. In larger fish of this species, pure fat deposits also infiltrate the flanks, and this probably leads to the higher percentage of fat in the German samples. In the United States, the Canadian species is sold as "ocean perch" (Table 8.4).

The fats of the ever-popular salmon are somewhat confusing, because farmed fish tend to have more fat (10%–16%) than wild fish (5%–8%) (see also Suzuki et al., 1986; Roch et al., 1988; Hardy and King, 1989; van Vliet and Katan, 1990), but basically (Table 8.5) salmon fillets have modest levels of 20:1 and 22:1, and 22:6n-3 exceeds 20:5n-3. This table also makes the point that in herring fillet, 22:1 usually exceeds 20:1, and because of the higher level of these two acids, the 20:5n-3 and 22:6n-3 are relatively low. The fatty acids of canned salmon (pink, *Oncorhynchus gorbuscha*) closely resemble those of the fillet in this case except for the detailed proportions among the 18:1, 20:1, and 22:1, which in both cases total over one-quarter of all fatty acids. These fatty acids are nutritionally "neutral" in terms of serum cholesterol. The levels of presumably functional 20:5n-3 and 22:6n-3 are virtually identical. The wild salmon are migratory, and actual fat levels vary with date and location of capture. Additional data on other salmon samples have been published

TABLE 8.1
Proximate Chemical Composition and Fatty Acid Contents of 37 Finfish Species of the Southeastern United States[a]

Species	Black Sea Bass	Bluefish	Gulf Butterfish	Channel Catfish[b]	Atlantic Croaker	Dolphin	Southern Flounder	Goosefish (Monkfish)	Gag Grouper	Yellowedge Grouper	White Grunt	Harvest Fish	Speckled Hind
No. of samples	2	4	4	3	4	3	2	2	3	2	3	3	3
Mean length (cm)	34.80	40.10	16.00	36.30	25.30	68.40	41.40	ND	70.40	74.55	33.20	18.40	47.60
Mean weight (kg)	0.61	0.93	0.10	0.55	0.20	2.83	1.01	ND	4.70	4.71	0.81	0.21	2.59
Proximate Composition (g/100 g)													
Protein	18.0	20.0	20.0	16.2	18.0	20.2	17.4	16.2	21.0	19.3	20.5	18.9	21.1
Moisture	81.3	77.5	77.5	82.1	77.3	79.3	82.4	84.5	76.8	79.2	80.9	78.0	75.5
Fat	0.7	2.4	3.9	2.4	2.9	0.8	0.7	0.6	2.2	1.3	0.7	2.4	4.7
Ash	1.4	1.1	1.2	1.0	1.0	1.2	0.9	0.9	1.3	1.1	1.1	1.2	1.1
Fatty Acid (g/100 g)													
14:0	0.005	0.064	0.137	0.045	0.046	0.005	0.009	0.003	0.060	0.020	0.007	0.057	0.149
15:0	—	—	—	—	—	—	—	—	—	—	—	—	—
16:0	0.101	0.491	0.820	0.377	0.667	0.115	0.098	0.065	0.482	0.188	0.100	0.516	1.305
18:0	0.043	0.154	0.237	0.102	0.171	0.079	0.027	0.027	0.152	0.064	0.051	0.186	0.329
16:1n-7	0.018	0.166	0.127	0.119	0.323	0.010	0.023	0.010	0.152	0.039	0.017	0.048	0.441
18:1n-9	0.060	0.346	0.632	0.489	0.364	0.056	0.043	0.049	0.317	0.096	0.066	0.447	0.726
18:1n-7	0.016	0.083	0.116	0.098	0.082	0.014	0.014	0.011	0.062	0.032	0.017	0.054	0.111
20:1n-9	0.004	0.035	0.102	0.030	0.022	0.002	0.003	0.003	0.020	0.036	0.003	0.047	0.066
22:1n-11 + 13	0.000	0.006	0.002	0.001	0.006	0.000	0.001	0.001	0.004	0.027	0.002	0.010	0.002
22:1n-9	0.003	0.002	0.035	0.001	0.003	0.000	0.000	0.002	0.003	0.006	0.000	0.014	0.013
18:2n-6	0.004	0.039	0.031	0.053	0.011	0.005	0.009	0.005	0.016	0.010	0.004	0.013	0.025
20:4n-6	0.024	0.033	0.072	0.069	0.054	0.022	0.013	0.015	0.051	0.033	0.048	0.056	0.051
22:4n-6	0.006	0.008	0.027	0.009	0.021	0.000	0.002	0.002	0.014	0.009	0.011	0.019	0.016
20:5n-3	0.027	0.083	0.127	0.076	0.125	0.022	0.018	0.025	0.065	0.036	0.033	0.053	0.087
22:5n-6	0.010	0.011	0.039	0.029	0.014	0.016	0.006	0.005	0.023	0.017	0.010	0.032	0.032
22:5n-3	0.012	0.050	0.100	0.053	0.057	0.009	0.014	0.007	0.039	0.031	0.014	0.046	0.065
22:6n-3	0.121	0.174	0.380	0.094	0.125	0.196	0.095	0.090	0.276	0.209	0.088	0.235	0.403

Continued

TABLE 8.1
(Continued)

Species	Crevalle Jack	Southern Kingfish	Ladyfish	Chub Mackerel	King Mackerel	Striped Mullet	Red Porgy	Silver Rag	Blue Runner	Rough Scad	Spotted Seatrout	American Shad
No. of samples	4	2	3	5	2	5	2	4	2	7	2	4
Mean length (cm)	45.60	29.20	41.30	23.70	94.80	37.00	35.30	16.00	26.30	21.70	39.00	40.10
Mean weight (kg)	4.95	0.30	1.04	0.19	5.53	0.75	0.91	0.05	0.41	0.13	0.69	1.12
Proximate Composition (g/100 g)												
Protein	21.1	18.7	22.7	21.8	21.7	21.0	22.0	21.1	21.3	19.8	19.4	19.1
Moisture	74.3	77.3	73.0	72.0	76.6	74.4	77.1	72.5	75.3	76.8	79.4	65.4
Fat	3.9	4.5	4.4	5.5	1.7	5.1	0.9	5.2	1.6	2.1	2.7	14.6
Ash	1.3	1.1	1.2	1.5	1.4	1.0	1.5	1.4	1.3	1.4	1.1	1.3
Fatty Acid (g/100 g)												
14:0	0.067	0.096	0.074	0.132	0.026	0.285	0.006	0.156	0.023	0.068	0.072	0.588
15:0	—	—	—	—	—	0.220	—	—	—	—	—	—
16:0	0.895	1.245	1.164	1.131	0.389	1.143	0.149	1.002	0.316	0.417	0.608	1.687
18:0	0.324	0.198	0.310	0.389	0.157	0.118	0.049	0.369	0.150	0.144	0.142	0.296
16:1n-7	0.230	0.695	0.310	0.167	0.050	0.726	0.016	0.133	0.034	0.095	0.324	0.341
18:1n-9	0.649	0.833	1.064	1.031	0.268	0.255	0.066	0.853	0.167	0.320	0.378	1.013
18:1n-7	0.135	0.114	0.112	0.190	0.050	0.125	0.015	0.153	0.037	0.051	0.086	0.307
20:1n-9	0.021	0.024	0.034	0.098	0.018	0.009	0.007	0.155	0.015	0.015	0.014	2.666
22:1n-11 + 13	0.004	0.008	0.002	0.007	0.002	0.002	0.002	0.030	0.002	0.004	0.001	2.663
22:1n-9	0.004	0.004	0.002	0.027	0.007	0.002	0.001	0.023	0.001	0.004	0.001	0.168
18:2n-6	0.085	0.016	0.026	0.063	0.014	0.060	0.004	0.044	0.009	0.022	0.020	0.177
20:4n-6	0.095	0.065	0.088	0.068	0.036	0.131	0.030	0.121	0.037	0.026	0.050	0.029
22:4n-6	0.023	0.025	0.026	0.012	0.008	0.016	0.011	0.054	0.008	0.004	0.020	0.017
20:5n-3	0.113	0.067	0.083	0.238	0.045	0.355	0.021	0.165	0.058	0.099	0.106	0.506
22:5n-6	0.029	0.018	0.024	0.045	0.017	0.007	0.015	0.062	0.017	0.020	0.021	0.015
22:5n-3	0.061	0.055	0.068	0.074	0.019	0.177	0.020	0.125	0.035	0.037	0.041	0.203
22:6n-3	0.258	0.175	0.251	0.720	0.131	0.136	0.193	0.546	0.308	0.317	0.175	0.887

Species	Atlantic Sharpnose Shark	Lemon Shark	Scalloped Hammer-Head Shark	Tiger Shark	Sheepshead	Red Snapper	Vermilion Snapper	Spot	Tilefish	Blueline Tilefish	Gray Triggerfish	Weakfish
No. of samples	3	2	2	2	3	2	2	2	2	2	2	2
Mean length (cm)	53.90	183.00	229.00	ND	49.30	44.70	48.20	21.60	62.40	63.10	36.30	72.40
Mean weight (kg)	6.10	37.05	106.60	104.33	3.15	1.54	1.57	0.20	2.74	3.21	1.11	3.56
Proximate Composition (g/100 g)												
Protein	23.7	19.7	23.1	19.2	21.4	20.4	21.3	18.2	17.6	19.9	20.7	19.3
Moisture	75.1	79.5	76.0	80.1	77.9	77.8	78.0	71.4	81.1	78.3	79.1	78.1
Fat	0.8	0.6	0.7	0.7	1.6	1.4	0.6	8.0	1.0	2.5	0.8	2.1
Ash	1.4	1.3	1.4	1.2	1.2	1.2	1.2	1.0	1.3	1.3	1.2	1.1
Fatty Acid (g/100 g)												
14:0	0.001	0.002	0.001	0.002	0.034	0.029	0.009	0.240	0.018	0.067	0.002	0.052
15:0	—	—	—	—	—	—	—	—	—	—	—	—
16:0	0.100	0.055	0.064	0.048	0.310	0.276	0.088	1.987	0.139	0.419	0.087	0.37%
18:0	0.085	0.044	0.064	0.073	0.086	0.107	0.036	0.492	0.040	0.129	0.071	0.089
16:1n-7	0.005	0.004	0.006	0.009	0.087	0.049	0.009	0.718	0.032	0.102	0.006	0.134
18:1n-9	0.038	0.037	0.036	0.089	0.284	0.199	0.045	1.400	0.129	0.406	0.065	0.277
18:1n-7	0.023	0.013	0.023	0.020	0.043	0.031	0.007	0.244	0.029	0.077	0.020	0.042
20:1n-9	0.003	0.002	0.003	0.003	0.011	0.015	0.003	0.085	0.021	0.076	0.002	0.079
22:1n-11 + 13	0.001	0.002	0.000	0.002	0.001	0.006	0.001	0.010	0.003	0.040	0.001	0.095
22:1n-9	0.000	0.000	0.001	0.000	0.003	0.002	0.002	0.011	0.006	0.022	0.000	0.008
18:2n-6	0.002	0.001	0.003	0.006	0.017	0.008	0.004	0.030	0.005	0.019	0.003	0.021
20:4n-6	0.047	0.025	0.030	0.045	0.063	0.032	0.014	0.093	0.018	0.042	0.061	0.020
22:4n-6	0.035	0.026	0.030	0.013	0.015	0.008	0.003	0.060	0.007	0.021	0.006	0.004
20:5n-3	0.013	0.006	0.011	0.007	0.051	0.040	0.014	0.347	0.022	0.060	0.024	0.066
22:5n-6	0.019	0.011	0.010	0.007	0.011	0.015	0.012	0.031	0.009	0.021	0.009	0.009
22:5n-3	0.020	0.010	0.021	0.011	0.037	0.022	0.006	0.153	0.026	0.075	0.015	0.025
22:6n-3	0.112	0.049	0.075	0.052	0.082	0.216	0.104	0.293	0.147	0.284	0.155	0.233

ND = not determined.

[a]For more detailed species identification, see Table 8.2.

[b]Wild catfish, not cultured.

Source: Pocket version of Gooch, J.A., et al. (1987). Proximate and fatty acid composition of 40 southeastern U.S. finfish species, NOAA Tech. Rep. NMFS 54, pp. 1–23, where data are expressed as fatty acid compositions.

TABLE 8.2.
Scientific Names of Fish Listed in Table 8.1

Species (Common Name)	Scientific Name
Bass, Black Sea	*Centropristis striata*
Bluefish	*Pomatomus saltatrix*
Butterfish, Gulf	*Peprilus burti*
Catfish, channel	*Ictalurus punctatus*
Croaker, Atlantic	*Micropogonias undulatus*
Dolphin	*Coryphaena hippurus*
Flounder, southern	*Paralichthys lethostigma*
Goosefish (monkfish)	*Lophius americanus*
Grouper, gag	*Mycteroperca microlepis*
Grouper, yellowedge	*Epinephelus flavolimbatus*
Grunt, white	*Haemulon plumieri*
Harvestfish	*Peprilus alepidotus*
Hind, speckled	*Epinephelus drummondhayi*
Jack, Crevalle	*Caranx hippos*
Kingfish, southern (whiting)	*Menticirrhus americanus*
Ladyfish	*Elops saurus*
Mackerel, chub	*Scomber japonicus*
Mackerel, king	*Scomberomorus cavalla*
Mullet, striped	*Mugil cephalus*
Porgy, red	*Pagrus pagrus*
Rag, Silver	*Ariomma bondi*
Runner, blue	*Caranx crysos*
Scad, rough	*Trachurus lathami*
Seatrout, spotted	*Cynoscion nebulosus*
Shad, American	*Alosa sapidissima*
Shark, Atlantic sharpnose	*Rhizoprionodon terraenovae*
Shark, lemon	*Negaprion brevirostris*
Shark, scalloped hammerhead	*Sphyrna lewini*
Shark, tiger	*Galeocerdo cuvieri*
Sheepshead	*Archosargus probatocephalus*
Snapper, red	*Lutjanus campechanus*
Snapper, vermilion	*Rhomboplites aurorubens*
Spot	*Leiostomus xanthurus*
Tilefish	*Lopholatilus chamaeleonticeps*
Tilefish, blueline	*Caulolatilus microps*
Triggerfish, gray	*Balistes capriscus*
Weakfish	*Cynoscion regalis*

(Ackman, 1989; Vanderstoep et al., 1990). The tuna (albacore, *Thunnus alalunga*) canned in oil is included to make the point that the vegetable oil components dominate the fatty acids in such products, whereas water-packed products have fish fatty acids only. For this reason, Table 8.6 presents data for a variety of canned retail fish products in summary form with 20:5n-3 and 22:6n-3 given in g/100 g. This, of course, has to be defined in terms of "can contents" or "drained weight of solids."

It is necessary to note that trade in fish products is international. For this reason, fatty acids of many Southern European species of fish and shellfish are included in Tables 8.7 and 8.8. Others from Northern Europe (e.g., Sweden; Ackman, 1989) will be found elsewhere (Paul et al., 1980).

TABLE 8.3
Weight Percent of Some Fatty Acids of Special Interest in Redfish Triglycerides and One Phospholipid[a]

	Norwegian Fillet			Canadian	
Fatty Acid	TG Only	PL Only	German, Red Muscle (7%–9% Fat)	Nape TG (13% Fat)	Muscle TG (1.5% Fat)
14:0	6.4	2.6	4.3	5.1	5.6
16:0	14.3	24.9	13.4	13.1	11.9
18:0	1.8	2.5	0.6	2.3	1.9
16:1	7.0	3.2	8.3	13.1	13.8
18:1	21.5	11.9	22.0	18.5	16.5
20:1	10.8	2.7	20.2	11.7	13.7
22:1	9.3	1.3	10.4[b]	15.2	19.9
18:2n-6	1.2	1.1	1.8	0.6	0.6
18:3n-3	0.8	0.4	0.9	0.3	0.2
18:4n-3	2.9	1.3	ND	0.8	0.9
20:4n-6	0.5	2.2	—	0.1	0.1
20:5n-3	10.2	13.8	11.2	9.6	7.4
22:5n-3	1.0	1.3	0.2	0.5	0.2
22:6n-3	6.6	25.6	1.8	4.5	2.4
Total	94.3	94.8	95.1	95.4	95.1

TG = triglycerides; PL = phospholipids. ND = not determined.

[a] Canadian redfish sample from *Sebastes (mantella) marinus.* German samples probably from the closely related Norway haddock *Sebastes viviparus.*
[b] Listed as 20:4n-6.

Source: Adapted from Ackman, R.G. (1976). Fish oil composition. In *Objective Methods for Food Evaluation*, National Academy of Science, Washington, DC, pp. 103–131.

B. Freshwater Fish

Freshwater fish represent a major part of aquaculture production across the globe. Their fatty acids are in some cases similar to those of marine fish, with 20:5n-3 and 22:6n-3 as reasonably important lipid components (Kinsella et al., 1977; Henderson and Tocher, 1987; Ackman, 1989). In other species such as the popular carp (see Table 8.8), there is more emphasis on the C_{18} fatty acids, although the fish diet has an important influence (Pigott, 1989).

The U.S. channel catfish *Ictalurus punctatus* accounts for 45% of all U.S. aquaculture production. Data from a recent thorough analysis (Nettleton et al., 1990) are included in Table 8.9. The mean fat content of the samples was 6.9%, and that for the U.S. Department of Agriculture (USDA) samples was 4.3%. On or above 2% or 3% fat, it is not unreasonable to convert this directly from g/100 g to weight percent fatty acid data, although more accurate conversions are possible (Exler et al., 1975). The species peculiarity of the high 18:1 is confirmed by the wild catfish sample of Table 8.1, but the 20:5n-3 and 22:6n-3 may reflect the fish meal component in the diet of the farmed fish. The carp from Italy (Table 8.8) is a noncarnivorous species with similar flesh characteristics. Coldwater fish such as the chub (*Coregonus zenithicus*) and whitefish (*Coregonus clupeaformis*), with 13.2% and 7.2% lipid, respectively, are more apt to be better sources of the 20:5n-3 and 22:6n-3 (Wang et al., 1990).

TABLE 8.4
Lipid Analysis: Amount of Total Lipid, EPA, DHA, and Total n-3 Acids in Seafood Available from Nova Scotia Sources

Seafood	Total Lipid (g/100 g)	EPA (20:5n-3) (g/100 g)	DHA (22:6n-3) (g/100 g)	Total n-3 (g/100 g)
Groundfish				
Cod	0.73	0.08	0.23	0.32
Cusk	0.67	0.03	0.09	0.13
Haddock	0.59	0.07	0.14	0.23
Hake, white	0.69	0.05	0.22	0.30
Halibut	2.04	0.16	0.22	0.45
Monkfish	0.42	0.03	0.07	0.11
Pollack, bluefish	1.10	0.08	0.18	0.26
Redfish, ocean perch	1.15	0.14	0.07	0.21
Turbot	10.0	0.34	0.28	0.84
Winter flounder, sole	0.45	0.07	0.12	0.22
Wolffish, catfish	0.59	0.11	0.06	0.20
Pelagic, estuarial				
Capelin	1.78	0.21	0.19	0.31
Eel, frozen	14.2	0.30	0.66	1.13
Gaspereau	3.49	0.10	0.27	0.51
Herring	12.0	1.05	1.29	3.12
Mackerel	20.6	1.45	2.16	5.10
Mackerel, smoked	21.2	1.47	2.30	5.25
Salmon, farm-reared	8.33	0.25	0.73	1.28
Smelt	1.2	0.12	0.18	0.32
Sturgeon	7.20	1.40	0.57	2.56
Swordfish, frozen	11.4	0.14	0.44	0.90
Trout, farm-reared	3.90	0.12	0.50	0.72
Mollusks, crustaceans				
Clam, surf	1.40	0.14	0.21	0.38
Crab, jonah	1.10	0.28	0.10	0.38
Crab, queen	0.75	0.20	0.09	0.30
Crab, rock	1.20	0.27	0.15	0.42
Lobster	1.20	0.24	0.12	0.38
Mussel	1.27	0.13	0.17	0.36
Oyster, American	1.60	0.11	0.09	0.29
Quahog, bay	0.62	0.11	0.09	0.23
Quahog, ocean	0.80	0.09	0.08	0.20
Scallop	1.00	0.19	0.18	0.44
Shrimp	2.43	0.28	0.16	0.50
Squid	2.00	0.27	0.64	0.93

C. Shellfish and Crustacea

Shellfish (Table 8.10) include both molluscs and crustaceans. Although phospholipids and triglycerides are included in Table 8.10, these are highly variable in proportions as the molluscs store glycogen for energy, not triglycerides. It is better to take the total animals in such cases, and the lipid contents are in the 0.5%–2.5% range. In crustaceans (lobster, crab, and shrimp), the edible parts are low in fat, 0.5%–1.5%, but in both types, a distinguishing feature is that 20:5n-3 ≥ 22:6n-3. It is worth recording that the shrimp has approximately 150 mg of cholesterol per 100 g (Krzynowek and Panunzio, 1989) but crab and lobster have only about 75 mg, and in edible muscle, the bivalves

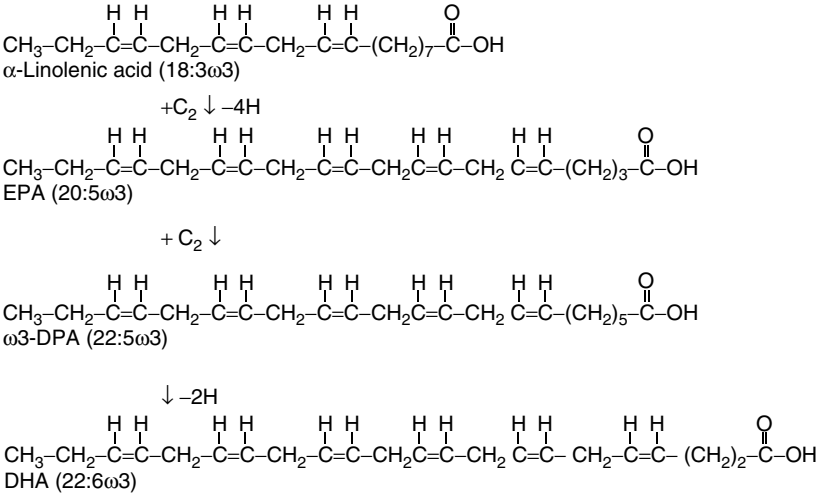

FIGURE 8.1 Principal fatty acids of the n-3 or linolenic acid (18:3n-3) family. Linolenic acid itself is a minor acid in marine animal fats, but the successor acids with five and six ethylenic bonds are two of the fatty acids most characteristic of marine animal fats and lipids. They have acquired considerable popular status as the "omega-3" fatty acids EPA and DHA.

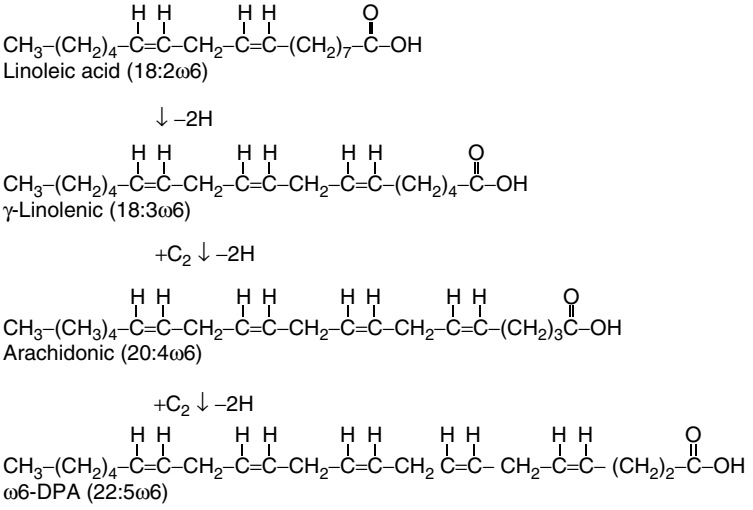

FIGURE 8.2 Condensed scheme of the elongation of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) and then to a DPA (22:5n-6). Note the retention of the basic n-6 ethylenic bonds, which establishes the "omega-6" relationship among these fatty acids.

usually have only about 35–45 mg (i.e., 40%–50% of total sterols). The most recent paper on this topic is that of King et al. (1990), but some other data are included in Ackman (1989). Northwest Pacific species are reviewed in some detail by Gordon (1982).

D. Australasia and the Tropics

The 1989 review of Ackman focuses on Australasian semitropical and tropical species, which have more 20:4n-6 than is common in coldwater marine species (Table 8.11). This extra 20:4n-6 in the diet may be of minor importance in most cases, but the view of Weber (1989) that the blood level of

TABLE 8.5
Weight Percent of Some Fatty Acids of Special Interest in Some Large-Volume Commercial Fish

Fatty Acid	Salmon Fillets		Herring Fillet	Pacific Salmon (Pink), Canned	Albacore Tuna (Solid White, Canned in Oil)
	Atlantic	Pacific			
14:0	5.0	6.0	6.1	5.6	0.2
16:0	15.9	19.2	10.8	14.0	11.4
18:0	2.5	3.3	1.4	2.9	4.0
16:1	6.3	6.2	7.3	5.2	0.2
18:1	21.4	17.3	10.3	12.7	21.0
20:1	10.6	3.3	13.4	13.9	0.4
22:1	8.7	6.6	21.3	11.1	0.2
18:2n-6	1.1	1.2	1.0	1.6	51.4
18:3n-3	0.6	1.3	2.0	1.3	7.3
18:4n-3	0.6	2.6	3.2	2.6	—
20:4n-6	0.5	1.3	trace	0.7	0.3
20-5n-3	4.6	8.2	7.5	9.0	0.2
22-5n-3	1.9	2.0	0.8	2.4	0.1
22:6n-3	11.9	12.5	6.8	14.1	1.6
Total	91.6	96.3	91.9	98.1	98.3

Source: Adapted in part from Ackman, R.G. (1976). Fish oil composition. In *Objective Methods for Food Evaluation*, National Academy of Science, Washington, DC, pp. 103–131; and Polvi, S.M. (1989). Diet and availability of omega-3 fatty acids in salmonids. MSc Dissertation, Technical University of Nova Scotia, Halifax, pp. 1–221.

selected acids such as 20:4n-6 and 20:5n-3 correlates with cardiovascular mortality requires that it be considered. Fortunately, these fish, by and large, are very lean (Evans et al., 1986; Brown et al., 1989; Childress et al., 1990) and contribute little fat to the diet.

Nevertheless, in view of the brief report that Japanese heavy fish eaters in Hawaii did not show a reduction in cardiac mortality compared to those who ate less fish (Curb and Reed, 1985), this imbalance in preformed long-chain n-6 vs. long-chain n-3 fatty acids may be important in total fish consumption. The most recent data for Queensland, Australia, is given in Table 8.12. In the totals for long-chain n-6 fatty acids, arachidonic acid (20:4n-6) is one-half to two-thirds of the figure presented for n-6. Linoleic acid was in the 1%–2% range of the total fatty acids, and 20:3n-6 was even less, so attention should focus on the three long-chain n-6 fatty acids in Table 18.2. The latitude in Australia is approximately the same as for Table 8.11; the data shows that it is the important factor in this brief comparison of fatty acids of warm water marine fish.

III. OILY FISH AND HEALTH CONSIDERATIONS

Among the numerous books dispensing advice, recipes, and fish composition data are those of Kinsella (1987) and Nettleton (1985, 1987). Although other information is available, it is indisputable that fish is in all respects a healthy food. Many people do not eat *any* fish (Must et al., 1988), and the consumer does not always understand quality considerations (Hadlett and Raab, 1990). Despite this, the evidence associating increased fish consumption with good circulatory health increased steadily (Leaf and Weber, 1988; Burr et al., 1989; Kinsella et al., 1990) after the results of the 20-year study in Zutphen were published (Kromhout et al., 1985). Biomedical citations on the topic regularly number more than 500 per year (Patrias, 1990).

TABLE 8.6
Lipid, EPA, DHA, and Cholesterol Contents in the Solids of Canned Fish Purchased Retail in Canada

Sl. No.	Sample and Number Cans in Sample	Gross Weight of Can and Contents (g)	Drained Weight of Solids (g)	Volume of H_2O (mL)	Volume of Oil (mL)	Lipid (g/100 g)	EPA (g/100 g)	DHA (g/100 g)	Cholesterol (mg/100 g)
1	Albacore solid white tuna in water, imported (2)	254	159	ND	ND	2.9	0.19	0.62	17.4
		256	154	ND	ND	2.8	0.18	0.57	14.2
2	Albacore solid white tuna in vegetable oil, imported (2)	261	158	42	7	5.7	0.29	0.84	11.4
		250	152	40	10	5.3	0.22	0.74	13.2
3	Flaked light tuna in vegetable oil, imported (2)	244	156	38	3	4.6	0.03	0.23	15.7
		246	145	44	7	7.7	0.03	0.20	11.8
4	Flaked light tuna in water, imported (2)	245	147	46	—	0.8	0.02	0.17	19.7
		246	150	48	—	0.7	0.02	0.15	26.0
5	Canned mackerel fillets (smoked), Canadian (2)	243	185	10	20	15.3	0.84	1.34	47.3
		210	155	5	23	16.7	0.90	1.42	438
6	Canned kipper snacks, fillets of smoked herring (2)	239	157	48	4	9.7	0.35	0.63	21.9
		238	155	50	3	8.5	0.26	0.44	16.0
7	Canned kipper snacks, herring fillets—wood-smoked flavor, Canadian (1)	116	71	22	3	9.0	0.39	1.13	15.1
8	Canned herring fillets in tomato sauce (imported) (1)	224	135	58	2	10.3	0.40	0.74	15.1

ND = Not determined, for abbreviations, see Table 8.4.

Source: Reproduced by permission from the Province of Nova Scotia.

TABLE 8.7
Weight Percentages of Some Fatty Acids of Mediterranean Marine Animal Lipids[a]

Fatty Acid	Ghiozzo (Goby)	Rondinella (Flying Fish)	Cefalo (Mullet)	Passera (Flounder)	Pagaro (Porgy)	Nasello (Hake)	Scorfano (Scorpion-Fish)	Buga (Bogue)	Cappone Imperiale (Gurnard)	Orata (Bream)	Tracina (Weaver)	Triglia (Mullet)	Suro (Scad)	Seppia (Sepia)	Calamaro (Squid)	Polpo (Octopus)
14:0	2.96	5.48	1.32	2.87	3.14	3.62	2.33	3.45	2.42	2.48	2.73	2.06	3.64	2.43	2.62	0.98
14:1	0.42	0.30	0.49	0.62	0.35	0.34	0.30	0.16	0.17	0.17	0.11	0.40	0.48	0.20	0.08	0.07
15:0	1.05	1.30	2.63	1.36	1.18	1.77	0.75	1.18	0.94	0.66	0.82	0.95	0.69	0.96	0.98	0.53
16:0	15.38	17.74	13.78	13.98	14.51	15.99	20.60	20.38	26.48	18.96	17.78	16.34	16.15	14.48	20.42	16.03
16:1	9.99	9.10	13.82	8.54	8.00	5.59	7.19	10.54	4.69	9.46	8.11	6.33	8.41	4.05	2.23	2.05
17:0	1.14	1.72	5.76	2.22	2.16	2.27	1.43	3.25	2.16	0.93	1.61	2.03	1.43	1.99	1.82	1.82
18:0	5.90	7.15	7.22	4.89	7.24	5.30	10.09	8.01	12.91	8.66	7.94	8.04	8.62	8.58	6.66	6.45
18:1	23.74	14.61	10.32	16.80	16.97	12.44	17.80	24.52	16.47	32.39	24.84	14.99	22.03	9.11	6.94	5.60
18:2n-6	3.27	1.62	1.91	2.64	1.86	2.16	1.44	1.03	1.39	2.86	2.33	1.75	2.09	1.20	0.67	0.64
18:3n-3	0.24	0.63	0.82	0.39	0.64	0.50	0.33	0.45	0.38	0.46	0.40	0.44	0.53	0.34	0.13	0.24
20:1	2.56	3.15	1.18	6.25	3.24	1.50	1.58	1.94	1.05	4.09	2.75	2.55	2.85	4.06	2.96	3.06
18:4n-3	0.95	0.79	1.66	1.03	0.91	1.09	0.29	0.52	0.39	1.70	0.83	0.49	1.02	0.22	0.15	0.08
20:2n-6	0.11	0.19	0.53	0.79	0.34	0.20	0.36	0.20	0.16	0.14	0.18	0.45	0.25	0.37	0.28	0.34
Unknown	0.03	0.08	0.05	0.10	0.03	—	0.03	—	0.12	—	—	0.02	0.03	0.04	0.03	—
20:3	0.25	0.26	0.21	0.20	0.33	0.11	0.28	0.10	0.43	0.30	0.10	0.37	0.32	0.33	0.16	0.12
20:4n-6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
or 22:1	4.07	3.37	3.59	2.60	4.30	1.97	7.71	2.93	3.37	3.00	3.31	6.81	2.79	3.22	3.22	6.67
20:4n-3	0.59	0.50	0.53	1.20	0.77	0.37	0.42	0.22	0.26	0.88	0.59	0.54	0.52	0.42	0.20	0.23
20:5n-3	11.65	8.05	16.50	14.78	7.48	6.75	5.45	7.55	4.92	4.91	5.25	7.88	9.71	19.94	14.57	13.75
22:4n-6	1.44	1.58	0.85	2.33	2.39	1.66	3.39	1.13	1.87	1.22	2.07	2.80	1.50	1.05	1.01	1.42
22:5n-6	0.55	0.96	0.61	0.61	1.33	1.58	1.39	0.23	0.84	1.02	0.78	1.36	0.62	0.55	1.12	0.71
22:5n-3	3.81	3.33	2.75	4.11	2.29	0.96	1.48	3.12	0.74	2.11	1.89	2.41	2.42	2.60	0.80	1.60
22:6n-3	5.83	13.75	5.75	6.91	17.04	30.98	12.06	5.99	13.82	1.89	13.22	17.78	11.77	20.90	30.44	21.57

[a]Approximately equivalent English/American names are given in parentheses for fish cited in original publication. The scientific names are as follows: Ghiozzo, *Gobius cobitis*; rondinella, *Cypsilurus rondeleti*; celalo, *Mugil cephalus*; passera, *Platichtys flesus flesus*; pagaro, *Pagrus pagrus*; nasello, *Merluccius merluccius*; scrofa, *Scorpaena scrofa*; buga, *Boops boops*; cappone imperiale, *Aspitrigla cuculus*; orata, *Sparus auratus*; tracina, *Trachinus draco*; triglia, *Mullus surmuletus*; suro, *Trachurus trachurus*; seppia, *Seppia officinalis*; calamaro, *Loligo vulgaris*; polpo, *Octopus vulgaris*.

Source: Forneris, G., Guidetti, L.M., and Sarra, C. (1981). *Riv. Soc. Ital. Sci. Aliment. 10*: 155–158.

TABLE 8.8
Weight Percentages of Some Fatty Acids of Italian Freshwater Animal Body Lipids[a]

Fatty Acid	Savetta	Salmerino (Char)	Anguilla (Eel)	Cavedano	Pesce gatto (Catfish)	Alborella (Chub)	Luccio (Pike)	Cobite (Roach)	Carpa (Carp)	Persico reale (Perch)	Lasca	Padogobio	Trota iridea (Rainbow trout)	Scardola (Rudd)
14:0	2.31	2.40	4.18	2.66	1.27	3.00	1.43	1.56	2.40	2.54	3.78	1.52	2.02	2.30
14:1	0.24	1.44	0.34	0.40	0.50	1.30	0.16	1.32	1.04	0.49	0.56	0.36	0.14	1.02
15:0	2.41	0.18	0.64	0.36	0.67	0.50	0.76	1.10	1.23	0.78	0.43	0.39	0.28	0.83
16:0	12.91	17.16	19.56	16.81	16.98	17.09	18.20	15.10	19.39	18.45	15.83	16.18	17.83	19.54
16:1	19.18	21.33	12.7	12.55	7.28	14.87	7.79	21.09	10.05	12.14	16.51	11.51	6.12	11.36
17:0	0.40	0.66	1.10	0.55	1.09	0.35	0.97	0.60	1.91	1.00	1.10	0.97	0.64	1.18
17:1	4.31	0.91	1.17	1.27	1.48	1.56	1.05	1.14	1.07	1.12	2.42	1.57	0.64	0.90
18:0	2.19	2.58	3.02	3.72	6.66	5.52	4.70	4.01	6.65	4.05	4.94	5.94	4.83	5.12
18:1	22.91	19.51	32.62	25.01	19.05	25.64	14.17	17.30	14.22	20.76	24.50	21.25	32.45	26.25
18:2n-6	2.67	6.21	1.38	10.38	8.02	8.02	4.71	3.72	5.62	5.53	3.66	5.60	14.33	4.83
18:3n-3	0.62	0.06	0.32	0.52	0.43	0.26	0.30	0.20	0.09	0.18	0.31	0.22	0.31	0.31
20:1	5.55	2.87	2.90	6.43	4.03	3.30	4.27	2.21	8.77	5.18	3.32	3.45	2.63	5.80
18:4n-3	1.19	0.57	0.61	2.78	0.76	0.44	1.04	1.83	1.49	0.86	1.43	1.03	0.35	1.49
20:2n-6	0.66	0.14	0.42	0.70	0.44	0.39	0.43	0.51	0.41	0.14	0.15	0.43	0.67	0.62
20:3	0.22	0.15	0.13	0.31	0.58	0.52	0.54	0.54	0.27	0.11	0.55	0.32	0.62	0.29
20:4n-6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
or 22:1	2.44	2.27	1.77	1.85	7.13	2.45	5.69	5.11	6.33	3.61	1.91	5.32	1.29	3.78
20:4n-3	0.76	0.28	0.63	0.96	0.45	0.28	1.19	0.67	1.09	0.44	0.67	0.58	0.51	0.78
20:5n-3	8.70	10.28	4.25	4.19	7.02	5.57	5.83	8.61	6.19	5.63	7.22	11.26	1.97	3.87
22:4n-6	0.30	0.28	0.68	0.23	1.41	0.22	0.81	0.85	0.87	0.55	0.42	1.23	0.27	0.97
22:5n-6	0.30	0.28	0.68	0.23	1.41	0.22	0.81	0.85	0.87	0.55	0.42	1.23	0.27	0.97
22:5n-3	2.47	2.07	3.04	2.37	3.97	1.38	2.84	3.78	1.79	5.92	2.76	4.88	0.80	1.56
22:6n-3	4.46	7.44	5.96	5.32	8.13	5.34	18.58	5.70	7.25	8.05	5.65	2.08	10.28	6.55

[a]Names of animal bodies given in Italian, approximately equivalent English names in parentheses; scientific names are as follows: Savetta, *Chondrostoma soetta*; salmerino, *Salvellinus alpinus*; anguila, *Anguila anguila*; cavedano, *Leuciscus cephalus*; pesco gatto, *Ictalurus melas*; alborella, *Alburnus alburnus*; luccio, *Exos lucius*; cobite, *Cobitis taenia*; carpa, *Ciprinus carpio*; persico reale, *Perca fluviatalis*; lasca, *Chondrostoma toxostoma*; padogobio, *Padogobius martensi*; trota iridea, *Salmo gairdneri* (now *Onchorynchus mykiss*); scardola, *Scardinius erythrophthalmus*.

TABLE 8.9
Weight Percentages of Some Fatty Acids of Special Interest in Oils and Lipids from Muscle of Freshwater Fish

	Catfish						
Fatty Acid	Minimum[a]	Maximum[a]	Nettleton et al. (1990) Mean	USDA[b]	Eel[b]	Chub[c]	Whitefish[c]
14:0	1.0	2.3	1.4	1.5	5.9	2.9	3.4
16:0	15.2	22.2	18.3	19.2	14.0	12.1	12.3
18:0	3.9	9.3	4.0	5.9	1.4	1.8	1.6
16:1	2.9	5.6	4.6	4.6	12.4	6.3	8.9
18:1	29.7	49.7	50.0	38.3	27.8	33.5	24.0
20:1	0.9	2.0	1.3	1.5	28.3	1.7	0.9
22:1	ND	ND	0.2	0.1	Trace	ND	ND
18:2n-6	10.0	15.7	12.0	10.9	1.0	7.5	9.2
18:3n-3	0.5	2.9	0.9	1.1	Trace	8.0	10.5
18:4n-3	0.4	1.0	0.1	0.8	ND	1.9	3.0
20:4n-6	0.8	5.5	0.5	2.8	0.5	2.2	2.5
20:5n-3	0.2	2.5	0.4	3.6	0.5	5.4	6.6
22:5n-3	0.2	1.3	0.6	1.7	0.8	1.8	2.0
22:6n-3	0.6	6.1	1.2	6.8	0.5	5.9	7.6

ND = not determined.

[a] From Ackman (1976).
[b] Agriculture Handbook. 8–15.
[c] From Wang et al. (1990).

TABLE 8.10
Weight Percentages of Some Fatty Acids of Special Interest in Total Lipids of Crustacean and Molluscan Meats and in a Specific Triglyceride and Phospholipid Source

	Crab			Oyster		Ocean quahaug		Pacific
Fatty Acid	King	Queen	Shrimp, Alaska	American	European[a]	TG	PL	Scallop[b]
14:0	1.4	0.5	2.5	3.5	5.2	3.9	1.1	1.8
16:0	9.2	14.0	16.0	28.9	22.4	23.6	19.6	19.3
18:0	4.3	2.4	2.6	3.6	5.2	5.0	9.0	6.3
16:1	5.0	5.9	5.8	4.2	3.4	7.8	3.1	2.2
18:1	15.0	21.7	19.0	8.2	6.9	11.7	7.5	5.0
20:1	3.5	2.6	2.4	5.0	3.4	5.3	2.8	0.8
22:1	3.9	0.6	1.6	0.3	3.4	0.1	ND	ND
18:2n-6	3.2	0.7	1.5	2.0	1.7	0.7	0.3	0.1
18:3n-3	3.3	0.2	1.4	3.3	—	0.2	2.2	1.2
18:4n-3	2.3	0.1	1.0	2.6	5.2	1.7	ND	2.2
20:4n-6	0.6	3.9	0.4	2.3	1.7	0.7	1.1	3.4
20:5n-3	21.5	30.5	22.0	11.2	17.0	20.1	13.8	26.0
22:5n-3	1.4	0.9	1.2	0.3	1.7	0.4	ND	0.6
22:6n-3	10.2	13.3	16.0	9.7	11.1	5.1	9.7	24.1

ND = not determined.

[a] From Paul et al. (1980).
[b] From King et al. (1990).

Source: Ackman, R.G. (1976). Fish oil composition. In *Objective Methods for Food Evaluation*, National Academy of Science, Washington, DC, pp. 103–131, except as specified.

TABLE 8.11
Some Typical Fish Fat Contents and Partial Fatty Acid Compositions for Fish from the Northwest Shelf of Australia

			Fatty Acid		
Common Name	**Species Name**	**% Lipid**	**20:4n-6**	**20:5n-3**	**22:6n-3**
Northern pearl perch	*Glaucosoma burgeri*	0.8	4.9	4.7	30.8
One-band sea perch	*Lutianus vittus*	1.0	4.4	3.8	31.4
Yellow-tailed angelfish	*Chaetodontoplus personifer*	1.2	14.3	2.3	11.8
Tropical halibut	*Psettodes erumei*	0.7	7.9	2.5	32.3

Source: Adapted from Evans et al. (1990).

TABLE 8.12
Lipid Content and Fatty Acid Analysis (Weight Percent) of Some Queensland (Australia) Fish

Common Name	**Scientific Name**	**Lipid %**	**Total Saturates**	**Total Mono-unsaturates**	**Total Poly-unsaturates**	**Long-Chain n-6[a]**	**Total n-3[b]**
Collared sea bream (Iodine bream)	*Gymnocranius audleyi*	2.7	31.1	18.1	41.5	14.5	22.5
Sweetlip emperor	*Lethrinus miniatus*	2.1	32.8	18.6	41.0	13.6	21.5
Venus tuskfish	*Choerodon venustus*	1.8	31.1	15.8	44.6	14.3	25.2
Chickenwire cod	*Epinephelus merra*	1.6	31.6	17.1	42.9	14.6	23.0
Yellowtail bream	*Acanthopagrus australis*	0.8	28.6	13.9	46.4	18.2	24.9
Longfin rock cod	*E. quoyanus*	0.5	30.5	15.8	45.3	13.0	27.0
Coral trout	*Plectroponus leopardus*	2.4	36.8	19.6	37.8	6.4	27.7
Red emperor	*Lutjanus sebae*	1.6	31.8	17.9	43.0	10.4	28.7
Parrot fish	*C. albigera*	1.3	29.5	13.7	46.5	17.8	29.9
Hussar Yellow stripey	*Lu. adetii*	4.9	33.5	22.1	38.3	5.7	28.8
Spanish flag	*Lu. carporonatus*	1.3	33.8	20.4	39.9	6.7	29.1

[a] Does not include 18:2n-6 or 18:3n-6.
[b] Includes 18:3n-3.

Source: Adapted from Belling, G.B., et al. (1997). *Lipids 32*: 621–625.

In the past decade, there have been several papers published in the medical literature stating even more clearly that fish consumption reduces the risk of *sudden* cardiac death (Shekelle and Stamler, 1993; Ascherio et al., 1995; Siscovick et al., 1995; Norday, 1996; Daviglus et al., 1997; Albert et al., 1998), and this term has been debated because of the impact of cardiac arrhythmia (Kang and Leaf, 1996) as a significant part of the mortality problem (Albert et al., 1998).

In addition, a diet rich in fish seems to prevent stroke (Keli et al., 1994). There is not enough space here to consider the other pathological conditions that may be influenced by dietary fish fats (Simopoulos, 1991, 1997), but papers continue to consider matters as diverse as asthma (Broughton et al., 1997) and perinatal neural development (Crawford, 1993). The concern here is the availability in our diets of the necessary long-chain fatty acids EPA, docosapentaenoic acid (DPA), and DHA shown in Figure 8.1. In an official report of the U.K. Cardiovascular Review Group Committee on

Medical Aspects of Food Policy (United Kingdom Department of Health, 1994) several recommendations stand out:

R.2.2 We recommend no further increase in average intakes of n-6 PUFA, and we recommend that the proportion of the population consuming n-6 PUFA in excess of about 10% of energy should not increase.

R.2.3 We recommend an increase in the population average consumption of long-chain n-3 PUFA from about 0.1 g/day to about 0.2 g/day (1.5 g/week).

R.3.1 We recommend that people eat at least two portions of fish, of which one should be oily fish, weekly.

This committee considered the competitive effects of plant n-6 and n-3 fatty acids. Here it is sufficient to say that there has been a severe imbalance in our intake of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). Holman (1997, 1998) has coined the term *omega-6-induced omega-3 deficiency* to describe the resulting lack of conversion of 18:3n-3 to the fish oil type of long-chain n-3 fatty acids. The now excessive Japanese intake of 18:2n-6 is discussed in detail by Okuyama et al. (1997) and in condensed form by Sugano (1996), who recommended that the plant (C_{18}) essential fatty acid dietary supply be in the proportion of 4:1 for n-6:n-3. A problem among the Japanese is that the traditional diet rich in fish and shellfish has now shifted to the Western diet and EPA, DPA, and DHA are now less common. The traditional Japanese diet was highly diversified in terms of seafood, but what about our Western diets? What oily fish were available and acceptable? Part of that question can be answered by Table 8.13. In 1848, fresh, salted, or smoked herring was a cheap food in the United Kingdom. Pilchards, mackerel, and salmon were also part of this diet. The much leaner "whitefish" such as cod, haddock, and pollack were less plentiful than later when steam trawlers were developed and sailed out as far as Iceland.

Burr et al. (1989) were responsible for introducing the term and recommending the consumption of *oily* fish and have expanded on that concept and the results. Salmon has a history of being thoroughly evaluated in a clinical context; for example, by Connor et al. (1983), by Nelson et al. (1991), and by Wander and Patton (1991). Gerhard et al. (1991) compared biochemical effects in humans of diets high in three Pacific fish species: Dover (*sic*) sole (lean), chincook salmon (mid range for fat), and sablefish (a fish high in fat but relatively lower in EPA and DHA). As expected the two "oily" fish produced more improvement in cardiovascular risk factors than the leaner sole. However, the latter showed improvement over the regular diet of the subjects. Even in Central Africa the availability of freshwater fish to one population group compared to a different diet group has produced similar results, illustrating that any fish is better than no fish (Pauletto et al., 1996).

The question usually asked by well-informed consumers interested in changing their diets is whether aquaculture (farmed) fish are as "good" as naturally harvested fish. Bell et al. (1998) have

TABLE 8.13
Estimates of Changes between 1848 and 1978 in *per capita* Consumption of Fish in the U.K. and of EPA and DHA in Grams/Week

	1848	1978
Oily fish	137	22
White fish	44	100
EPA	1.7	0.2
DHA	1.2	0.2

Source: Adapted from Ackman, R.G. (1988).

examined the muscle from stocks of Atlantic salmon farmed in Scotland. Doubling the dietary fat content from 16% to 30% in diets fed to these salmon increased the flesh lipid from about 8% to about 11% depending on the edible part examined. The percentages of EPA and DHA in the total fatty acids of the muscle lipid averaged between 5.6% and 11.3%, respectively, which is similar to their analysis of wild Atlantic salmon (3.5% lipid, EPA 5.9%, DHA 12.9%). This comparison shows the fallacy of merely looking at the percentage of fatty acids, and in this species, at least, the farmed salmon is the better source of omega-3 fatty acids. Some vegetable products such as canola oil can give the lipids of farmed salmon increased linoleic acid (18:2n-6) in the total fatty acids, but the wild salmon examined by Bell et al. (1998) had only 1.2% of this fatty acid, a figure common in muscle lipids of coldwater marine fish. A new technology, x-ray tomography, has been applied to determine the fat in Atlantic salmon flesh (Bjerkeng et al., 1997). High-energy (i.e., high fat) diets with 32% and 39% fat gave only marginal differences in whole body fat (15.6 vs. 17.7%), suggesting that the fish diet is overloaded if maximizing fat were an objective of the dietary difference. Recently, Aursand et al. (1997) could also give the n-3 fatty acid content through quantitative nuclear magnetic resonance (NMR) technology. Some consumers worry about quality or contamination, but the North Atlantic salmon aquaculture industry is constantly improving quality control (Sigurgisladottir et al., 1997). Lipids are less likely to be part of the quality image than bacterial or protein-related changes in unfrozen fresh fish. For example, the gilthead seabream *Sparus aurata* had 7.69 g of fat/100 g flesh, and 5%–10% of this fat was hydrolyzed to free fatty acids in 24 days on ice. The thiobarbituric acid (TBA) index increased only slightly in this period, from 0.67 to 1.07, so rancidity was not a problem in the shelf life of this species (Kyrana et al., 1997). The free fatty acids, probably from phospholipids, would also be completely available and digestible. In frozen storage, similar processes are very slow in lean fish such as cod (Hardy et al., 1979) and are less obvious in oily fish (Polvi et al., 1991), although some oily fish such as Atlantic mackerel (*Scomber scombrus*) eventually can go rancid if tocopherol is depleted (Ackman and Gunnlaugsdottir, 1992; Bhuiyan et al., 1993; Ackman and Timmins, 1995). Only fish such as Atlantic salmon show to some degree changes in muscle fatty acids with changes in diet. Table 8.14 shows that most phopholipids in farmed fish muscle resist changes from dietary fatty acids, and that the depot fat triacylglycerols are only moderately responsive to extreme changes in dietary fats. This is because each species of fish has some typical composition factors affecting triacylglycerols that could be called "species specific." In the United States, menhaden oil (from *Brevoortia tyrannus*) EPA usually exceeds DHA, and menhaden oil or meal are extensively used in farmed fish product diets as well as for, for example, fowl and pigs. However, the U.S. channel catfish (*I. punctatus*) does not respond with increased EPA in the depot fat (see Table 8.9). It is unfortunate that this freshwater species may be included in U.S. fish consumption statistics, since conclusions drawn for fish are often thought to be for marine species. Despite a fat content of 6%–7%, the amount of n-3 fatty acids is very low (i.e., roughly 100 mg/100 g fish). The term *oily* fish must therefore be used with care.

Seals are omnivores, eating fish or invertebrates, and they accumulate fish oil fatty acids in their depot fat. Even seal oil tends to be species specific in fatty acid composition, possibly reflecting diet, but seal oil can be regarded as a high-quality "biofiltered" fish oil safe for human consumption (Ackman, 1997), and it has joined other encapsulated oils rich in omega-3 fatty acids for those who do not like fish but wish to partake of the benefits of fish as a healthy food. The n-3 DPA (22:5n-3) of seal oil may be unexpectedly potent and beneficial in the biochemistry of the blood vessel wall (Kanayasu-Toyoda et al., 1996). Seal oil is richer in this DPA than are most fish oils.

There is increasingly some confusion over "omega-3" fatty acids because of aggressive health claims in the marketing of flaxseed (linseed, *Linum usitatissimum* L.) oil and its products. The oil contains more than 50% of 18:3n-3 or α-linolenic acid. This has only recently been accepted as truly an "essential" fatty acid in C_{18} terminology (Holman, 1998). Even by 1992 (see British Nutrition Foundation Report, 1992), it was not considered as important as linoleic acid (18:2n-6). Although both are important in our diets (Ackman and Cunnane, 1991), public awareness of the benefits of consuming fish and shellfish for preformed and preferred EPA and DHA suffers from this public relation problem. Only recently (Leaf et al., 1995; Garland et al., 1998) has there been research

TABLE 8.14
Weight Percentage Fatty Acid Compositions of Total Phospholipids and Triacylglycerols in Muscle of Atlantic Salmon Fed Four Experimental Diets Supplemented with One of Herring Oil, Canola Oil, EPA/DHA Concentrate, or Egg Lipid

	Phospholipds				Triacylglycerols			
Fatty Acid	Herring Oil	Canola Oil	EPA/DHA Conc.	Egg Lipid	Herring Oil	Canola Oil	EPA/DHA Conc.	Egg Lipid
14:0	3.3	1.6	2.1	1.6	8.7	2.7	4.8	3.3
16:0	16.0	12.1	13.9	12.8	18.6	13.0	15.9	14.3
18:0	3.0	1.9	2.8	2.7	2.8	2.9	2.4	2.4
16:1n-7	5.4	1.8	3.3	3.4	9.5	3.0	5.7	5.9
18:1n-9	10.6	25.1	9.2	25.5	13.5	41.1	9.3	40.6
18:1n-7	2.0	2.8	1.1	2.3	2.7	2.2	1.8	3.2
Σ20:1	5.4	2.1	2.4	2.3	12.4	4.5	4.3	5.0
Σ22:1	2.8	0.7	1.0	0.9	11.9	2.7	3.7	3.6
ΣSat + mono	49.2	48.2	35.8	52.5	80.0	72.1	47.9	78.3
18:2n-6	4.5	11.2	4.9	7.7	7.6	16.8	9.4	12.6
18:3n-3	0.9	3.5	0.7	0.7	1.0	4.4	1.1	0.7
18:4n-3	1.3	1.3	2.5	0.5	1.3	1.3	3.2	0.6
20:4n-6	0.8	1.3	0.7	3.7	0.3	0.3	0.6	1.0
20:5n-3	6.8	3.5	8.6	2.6	2.5	0.8	13.2	0.9
22:5n-6	0.3	0.4	0.4	1.7	<0.1	0.1	0.3	0.3
22:5n-3	1.9	1.9	2.8	1.3	1.0	0.4	2.7	0.6
22:6n-3	33.6	27.2	42.6	30.2	5.5	2.9	19.2	4.2
Others	0.7	0.0	1.0	0.1	0.8	0.4	2.4	0.8

Source: Adapted from Polvi, S.M., and Ackman, R.G. (1992). *J. Agric. Food Chem. 40*: 1001–1007.

directed to assessing body depot fat acid status and the relationship to longer-chain dietary fatty acids of the n-6 and n-3 types, as well as to those circulating throughout the body (Bjerve et al., 1993).

Even greater confusion is created by the increasing sale and restaurant use of surimi products. Although the protein is still nutritionally valuable, the lipids may be reduced by processing. Analyses of finfish surimi in USDA Handbook 8-15 (now found under USDA Nutrient Database for Standard Reference, Release No. 12, at http://www.nal.usda.gov/fnic/foodcomp/) do not indicate the content of EPA + DHA. Imitation Alaska king crab, on the other hand, had 1.3% lipid and a total of 0.6% EPA + DHA. Excellent reference sources for most considerations of fish handling, composition, and quality will be found in Martin and Flick (1990), Burt et al. (1992), and Ruiter (1995).

IV. FISH AS FOOD

The seminal research reported by Dyerberg and Bang (1979) caused a sensation in the fatty acid world, and reports by Dyerberg (1981) and Dyerberg et al. (1981) enhanced the acceptability of longer-chain omega-3 fatty acids of marine origin. Later in an international meeting in London, United Kingdom, Ackman (1988b) reviewed "The Year of the Fish Oils" in 1987, suggesting the maturation stage of a new science. The enormously productive research in medicine in the next decade prompts an update mainly of 1990 and on as a decade of progress, and the controversy that has sprung up about fish as food during this decade. Owing to wider implications, some revision in the viewpoints is needed as being solely concerned with public acceptance of fish as food.

A. Omega-3 Fatty Acids and Health

It has to be recognized that the originator of "lies, damned lies, and statistics," Benjamin Franklin, was a far-sighted person. In a recent review article, Hooper et al. (2006) concluded that "Long-chain and shorter-chain omega-3 fats do not have a clear effect on total mortality, combined cardiovascular events, or cancer."

The media did not miss this opportunity for publicity, especially when furnished with the reactions of other scientists. Among the torrents of objections some pointed out that simply removing one study from the multiplicity included would reverse most of the negative conclusions on the basis of statistical analyses. Today, many of the problems, including some negative results for omega-3 and certain ailments or clinical conditions, are genuine, but apply to small groups. An example is provided by ventricular tachycardia and fibrillation patients, as a study (Raitt et al., 2005) showed that fish oil may "act in a proarrhythmic way in some patients." More or less the same conclusions were reached by Brouwer et al. (2006). Also, Iso et al. (2006) followed 41,578 middle-aged men and women free of prior diagnoses and habitual fish consumers, ranging from 20 g/day to the highest with a median consumption of 180 g/day for 5 years and found that strong inverse associations existed between dietary intake of n-3 fatty acids and risk of definite myocardial infarction and nonfatal coronary events (Figure 8.3). Compared with a modest fish intake of once per week or ≈20 g/day, a higher intake was associated with a substantially reduced risk of coronary heart disease, primarily nonfatal cardiac events, among middle-aged persons.

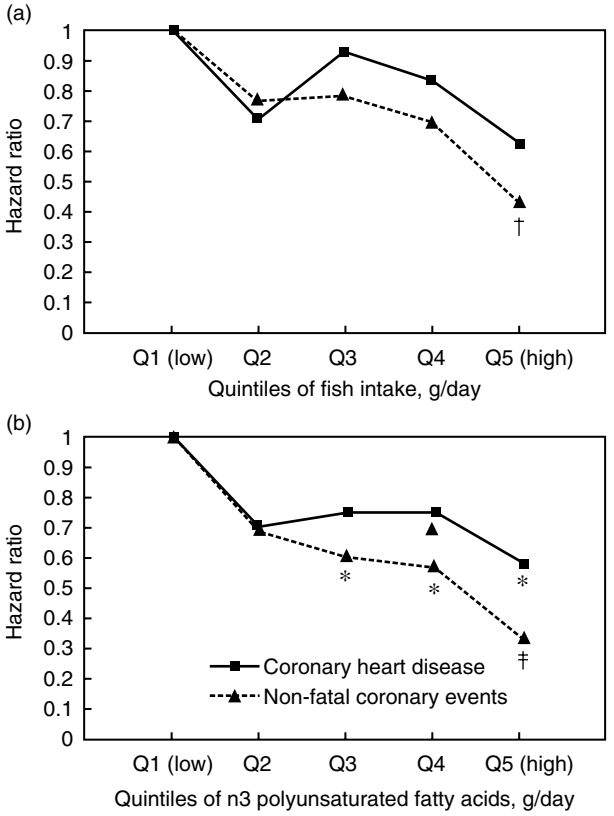

FIGURE 8.3 Comparison of hazard ratio for quintiles of fish consumed (a) and n-3 PUFAs (b) for men and women. The term quintile refers to the five diet levels of habitual fish consumers, ranging from 20 g/day to the highest with a median consumption of 180 g/day. Subjects were middle-aged Japanese men and women. (From Iso, H., et al. (2006). Circulation 113: 195–202. With permission from the American Heart Association.)

TABLE 8.15
U.S. Government[a] Listing of Food Components in Fish, Salmon, Atlantic, Wild, Raw (Scientific Name *Salmo salar* L.)

Nutrient	Units	Value per 100 g of Edible Portion	Number of Data Points
Proximates			
Water	g	68.50	21
Energy	Kcal	142	0
Energy	kJ	594	0
Protein	g	19.84	9
Total lipid (fat)	g	6.34	7
Ash	g	2.54	6
Carbohydrate, by difference	g	0.00	0
Fiber, total dietary	g	0.0	0
Minerals			
Calcium, Ca	mg	12	3
Iron, Fe	mg	0.80	1
Magnesium, Mg	mg	29	1
Phosphorus, P	mg	200	1
Potassium, K	mg	490	3
Sodium, Na	mg	44	2
Zinc, Zn	mg	0.64	1
Copper, Cu	mg	0.250	1
Manganese, Mn	mg	0.016	1
Selenium, Se	mcg	36.5	0
Vitamins			
Vitamin C, total ascorbic acid	mg	0.0	0
Thiamin	mg	0.226	11
Riboflavin	mg	0.380	6
Niacin	mg	7.860	13
Pantothenic acid	mg	1.664	5
Vitamin B_6	mg	0.818	4
Folate, total	mcg	25	1
Folic acid	mcg	0	0
Folate, food	mcg	25	1
Folate, DFE	mcg DFE	25	0
Vitamin B_{12}	mcg	3.18	10
Vitamin A, IU	IU	40	1
Vitamin A, RAE	mcg RAE	12	1
Retinol	mcg	12	1
Amino Acids			
Tryptophan	g	0.222	0
Threonine	g	0.870	0
Isoleucine	g	0.914	0
Leucine	g	1.613	0
Lysine	g	1.822	0
Methionine	g	0.587	0
Cystine	g	0.213	0
Phenylalanine	g	0.775	0
Tyrosine	g	0.670	0
Valine	g	1.022	0
Arginine	g	1.187	0

TABLE 8.15 (Continued)

Nutrient	Units	Value per 100 g of Edible Portion	Number of Data Points
Histidine	g	0.584	0
Alanine	g	1.200	0
Aspartic acid	g	2.032	0
Glutamic acid	g	2.962	0
Glycine	g	0.952	0
Proline	g	0.702	0
Serine	g	0.809	0
Lipids			
Fatty acids, total saturated	g	0.981	0
14:0	g	0.137	2
16:0	g	0.632	2
18:0	g	0.212	2
Fatty acids, total monounsaturated	g	2.103	0
16:1 undifferentiated	g	0.251	2
18:1 undifferentiated	g	1.351	2
20:1	g	0.223	2
22:1 undifferentiated	g	0.279	1
Fatty acids, total polyunsaturated	g	2.539	0
18:2 undifferentiated	g	0.172	2
18:3 undifferentiated	g	0.295	1
18:4	g	0.083	1
20:4 undifferentiated	g	0.267	2
20:5n-3	g	0.321	2
22:5n-3	g	0.287	2
22:6n-3	g	1.115	2
Cholesterol	mg	55	0

[a] NDB No: 15076, http://www.na;/usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl

This confirms other studies that discriminate between definite myocardial infarction and nonfatal coronary events. The most recent reviews, both in the form of a brief editorial (Deckelbaum and Akabas, 2006) and a lengthy review (Wang et al., 2006), are in favor of the longer-chain omega-3 fatty acids, but not α-linolenic acid. This is for reducing the "rates of all-cause mortality, cardiac and sudden death, and possibly stroke. The evidence for the benefits of fish oil is stronger in secondary—than in primary—prevention settings." Marine omega-3 fatty acids cannot prevent death, but do reduce the probability. Obviously fish as a healthy food is generally supported by these recent findings, including the low C-reactive protein concentrations found in Japanese with a diet rich in marine products (Niu et al., 2006).

B. New Market Exploitation

The aging populations in North America and Europe are often made up of persons who have survived cardiovascular disease or will never develop it. Instead their interest, when they or their relatives recognize it, is "cognitive decline with age," typified by diagnosis of Alzheimer's disease. This is even harder to recognize and control than cardiovascular disease, but is unlikely to convert nonfish consumers to a new diet, so they will follow supplement or nutraceutical routes to omega-3

treatment. In a large community study of over 65 population of Chicago, Morris et al. (2005) compared different rates of fish consumption, and found that higher fish intake correlated with slower cognitive decline.

What does the average consumer really know about fish as food? Table 8.15 presents the case for Atlantic salmon. The U.S. Government did not bother to state whether it was a steak or a fillet, tissues with quite different lipid compositions (Zhou et al., 1995). Among the fatty acids, "undifferentiated" means that two or more isomers were lumped together. The three critical longer-chain omega-3 fatty acids, EPA (20:5n-3), DPA (22:5n-3), and DHA (22:6n-3) are differentiated and correspond to those expected. The lipid content (6.34%) is about right for most wild Atlantic salmon returning to a river to spawn. Since the sample tissue is not known it could be estimated that about 5.5 g will be triacylglycerides (oil) and the balance, about 1%, phospholipids. Roughly, the fatty acids correspond to those of the farmed Atlantic salmon (see Figure 3.3 in Chapter 3 of this book). It is presumed to be all muscle with at worst a few pinbones. Note that no man-made organic compounds, persistent pesticides, or dangerous metals are included. Salmon acquire environmental chemicals associated with health risks from both their diets and the ocean waters in which they live. Geography, however, can be a factor in eating fish.

A particularly appropriate paper on the subject of benefits vs. risks is that of Foran et al. (2005). This paper provides a U.S. Government data table of 16 contaminants and salmon was provided for analyses from Northern Europe, South America (Chile), Eastern and Western Canada, and finally all five wild Pacific species were included. Their opinion is that benefit–risk ratio can be optimized by selective shopping on the basis of geographical origins. The inevitable conclusion provided suggests that the risk from exposure to contaminants in farmed and wild salmon is at least partially offset by the associated fatty acid health benefits.Those persons not at significant risk for sudden cardiac death associated with coronary heart disease but concerned with health impairments such as reduction in IQ and other cognitive and behavioral effects, can minimize contaminant exposure by choosing the least contaminated wild salmon or by selecting other sources of longer-chain n-3 fatty acids. However, young children, women of child-bearing age, pregnant women, and nursing mothers, receive the usual cautions with respect to consuming fish. This period of pregnancy and lactation has long been recognized as unique and requiring special dietary considerations (Lichtenstein et al., 2006). The new factor of DHA requirement in early childhood, even into school years, has been considered by Genuis and Schwalfenberg (2006), who carried their overview of the problem into the time of pediatric health problems. They were careful to suggest that only refined fish oil materials be considered, even in the postnatal period, as an alternative to fish.

A rhetorical question (see title in references) asked by J.-M. Bourre (2005) clarifies some of the French viewpoints on fats in foods. That country was for decades held to be the stronghold of gourmands who put desirable flavors above nutritional value. Now a majority of the population has access to new foods strong in either principle, and this paper evaluates their position on fish fed different diets, as well as other matters such as eggs, ducks, and dairy products.

The current levels of recommended intakes of longer-chain omega-3 fatty acids will need to be adjusted in most western countries to cope with the neurological changes in an aging population. Alzheimer is in fact now better known than many other medical pioneers. Research is very difficult but omega-3 fatty acids, particularly DHA, have been obvious candidates. Recent progress by Florent et al. (2006) at the cellular level is able to explain how DHA might function by interacting with protein processes, rather than on a purely lipid basis. "Animal fats" otherwise remain out of the picture, but Colombani (2006) is worth reading in view of the statistical comments above, if only to find out that the U.S. Government made an enormous error (tenfold) in calculating a recommendation suggesting limits on cholesterol consumption. A valuable lesson! An animal fat that is being ignored is the "EGG, Chicken, whole, hard cooked." Thus, the description in USDA Agriculture Handbook No. 8-1 says that 50 g, edible portion, contains 0.019 g of DHA. In view of the low mass of children a steady diet of at least one egg at breakfast may have provided past generations with enough DHA for normal child development. The contemporary cereal breakfasts of today do not,

possibly, account for the attention deficit disorder and other problems in an age group that often formerly also received cod liver oil supplementation for vitamins A and D, unwittingly consuming EPA and DHA in large amounts.

In 2002, Burdge et al. upset the essentiality of α-linolenic acid by showing that 33% of a labeled dose given to a human was exhaled as CO_2 in the first 24 h. More recently "Long-Chain n-3 PUFA: Plant v Marine-Sources" (William and Burdge, 2006) really focuses sharply on whether the well-fed human can benefit much from supplemental α-linolenic acid when only 8% of a label reaches the EPA stage of elongation, and only 0.1% reaches the DHA stage. Women do better, producing 9% DHA, in keeping with the demands that can be made by the fetus and neonate lactation (Burdge and Calder, 2005). These very recent presentations should be compared with the view of Sinclair et al., as recently as 2002. Minor roles for α-linolenic acid in the body are given by this group, but progress with it since then has not been rapid. The conversion of α-linolenic acid to EPA is good in humans, but as continued by a recent study conversion to DHA would add only minimal new DHA (Goyens et al., 2006).

The "plant" title quoted above adds a modest element of confusion to these topics since Martek Biosciences Corporation and other firms are now successfully marketing DHA produced by culturing algae, and so could more legitimately make a vegetarian claim. Martek has gone as far as advertising DHA with a trade mark sign on the Martek DHA Omega-3 but then some fish may also accumulate most of their DHA from algae. More commonly, marine plants biosynthesize EPA and not DHA. Another example of scientific findings being exploited in a dubious manner would be a health claim for tuna, a popular food in the United States, if marketing of albacore and other popular species were to use results from a pelagic tuna species *Thunnus tonggol* (Bleeker). Saito (2006) of Japan has published comprehensive analyses on it recently, showing that the neutral body lipids (oils) may have 20% DHA. Similarly, there are extravagant claims for products made from Antarctic krill *Euphausia superba*. There are enormous populations of this quite large species that happens to store energy in the form of phosphatidyl choline (Lee et al., 2006). Unlike the muscle phospholipids of fish, rich in DHA, and because there is a direct link to feeding on algae, these lipids have an excess of EPA over DHA (Stubing et al., 2003). The menhaden fish of the U.S. east coast has a similar algal diet but uses oil for its energy store, resulting in the initially famous 180:120 ratio for EPA/DHA in fish oil supplements. The *E. superba* has however been merchandized as more digestible than competing omega-3 supplements on the basis of triacylglyceride fish oils. Canada-based Neptune Technologies has also announced results from a clinical trial that reports a daily dose of its krill oil can lower bad cholesterol levels by 34% and boost good cholesterol by 44%.

C. Short-Chain or Long-Chain

The controversy over "n-3" or "omega-3" fatty acids needed for good health falls into place in many articles debating whether the C18 α-linolenic acid has some merit in itself, or functions mainly as a precursor of the C20 EPA and the C22 DHA, or whether the latter two are always needed preformed. An excellent article on this has just appeared in the *American Journal of the Clinical Nutrition* (Gebauer et al., 2006). This takes a broad viewpoint, even admitting that fish oil supplements are a valuable alternative to eating fish and shellfish only.

a. Alternate Sources and Delivery Systems

Medical people have sometimes narrow viewpoints. At the same time as some emphasize the long-chain omega-3 PUFA of (mostly) marine origin, other dedicated scientists are working to persuade oilseed plants to undergo genetic modifications to become pharmacologically active sources of long-chain PUFAs. An overview of the changes in dietary fats possible and considered desirable includes the current human targets of several national and international bodies and makes thoughtful reading (Griffiths and Morse, 2006).

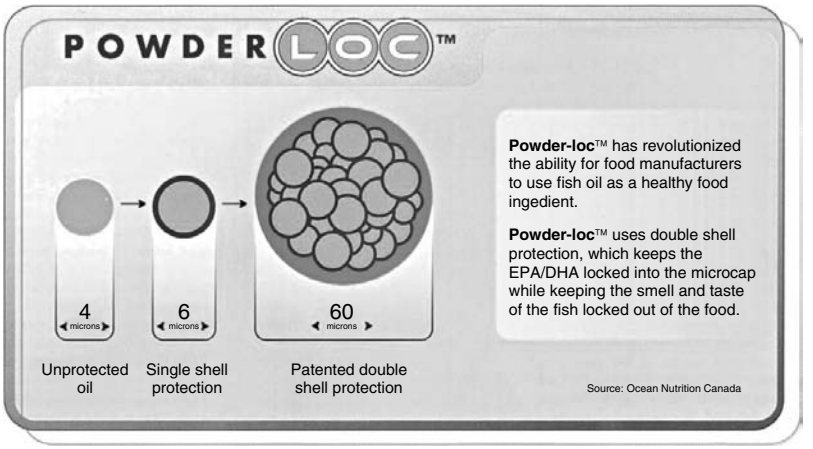

FIGURE 8.4 Microencapsulation can protect the long-chain omega-3 fatty acids of marine oils and concentrates from oxidation and production of off-flavors. A particularly strong microcapsule is the POWDER LOC™ of Ocean Nutrition Canada.

"Fortification" of foods is not new (Muggli, 2006). In fact the addition of DHA produced by Martek Biosciences Corporation to infant formulas, thus copying mother's milk, has been standard for some years. Extravagant claims of health benefits from fatty acids have been the norm for many years but may now become less common as regulations get tighter (D'Innocenzo, 2006). "Nutraceuticals" can include a variety of fats and oils. These are considered by Shahidi and Senanayzke (2006) in a new book edited by F. Shahidi (2006), who also contributed other material to the book *Nutraceuticals and Specialty Lipids and Their Co-Products*.

A new type of fish oil supplement that may be unnoticed for health reasons unless eaten regularly is now on the market, in foods and baked goods enriched with fish oil in microcapsules. It is important that no trace of oil or oil concentrate rich in omega-3 fatty acids be left on the outside to oxidize. Moreover, the encapsulating shell must be strong enough to stand up to handling and mixing as well as any baking process. The patented POWDER-LOC of Ocean Nutrition Canada (Figure 8.4) illustrates how a double shell achieves these objectives. The type of enrichment of food fortification is featured in articles in many trade publications (Wade, 2006; Zegler, 2006), but more sober reflection reveals the problems (Williams, 2005). The health claims allowed in Canada for functional foods and nutraceuticals are discussed in a recent trade publication (D'Innocenzo, 2006). They are very strict compared to those allowed in the United States. So far Health Canada has only permitted five generic health claims (sodium, potassium, and hypertension; calcium, vitamin D, and osteoporosis; saturated fat, *trans* fat, and heart disease; vegetables and fruits and some types of cancer; and sugar alcohol and dental caries) that may be used to communicate the benefits of functional foods and nutraceuticals. Examples of health claims would be "A diet low in saturated and *trans* fat reduces risk of heart disease. Product is low in saturated and *trans* fat," or "A diet with adequate calcium and vitamin D, and regular physical activity reduces risk of osteoporosis."

D. The Safety Factor

A recent advertisement in a daily newspaper merchandizing insert displayed two bottles side-by-side, containing capsules of fish oil supplements. One label read "Salmon and Fish Oils, 1000 mg, 180:120" (180 Softgel Capsules). The other, of identical size and from the same company, read "*Wild* Salmon & Fish Oils, 1000 mg, EPA 180.DHA 120" (150 softgels) and carried a small symbol in the lower left corner of a fish followed by "MEG-3." The latter is part of a licensed trademark of

the firm Ocean Nutrition Canada, and indicates a highly refined and purified fish oil product meeting the standards of the Council for Responsible Nutrition. That the unspecified salmon oil, and for that matter both salmon oils, may not be of good quality is a paradox. Yet both products contain marine long-chain omega-3 fatty acids that are esteemed by the public and most scientists as healthy nutritional supplements.

Unfortunately an activist minority have taken it on themselves to claim that farmed salmon, as distinct from the same species caught in the wild, are dangerous sources of man-made organic chemical of the pesticide, herbicide, and flame retardant type (DeMont and Nicklen, 2004). Mercury is another favorite threat in their activities, mostly as methylmercury, naturally present in ocean water, and the authorities do frown on it in the diet of pregnant women (Smith and Sahyoun, 2005; Wilson, 2004).

Unfortunately, public information on foods is often no better, for example, than a newspaper article "Is it safe to eat salmon?" Calamai (2006). Citing several scientific publications did not really clarify the issues because the authors cited were themselves often at odds. One effort to control and resolve these issues with omega-3 fatty acid supplements has been successful in North America. The Council for Responsible Nutrition was a typical response of U.S. industries to voluntarily control a problem faster than the U.S. Government could take action. The voluntary specifications for long-chain omega-3 EPA and DHA products published by the Council for Responsible Nutrition are shown in Table 8.16.

It should be understood that there are hundreds of isomers of chlorinated hydrocarbons involved. They can be distinguished often in tiny amounts only by specialized laboratories. To facilitate rapid understanding of results certain "congeners" are selected as representative, important, and widely distributed. They may also be found in familiar terrestrial foodstuffs. Jacobs et al. (2004) explore these factors for the whole regional food chains, and not just for fish. Other sources of information that may be useful include the U.S. Pharmacopoeia and the corresponding pharmaceutical bodies in other countries, including the one for the whole of Europe. Measuring toxic chemicals by chemical analysis is an exact science. Deciding on how toxic they are in the normal human adult body is biochemistry hardly ever exposed to the public. Some are very fat soluble and from a meal may be mostly deposited at once in our depot fat, then being very slowly released. How does this compare humans with rats, mice, and rabbits? One of the few efforts to expose this aspect of mercury toxicity is that of Flores (2005) in a REUTERS (know.now) release. In this document Dr William E. M. Lands, a retired professor of biochemistry, pointed out that mammals, including humans, are protected by variable levels of the desirable element selenium, and concluded that the benefits of eating seafood far outweighs the risks of the little bit of mercury that are found in the seafood.

TABLE 8.16
The Voluntary Specifications for Long-Chain Omega-3 EPA and DHA Products[a]

Arsenic: Maximum 0.1 ppm
Cadmium: Maximum 0.1 ppm
Lead: Maximum 0.1 ppm
Mercury: Maximum 0.1 ppm (ONC's specification is maximum 0.01 ppm)
Dioxins and Furans: Maximum 2 pg WHO-PCDD/F-TEQ/g
Dioxin-like PCBs: Maximum 3 pg WHO-PCB-TEQ/g
PCBs: Maximum 0.09 ppm (Sum of IUPAC congeners 28, 52, 101, 118, 138, 153, and 180)

[a] Information courtesy of Ocean Nutrition Canada. WHO, World Health Organization; PCDD, polychlorinated dibenzo-*p*-dioxides; PCDF, polychlorinated dibenzofurans; TEQ, toxic equivalents; PCB, polychlorinated biphenyls; IUPAC, International Union of Pure and Applied Chemistry.

In 1994, the U.K. Committee on Medical Aspects of Food Policy, Department of Health issued a special report on dealing with omega-3 fatty acids and cardiovascular disease. The report includes the now famous recommendation to eat two servings of fish a week, one being an oily fish. Those who are currently pondering whether they should eat fish for good cardiovascular health, at a time when toxic contents receive wide publicity, will find two publications extremely useful. U.K. Scientific Advisory Committee on Nutrition, Committee on Toxicology (2004) issued science-based advice on the benefits and risks of fish consumption. This document brings together the nutritional considerations from the U.K. Scientific Advisory Committee on Nutrition (SACN) on fish consumption with the toxicological considerations from a Toxicology Committee on the contamination in fish. In May, 2004, the Joint Health Claims Initiative (JHCI) released a final draft of a health claim submission along with the paper "Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish" by Tocher (2003). This document was later approved on November 2, 2005 as a final report (U.K. Joint Health Claims Initiative, 2005). The JHCI is a U.K. body joining consumer organizations, enforcement authorities, and industry trade associations. In some aspects, it acts like the Council for Responsible Nutrition in North America.

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9 Fatty Acids in Vegetables and Vegetable Products

Geza Bruckner and Andrew C. Peng

CONTENTS

I. INTRODUCTION

Consuming a diet high in fruits and vegetables is associated with lower risks for numerous chronic diseases, including cancer and cardiovascular disease (Block et al., 1992; Ness and Fowle, 1997). In 1990, the Dietary Guidelines Committee recommended three or more servings of vegetables each day (Report of the Dietary Guidelines Advisory, 1990), and the National Cancer Institute in 1991 and the Produce for Better Health Foundation jointly established the 5 A Day for Better Health Program (Heimendinger et al., 1996). Per capita consumption of fresh and frozen vegetables has increased steadily over the past two decades (data for 1970–1988). Consumption of fresh vegetables increased by 40% (from 64.1 to 89.8 lb per capita), and that of frozen vegetables, more than 32%

(from 13.5 to 17.9 lb) (Institute of Food Technologists, 1990; Putnam, 1990). However, since 1990 the level of vegetable consumption has increased at a much slower rate. The proportion of adults who consumed fruits and vegetables at least five times per day increased from 19.0% in 1990 to 22.1% in 1994 and to 22.7% in 1996. The geometric mean intake of fruits and vegetables increased from 3.3 times per day in 1990 to 3.4 times per day in both 1994 and 1996, whereas the arithmetic mean increased from 3.7 times a day in 1990 to 3.9 times per day in both 1994 and 1996. Although the trend toward increased consumption of fruits and vegetables is apparent, the overall frequency of fruit and vegetable consumption changed little from 1994 to 2000, if the true goal is to achieve five per day. If increases are to be achieved, additional efforts and new strategies will be needed.

Although we know the overall health benefits of increased vegetable consumption, we know very little about the specific components responsible for these health effects. It is known that increased consumption of mono- and polyunsaturated fatty acids and decreased consumption of *trans* and saturated fats are linked to positive health outcomes (Lemaitre et al., 2006). The fatty acid composition of vegetables is generally accepted as "healthful," containing more mono- and polyunsaturated fatty acids than saturated fatty acids.

Vegetable is defined in Webster's dictionary as "an herbaceous plant cultivated for food, and the edible part or parts of such plants as prepared for market or table." In other words, vegetables form one of the legs of MyPyramid (http://www.mypyramid.gov/) and the USDA recommends increased consumption of vegetables with an emphasis on dark green and colored vegetables.

Vegetables are a good source of lipids, simple and complex carbohydrates (especially soluble and insoluble fiber), proteins (beans, peas, and sweet corn), minerals (leafy vegetables and root crops), and secondary metabolites for human nutrition. Additionally, a great deal of attention has recently been focused on the health benefits of phytochemicals (Heber, 2004). Lipids are a major constituent of foods; fresh vegetables may contain 0.1%–1.0% lipids on fresh weight basis (Haytowitz and Matthews, 1984). Their presence, quantity, and composition are important not only for organoleptic satisfaction but also for nutrition and keeping quality. A small amount of lipid present in a food system makes it more palatable and satisfying, facilitates the utilization of proteins and fat-soluble vitamins, provides the essential fatty acids for body needs, and serves as the structural material of cell membranes in all tissues (Gurr and James, 1975). Each fatty acid plays a significant role in normal biological functions and in relation to major problems in health and agriculture (King, 1970; Hansen, 1990).

The type and quantity of fatty acids in the lipid molecule and the position and distribution of these fatty acids in the lipids determine the chemical, physical, and functional properties (Dugan, 1976). Knowing fatty acid composition may help to facilitate new food product development. Some medium-chain fatty acids (C_{12}–C_{18}) have antimicrobial properties (Jay, 1986; Beuchat and Golden, 1989). The major fatty acids of commercial vegetable crops are lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids, with palmitic, oleic, and linoleic acids predominating. Generally, lipids from vegetable sources are liquid (oil) at room temperature and less saturated than animal fats (Hitchcock and Nichols, 1971).

The analysis and evaluation of vegetable lipids and fatty acid composition are influenced by production conditions, cultivar, maturity, season, cultural practices, processing, storage, packaging, and analytical procedures (Kinsella et al., 1975). The fatty acid composition is generally analyzed by gas–liquid chromatography and mass spectrometry. As with any analytical technique there are problems and limitations (Ackman et al., 1967); however, these are the most widely used analytical tool. For vegetables not cited in this chapter, information on their fatty acid composition may be found at the following USDA website: http://www.nal.usda.gov/fnic/foodcomp/search/.

II. SOLANACEOUS CROPS

The vegetables in this group belong to the Solanaceae family.

A. Potatoes (*Solanum tuberosum*)

The tuber-bearing species are herbaceous dicotyledonous annual plants and reproduce asexually by seed tubers. The total lipid content of potatoes is relatively low, approximately 0.1%–0.13% of the fresh weight (Galliard, 1968, 1973; Haytowitz and Matthews, 1984) or about 0.21%–0.81% of the dry weight (Mondy et al., 1963; Cotrufo and Lunsetter, 1964; Lepage, 1968). Galliard (1973) found no varietal difference in total lipid content. Potato tubers are composed of 16.5% neutral lipids, 38.0% glycolipids, and 45.5% phospholipids (Lepage, 1968). The potato microsomes contain 15.6%–19.0% neutral lipids, 35.1%–42.7% glycolipids, and 41.7%–45.9% phospholipids, whereas potato mitochondria have 8.1%–9.0% neutral lipids, 6.4%–11.8% glycolipids, and 79.2%–85.5% phospholipids (Lepage, 1968; Pun et al., 1980). The polar structural lipids are the major lipid components.

The fatty acid composition varies with cultivar, growth, maturity, storage and temperature, and lipid class (Mondy et al., 1963; Cotrufo and Lunsetter, 1964; Galliard, 1968, 1972, 1973; Lepage, 1968; Schwartz et al., 1968; Berkeley and Galliard, 1974; Galliard et al., 1975), as well as with

TABLE 9.1
Fatty Acid Composition of Pontiac and Ontario Potato Cultivars during Storage (%)

	Pontiac		Ontario	
Fatty Acid	**2 Weeks**	**16 Weeks**	**2 Weeks**	**16 Weeks**
10:0	tr	tr	—	0.2
11:0	0.1	0.2	tr	tr
12:0	0.1	0.2	0.1	0.1
13:0	0.2	—	—	—
14:0	0.2	0.7	0.3	0.5
15:0	1.2	1.2	0.4	0.8
15:1	0.1	tr	0.2	0.2
16:0	25.5	39.7	17.6	12.2
16:1	0.1	0.7	0.8	1.8
16:2	tr	—	0.7	0.2
17:0	tr	tr	0.4	0.6
17:1	tr	0.8	0.1	0.1
18:0	2.2	4.9	8.2	11.1
18:1	0.8	1.2	1.7	2.8
18:2	50.7	7.2	27.5	27.9
18:3	15.9	37.4	37.2	32.2
20:0	1.0	2.2	2.4	3.9
20:1	—	0.8	—	—
20:2	0.1	—	0.3	0.3
21:0	—	tr	0.2	0.5
22:0	0.3	—	0.6	1.2
23:0	—	0.4	0.2	0.8
24:0	—	—	0.7	1.3
24:1	0.4	—	—	—
24:2	—	1.6	—	—
U/S[a]	2.4	1.0	2.6	2.7

tr = trace.

[a] U/S = unsaturation ratio = *(*18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Mondy, N.I., et al. (1963). *J. Agric. Food Chem. 11*: 328–329. With permission. Courtesy of American Chemical Society.

TABLE 9.2
Fatty Acid Composition of Lipids Extracted from 12 Brands of Potato Chips

Fatty Acid	Composition (%)
12:0	0.0–0.1
14:0	0.2–0.5
16:0	6.7–14.5
16:1	0.1–0.4
18:0	0.7–1.6
18:1	6.8–18.9
18:2	2.4–21.9
18:3	0.0–0.8

Source: From Sheppard, A.J., et al. (1978). *J. Agric. Food Chem. 26*: 346–348. With permission. Courtesy of American Chemical Society.

subcellular particles (Schwertner and Biale, 1973; Pun et al., 1980). Table 9.1 shows the fatty acid composition as affected by storage time and cultivar for potatoes stored at 4.4°C (Mondy et al., 1963). The Pontiac cultivar decreased in linoleic acid and increased in palmitic acid, whereas the Ontario cultivar decreased in palmitic and linolenic acids. The potato fatty acids are highly unsaturated. The unsaturation ratio (18:1 18:2 18:3)/(16:0 18:0) for Pontiac stored at 4.4°C was 2.4 after 2 weeks and 1.0 after 16 weeks; for Ontario, it was 2.6 and 2.7, respectively.

Pun et al. (1980) reported that potato microsomal and mitochondrial lipids consisted of palmitic (24.7% and 19.4%), stearic (6.8% and 4.7%), oleic (2.0% and 2.4%), linoleic (49.6% and 59.6%), and linolenic (15.4% and 12.1%) acids, and the unsaturation ratio was 2.1 and 3.1, respectively.

In raw and processed potatoes, phospholipids are the major lipid component, and palmitic and linoleic acids are the most common fatty acids, as shown in Table 9.2 (Pun and Hadziyev, 1978; Lilja and Lingnert, 1989).

The total lipid content of deep-fat-fried foods is greatly increased relative to the unfried products; for example, the average lipid content of potato chips is 40.4% and that of French fries is 13.5% (Smith et al., 1985) as compared with 0.1%–0.13% for raw potatoes. The fatty acid composition of potato chips is primarily reflective of the oil used for frying. Sheppard et al. (1978) investigated 12 brands of potato chips in the Washington, DC, and Davis, CA, areas and found a wide range of fatty acid content (Table 9.2); the chips were high in palmitic, oleic, and linoleic acids. The amount of unsaturated fatty acids was much lower than those reported by Smith et al. (1985) (Table 9.3), who studied nine brands of potato chips in the Davis and Sacramento areas of California. The storage of Norchip potato chips at 24°C for 10 weeks showed no significant changes in fatty acids (Robertson et al., 1972). However, the fatty acid composition of potato chips is altered by the use of different frying oils (Tables 9.4 through 9.6).

B. Tomatoes (*Lycopersicon esculentum*)

Tomato is an herbaceous perennial in the tropics and an annual plant in the Temperate Zone. Per capita consumption in America is 15.2 lb for fresh tomatoes and 61.0 lb for processed tomatoes (Putnam, 1990). The total lipids of red fresh tomatoes are 0.06%–0.21% (Ueda et al., 1970a; Haytowitz and Matthews, 1984; Peng, 1990).

Fatty acid composition of neutral lipids (Ueda et al., 1970a, b), glycolipids (Minamide and Ogata, 1972a), and phospholipids (Minamide et al., 1970a; Minamide and Ogata, 1972b) of

TABLE 9.3
Fatty Acid Composition of Lipids Extracted from Nine Brands of Potato Chips

Fatty Acid	Composition (%)
14:0	0.8–1.0
16:0	20.0–25.9
16:1c	0.6–0.8
18:0	2.3–2.6
18:1t	0.0–0.2
18:1c	16.1–17.9
18:2ct	0.8–1.4
18:2ω6	47.7–51.5
18:3ω3	0.3–0.4
20:1	0.1–0.2
22:1	0.0–0.2
Total (mean)	
Saturated	27.8
Monounsaturated	17.5
Polyunsaturated	50.3
Trans-unsaturated	1.3

Source: From Smith, L.M., et al. (1985). *J. Am. Oil Chem. Soc. 62*: 996–999. With permission. Courtesy of the American Oil Chemists' Society.

TABLE 9.4
Fatty Acid Composition of Oil Extracted from Potato Chips after Frying in Partially Hydrogenated Sunflower Oil

Hr Oil Heated	Storage Time (Week)	Fatty Acid (%)						
		16:0	18:0	18:1	18:2	18:3	20:0	22:0
2	0	7.0	5.4	47.4	36.8	0.5	0.4	0.4
2	4	7.2	5.5	48.4	37.2	1.1	0.4	0.5
2	6	7.1	5.3	46.6	38.1	1.0	0.6	0.3
2	11	7.1	5.5	48.2	37.6	0.6	0.5	0.4
12	0	7.2	5.8	47.2	35.5	0.5	0.4	0.5
12	4	7.3	5.9	48.2	36.1	0.9	0.4	0.5
12	6	7.1	5.5	45.2	36.7	0.5	0.4	0.3
12	11	7.2	5.8	48.4	36.0	0.6	0.4	0.5
23	0	7.3	6.0	47.4	35.0	0.4	0.5	0.4
23	4	7.1	5.9	47.3	34.8	0.9	0.4	0.5
23	6	7.2	5.8	45.6	33.2	0.9	0.5	0.3
23	11	7.2	5.7	46.9	35.1	0.5	0.5	0.4

Source: From Robertson, J.A., et al. (1972). *Am. Potato J. 49*: 444–450. With permission.

TABLE 9.5
Fatty Acid Composition of Oil Extracted from Potato Chips after Frying in Cottonseed-Corn Oil Mixture (70:30)

Hr Oil Heated	Storage Time (Week)	Fatty Acid (%)						
		14:0	16:0	18:0	18:1	18:2	18:3	20:0
2	0	0.5	16.8	2.2	19.1	52.9	0.7	0.4
2	4	0.5	17.0	2.3	19.4	54.0	0.8	0.4
2	6	0.4	15.2	2.1	17.4	48.2	0.8	0.4
2	10	0.5	17.2	2.2	19.6	54.3	0.3	0.4
12	0	0.5	17.4	2.3	19.5	52.5	0.7	0.5
12	4	0.5	16.7	2.2	18.8	49.4	0.7	0.3
12	6	0.5	16.8	2.2	18.7	49.5	0.8	0.4
12	10	0.5	17.1	2.3	19.4	51.8	0.4	0.3
23	0	0.5	17.6	2.3	19.6	50.5	0.3	0.3
23	4	0.5	18.1	2.4	20.3	50.3	0.7	0.4
23	6	0.5	17.1	2.2	19.1	48.5	0.7	0.4
23	10	0.5	16.4	2.2	18.4	47.0	0.3	0.4

Source: From Robertson, J.A., et al. (1972). *Am. Potato J. 49*: 444–450. With permission.

TABLE 9.6
Fatty Acid Composition of Potatoes in Various Preparations

Fatty Acids (g/100 g)	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Poly-unsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Mono-unsaturated
Baked without salt	0.001	0.004	0.001	0.022	0.001	0.005	0.001	0.043	0.013	0.058	0.030	0.003
Dehydrated flakes without milk	0.003	0.019	0.004	0.091	0.005	0.020	0.006	0.180	0.057	0.238	0.138	0.011
Raw	0.001	0.003	0.001	0.016	0.001	0.004	0.001	0.032	0.010	0.043	0.026	0.002
Canned	0.001	0.007	0.001	0.031	0.002	0.007	0.002	0.063	0.020	0.085	0.051	0.005

Source: USDA National Nutrient Database for Standard Reference.

field tomatoes is primarily palmitic, stearic, oleic, linoleic, and linolenic acids. The unsaturated fatty acids in phospholipids are increased during ripening on the plant and decreased in the after-ripening stage (Minamide and Ogata, 1972b). Ueda et al. (1970a) investigated ten cultivars of tomatoes for processing; the main fatty acids of average-weight tomatoes are listed in Tables 9.7 and 9.8. The neutral lipids contained more unsaturated acids with a higher unsaturation ratio than the polar lipids (glycolipids and phospholipids), but polar lipids had higher palmitic and linolenic acids.

Minamide et al. (1970b) studied the fatty acid composition of greenhouse tomato phospholipids and found a higher fatty acid content and higher unsaturation ratios. The unsaturated fatty acids are increased during ripening after harvest. Three greenhouse tomato cultivars—GS244, MR13, and Jumbo—were examined (Peng, 1990). Except for GS244, glycolipids are generally higher in concentration (40.4%–43.9%) than the other two lipids. Fatty acids showed a typical composition of plant lipids, palmitic, stearic, oleic, linoleic, and linolenic acids (Table 9.9). There appears to be higher unsaturation in polar lipids than in neutral lipids.

TABLE 9.7
Average Fatty Acid Composition (%) of the Neutral Lipids of Tomatoes for Processing

Fatty Acid	Cultivar									
	Epoch	H-1409	H-1370	Anahu	Kurikoma	VF-36	T-613-1	Nozomi-2	Chico	Texto-2
14:0	2.9	3.8	3.4	3.9	3.9	2.9	2.5	3.1	3.3	2.8
16:0	17.9	19.8	19.3	19.4	18.0	20.2	20.6	21.2	20.4	21.4
16:1	2.3	1.9	1.9	1.5	1.9	1.4	1.6	1.2	2.0	1.8
18:0	3.9	4.1	3.9	3.9	3.3	4.4	3.3	6.0	3.8	3.5
18:1	7.2	8.3	8.0	6.1	6.1	6.3	7.0	9.0	4.7	7.1
18:2	47.3	39.4	39.3	42.3	46.4	46.3	44.0	44.1	41.4	41.5
18:3	10.6	12.7	14.7	15.0	12.5	15.0	16.4	9.2	17.9	15.6
20:0	2.9	2.2	2.2	2.0	1.4	—	3.7	2.9	2.0	1.7
22:0	3.7	4.6	5.2	4.8	4.5	2.1	1.9	2.1	3.2	2.4
U/S[a]	3.0	2.5	2.7	2.7	3.0	2.7	2.8	2.3	2.6	2.6

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Ueda, Y., et al. (1970a). *J. Jpn. Soc. Hort. Sci. 39*: 385–389. With permission. Courtesy of the Japanese Society for Horticultural Science.

TABLE 9.8
Average Fatty Acid Composition (%) of the Polar Lipids of Tomatoes for Processing

Fatty Acid	Cultivar									
	Epoch	H-1409	H-1370	Anahu	Kurikoma	VF-36	T-613-1	Nozomi-2	Chico	Texto-2
16:0	31.6	31.4	30.3	29.1	29.4	31.4	31.9	26.7	29.7	28.5
16:1	—	1.4	1.5	1.0	2.6	—	—	—	2.0	1.0
18:0	4.1	4.9	5.3	4.7	4.7	4.0	4.3	3.4	4.6	3.9
18:1	4.8	5.4	6.9	3.9	6.3	3.5	5.6	4.5	3.2	5.3
18:2	40.6	35.9	32.5	34.4	35.2	35.6	36.0	43.3	36.6	39.4
18:3	16.4	18.8	21.7	24.0	19.5	23.9	20.5	20.2	22.5	20.1
22:0	2.0	1.0	1.0	1.5	1.5	—	—	1.7	2.0	1.0
U/S[a]	1.7	1.6	1.7	1.8	1.8	1.8	1.7	2.3	1.8	2.0

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Ueda, Y., et al. (1970a). *J. Jpn. Soc. Hort. Sci. 39*: 385–389. With permission. Courtesy of the Japanese Society for Horticultural Science.

Tomato processing waste consists of skins, seeds, and pulp. The seeds contain 22%–30% crude fat, 16%–22% crude fiber, and 23%–34% crude proteins (Lazos and Kalathenos, 1988), which could be used as essential raw material sources (Al-Wandawi et al., 1985). The fatty acids of tomato seed oil from processed tomato waste are found to be higher in unsaturated fatty acids (Peng, 1983), as presented in Table 9.10.

C. Pepper (*Capsicum annuum*)

The pepper, similar to the tomato, is a perennial in the tropics and an herbaceous annual in the Temperate Zone. The total lipid content is 0.40%–0.45% of fresh weight (Kinsella, 1971; Haytowitz and Matthews, 1984). Wang and Baker (1979) studied chilling injury on pepper fruits and found

TABLE 9.9
Fatty Acid Composition (%) of Greenhouse Tomatoes

Fatty Acid	Cultivar											
	Total Lipids			Neutral Lipids			Glycolipids			Phospholipids		
	GS244	MR13	Jumbo	GS244	MR13	Jumbo	GS244	MR13	Jumbo	GS244	MR13	Jumbo
14:0	2.1	0.8	1.8	3.6	4.5	4.4	1.5	1.7	0.6	1.3	0.3	0.3
16:0	11.6	16.4	15.5	17.9	12.3	14.5	14.4	14.2	14.7	15.7	15.3	18.1
16:1	1.9	0.5	1.3	3.0	2.1	2.9	0.7	0.9	1.6	0.7	2.1	1.3
18:0	7.1	4.3	8.0	4.2	6.4	7.1	4.2	8.2	4.3	3.8	8.2	4.6
18:1	6.1	3.1	4.1	5.6	8.5	9.3	4.1	8.2	7.0	2.7	5.2	2.9
18:2	17.3	26.2	22.5	13.3	15.5	15.8	15.2	19.3	20.1	27.3	25.0	29.4
18:3	19.4	24.3	20.9	2.4	6.5	9.0	23.9	23.8	23.9	21.6	21.6	22.1
20:0	—	1.4	3.2	0.7	2.8	3.6	—	1.2	0.7	—	1.9	1.4
21:0	0.3	2.1	0.7	0.8	0.3	0.3	1.1	1.2	1.1	1.6	0.9	1.4
22:0	1.0	2.4	1.2	2.6	2.0	2.0	2.0	1.3	1.9	2.5	1.3	1.7
22:1	2.7	2.6	1.1	2.9	1.1	1.2	—	1.0	2.6	—	1.3	1.6
23:0	0.8	2.4	2.1	2.3	1.4	1.5	2.1	1.6	3.0	2.4	1.9	2.7
24:0	1.2	4.6	2.9	—	4.9	1.6	0.8	3.2	3.6	—	3.6	3.5
U/S[a]	2.3	2.6	2.0	1.0	1.6	1.6	2.3	2.3	2.7	2.6	2.2	2.4

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Cir. 121*: 21–23.

TABLE 9.10
Fatty Acid Composition (%) of Tomato Seed Oil

Fatty Acid	Composition (%)
14:0	1.8
15:0	1.3
16:0	15.2
16:1	1.5
17:0	2.0
18:0	3.6
18:1	34.1
18:2	27.1
18:3	2.6
20:0	1.0
20:1	1.8
22:0	1.0
22:1	1.5
24:0	0.7
24:1	0.7
U/S[a]	1.6

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From A.C. Peng, unpublished data (1983).

TABLE 9.11
Fatty Acid Composition (%) of Pepper

Fatty Acid	Neutral Lipids	Glycolipids	Phospholipids
12:0	0.1	tr	—
14:0	0.4	0.4	tr
16:0	15.1	11.5	19.8
16:1	0.1	0.5	—
18:0	3.2	15.7	6.0
18:1	8.6	5.3	1.8
18:2	70.0	30.5	64.5
18:3	2.5	36.1	7.9
U/S[a]	4.4	2.6	2.9

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Kinsella, J.E. (1971). *J. Food Sci. 36*: 865–866. With permission. Courtesy of Institute of Food Technologists.

that the degree of unsaturation of 18-carbon fatty acids in the polar lipids increased. The fatty acid composition is quite typical of plant lipids and consisted mainly of palmitic, stearic, oleic, linoleic, and linolenic acids (Kinsella, 1971). The neutral lipids and phospholipids are very high in linoleic acid, and glycolipids are rich in linolenic acids (Tables 9.11 and 9.12).

D. Eggplant (*Solanum melongena*)

The eggplant, a member of the deadly nightshade family, is a vegetable of dubious origin. Some say it originated in China some 4000 years ago, and then it was introduced into the Mideast by Arab traders in the eighth century. Others say it originated and was domesticated in India, and then brought home by members of Arab armies in the seventh century. Others yet say it has been grown and eaten as a vegetable in Iran since 1500 BC. The fatty acid composition of eggplant in various preparations is shown in Table 9.13.

III. CRUCIFERS

All vegetable crops in this group belong to the Cruciferae family.

A. Cabbage (*Brassica oleracea*)

The cabbage is a dicotyledonous biennial plant. It is the most important member of the cole crops in our daily diet. It has significant nutritional value and is valuable as a fresh or processed vegetable. The lipid content of cabbage is very low, 0.10%–0.21% (Wheeldon, 1960; Pederson and Albury, 1969; Peng, 1974a, b, 1975, 1982a; Salunkhe et al., 1974), but it is significant to keeping quality. Cabbage lipid composition has been studied (Wheeldon, 1960; Laseter et al., 1968; Komaitis and Melissari-Panagiotou, 1990). In our laboratory, we have investigated cabbage lipid components and their fatty acid composition (Table 9.14). The lipids isolated from the cabbage leaves are predominantly neutral lipids (51.0%), followed by glycolipids (40.8%) and phospholipids (8.2%). It is apparent that cabbage lipids contain more unsaturated fatty acids than saturated. Neutral lipids have a greater degree of unsaturation than polar lipids. The distribution of the fatty acids is mainly

TABLE 9.12
Fatty Acid Composition of Pepper in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated	Phytosterols
Banana											
Raw	0.015	0.036	0.002	0.007	0.025	0.240	0.003	0.243	0.048	0.027	3
Green											
Canned	0.001	0.036	0.002	0.011	0.018	0.146	0.015	0.161	0.045	0.020	
Cooked without salt		0.022	0.001	0.007	0.012	0.097	0.010	0.107	0.029	0.013	9
Frozen		0.023	0.001	0.007	0.013	0.102	0.010	0.113	0.031	0.014	
Raw	0.001	0.050		0.008	0.008	0.054	0.008	0.062	0.058	0.008	9
Dehydrated	0.009	0.331	0.021	0.105	0.181	1.462	0.145	1.607	0.447	0.202	
Red											
Canned	0.001	0.033	0.002	0.011	0.018	0.146	0.015	0.161	0.045	0.020	
Cooked without salt		0.022	0.001	0.007	0.012	0.096	0.010	0.106	0.029	0.013	9
Frozen	0.001	0.023	0.001	0.007	0.013	0.102	0.010	0.113	0.031	0.014	
Raw		0.056	0.004	0.004	0.004	0.100	0.056	0.156	0.059	0.007	
Jalapeno											
Canned	0.006	0.072	0.002	0.013	0.05	0.49	0.024	0.514	0.097	0.053	
Raw	0.004	0.045	0.001	0.008	0.031	0.304	0.015	0.319	0.062	0.033	
Red chili											
Canned	0.010	0.008		0.001	0.005	0.052	0.002	0.055	0.010	0.006	
Raw	0.002	0.033		0.007	0.024	0.228	0.011	0.239	0.042	0.024	
Pimento											
Canned	0.001	0.033	0.002	0.011	0.018	0.146	0.015	0.161	0.045	0.020	9

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.13
Eggplant Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated	Phytosterols
Cooked without salt	0.001	0.032	0.002	0.012	0.018	0.078	0.015	0.093	0.044	0.02	
Raw		0.025	0.002	0.009	0.014	0.063	0.013	0.076	0.034	0.016	7

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.14
Fatty Acid Composition (%) of Cabbage Lipids

Fatty Acid	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
11:0	—	—	—	4.5
12:0	1.6	5.1	7.9	2.5
13:0	0.6	2.1	6.0	1.5
13:1	2.0	3.7	7.1	3.9
14:0	2.9	5.4	7.1	2.2
14:1	1.2	2.5	2.6	1.2
15:0	2.3	3.7	2.4	1.6
15:1	1.4	1.5	0.7	0.3
16:0	7.5	13.3	17.1	26.2
16:1	2.6	2.3	1.1	1.4
17:0	0.6	1.1	1.2	tr
17:1	1.0	1.0	1.5	tr
18:0	5.4	3.6	3.5	3.4
18:1	10.8	7.0	4.2	10.1
18:2	14.0	6.7	4.1	14.2
20:0	1.1	—	—	2.0
18:3	16.9	24.0	26.4	16.3
21:1	0.9	4.9	—	2.5
22:0	0.5	1.0	2.9	3.2
?	2.1	—	—	—
23:0	0.4	9.9	3.7	—
24:0	21.1(?)	0.3	—	—
24:1	1.9	—	—	—
U/S[a]	3.2	2.2	1.7	1.4

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1974b). *Lipids 9*: 299–301.

palmitic, stearic, oleic, linoleic, and linolenic acids (Peng, 1974b). The varietal influence on the total lipid content and fatty acids examined by our laboratory (Table 9.15) indicates that the distribution of fatty acids varies but follows a typical plant lipid pattern (Hitchcock and Nichols, 1971; Peng, 1982b).

TABLE 9.15
Fatty Acid Composition (%) of Four Cultivars of Cabbage

Fatty Acid	King Cole	Golden Acre Yellow Resistant	HiDri 364 × 326	HiDri 364 × 328
9:0	2.3	—	0.5	tr
10:0	1.5	—	2.3	tr
11:0	1.3	—	1.1	tr
12:0	0.9	1.6	0.9	tr
13:0	0.5	0.6	1.3	—
?	tr	2.0	1.0	—
14:0	1.8	2.9	2.2	tr
14:1	tr	1.2	0.7	tr
15:0	2.5	2.3	2.4	1.1
15:1	1.5	1.4	1.8	tr
16:0	20.8	7.5	14.1	14.0
16:1	4.5	2.6	5.4	1.4
17:0	0.9	0.6	1.2	1.1
17:1	12.4	1.0	1.6	0.5
18:0	6.2	5.4	3.2	2.6
18:1	3.4	10.8	14.6	12.6
19:0	—	—	2.2	0.6
18:2	0.9	14.0	14.2	21.1
20:0	0.8	1.1	2.0	0.8
18:3	1.1	16.9	17.1	26.2
21:0	1.8	0.8	1.7	1.0
21:1	2.5	0.9	—	1.3
22:0	4.2	0.5	3.0	1.7
?	—	2.1	—	—
22:1	7.1	—	tr	2.0
23:1	5.2	tr	—	—
24:0	5.9	21.1	—	4.5
24:1	8.9	1.9	—	—
U/S[a]	0.2	3.2	3.6	3.6

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng (1982f).

Leafy vegetables are perishable. Storage is the only means to extend the supply the year round. Cabbage is usually stored after harvest before it reaches the consumer's table. However, it is still physiologically active, with enzymatic and respiratory processes continuing; the storage temperature is crucial, for higher temperatures induce greater physiological activity, requiring more energy. In our laboratory, we found that total lipids decreased more rapidly when cabbage was stored at 7°C than when it was stored at 1°C (Peng, 1979). Fatty acids vary considerably in their quantitative distribution at different storage temperatures (Table 9.16). Total unsaturated fatty acids appear to change more than the saturated acids during storage, and the rate of change is higher at 7°C than at 1°C.

Dickson and Stamer (1975) developed several new high-solid cabbage inbreds that had higher dry matter than the standard cultivars, and a kraut made from such hybrids resulted in less juice. Our laboratory analyzed two of the hybrids, "364 × 326" and "364 × 328." Each high-solid cabbage

TABLE 9.16
Fatty Acid Composition (%) of Total Cabbage Lipids after Storage

		7°C Storage (months)		1°C Storage (months)		
Fatty Acid	**0**	**2**	**3**	**2**	**3**	**4**
8:0	—	1.0	1.2	1.9	1.5	1.7
9:0	2.3	0.5	0.8	1.1	1.7	2.3
10:0	1.5	tr	0.5	0.5	1.2	1.5
11:0	1.3	tr	0.8	tr	1.2	0.9
14:0	1.8	1.8	1.8	1.6	1.8	2.1
15:0	2.5	3.4	2.8	2.9	3.1	3.3
15:1	1.5	1.4	1.5	0.6	1.0	1.2
16:0	20.8	16.6	21.3	26.0	23.7	26.8
16:1	4.5	3.4	1.3	2.4	4.1	4.0
17:0	0.9	1.9	1.0	1.4	1.1	1.2
?	12.4	11.9	6.4	10.4	12.4	13.4
18:0	6.2	6.7	4.9	4.2	5.6	7.1
18:1	3.4	13.0	13.8	5.7	7.8	8.9
18:2	0.9	1.9	3.3	2.3	1.6	1.3
18:3	1.1	6.5	9.7	4.5	2.2	2.2
21:0	1.8	2.0	1.9	2.6	1.6	tr
21:1	2.5	1.5	2.1	3.4	—	—
22:0	4.2	1.4	2.0	5.3	2.3	2.4
22:1	7.1	1.5	2.6	5.1	2.5	2.3
23:1	5.2	9.8	2.8	3.4	3.0	—
24:0	5.9	2.9	3.8	5.6	5.4	5.2
24:1	8.9	1.7	2.8	5.2	3.9	2.5
U/S[a]	0.2	0.9	1.0	0.4	0.4	0.4

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1979). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Res. Circ. 250*: 45–47.

contains a different composition and distribution of lipid components. Neutral lipids are the predominant class—63.3% in "364 × 326" and 41.6% in "364 × 328"; phospholipids content is 22.6% and 39.0%; and glycolipids content is 14.1% and 19.4%, respectively (Peng, 1982a). The fatty acid composition of each class is reported in Table 9.17, in which palmitic, palmitoleic (16:1), stearic, oleic, linoleic, and linolenic acids constitute the major fatty acids. Among three lipid classes, phospholipids are higher in unsaturated fatty acids.

Lactic acid fermentation of cabbage has long been a traditional Chinese household skill for preserving this vegetable for off-season use. It produces a palatable, microbiologically safe, economical, and appetizing food that has been enjoyed by Asians for centuries. Vorbeck et al. (1963) reported lipid alterations during the fermentation of cabbage. Peng (1975, 1979) studied lipid changes of cabbage fermented to sauerkraut. Cabbage was fermented, canned, heat-processed, and stored at room temperature, and the total lipids, lipid classes, and fatty acids were analyzed at each stage of storage. Total lipids increased from 0.16 in the fresh cabbage to 0.22% in the sauerkraut after fermentation and then decreased to 0.17% after 8 months of storage (Table 9.18). It was found that the loss of 10.3% in neutral lipids from the original concentration was equal to the combined gains of 0.2% in glycolipids and 10.1% in phospholipids. The overall total lipid content was not altered.

The relative quantitative changes of fatty acids in total lipids are shown in Table 9.19. During fermentation and processing, palmitic, linoleic, and linolenic acids increased slightly, and a further

TABLE 9.17
Fatty Acid Composition (%) of High-Solids Cabbage

	Cultivar 364 × 326			Cultivar 364 × 328		
Fatty Acid	**Neutral Lipids**	**Glycolipids**	**Phospholipids**	**Neutral Lipids**	**Glycolipids**	**Phospholipids**
12:0	4.8	4.9	1.2	1.9	3.1	1.2
14:0	6.1	3.9	1.0	6.5	2.0	1.5
15:0	5.3	2.9	1.5	4.7	2.9	1.8
16:0	18.8	22.5	17.6	17.7	20.4	15.2
16:1	4.8	4.6	4.2	3.2	2.0	3.5
17:0	1.4	—	0.6	2.2	2.5	2.9
18:0	3.2	2.9	2.7	4.4	4.6	4.0
18:1	10.2	9.0	16.2	5.1	9.3	11.7
18:2	6.8	7.1	15.9	5.7	6.8	18.1
18:3	8.5	8.6	17.5	8.8	8.7	22.2
22:0	—	—	2.4	4.7	—	1.5
U/S[a]	1.2	1.0	2.4	0.9	1.0	2.7

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1982a). *J. Food Sci. 47*: 1036–1037.

TABLE 9.18
Lipid Content of Sauerkraut after Fermentation, Processing, and Storage

	Total Lipids		Neutral Lipids		Glycolipids		Phospholipids	
Storage Time	**Wt (g)**	**%**	**Wt (g)**	**%**	**Wt (g)**	**%**	**Wt (g)**	**%**
Cabbage	0.32	0.16	0.16	51.0	0.13	40.8	0.03	8.2
0 months	0.44	0.22	0.25	55.8	0.16	36.8	0.03	7.3
2 months	0.45	0.22	0.26	58.2	0.16	34.8	0.03	7.0
4 months	0.46	0.23	0.22	46.9	0.17	38.4	0.07	14.7
8 months	0.34	0.17	0.10	29.2	0.16	47.8	0.08	23.0
12 months	0.35	0.17	0.14	40.7	0.15	41.0	0.06	18.3

Source: From Peng, A.C. (1975). *J. Sci. Food Agric. 26*: 1325–1332.

increase in these acids occurred in storage. On the other hand, stearic and oleic acids declined after 2 months but reappeared after 8 and 12 months of storage. High levels of lignoceric (24:0) and nervonic (24:1) acids could be an artifact during fermentation and storage that certainly affected the percentage of distribution of fatty acids (Peng, 1973, 1974a, 1975). A higher unsaturation ratio indicates the presence of higher unsaturated fatty acids.

Table 9.20 records the relative gross changes in fatty acids in the neutral lipid fraction. Except for palmitic, stearic, oleic, linoleic, and linolenic acids, fatty acids are found in smaller amounts after fermentation and processing. As storage time increases, palmitic and oleic acids are maintained at about the same level, whereas the relative changes of stearic, linoleic, and linolenic acids are varied. Higher unsaturated fatty acids are observed.

The glycolipid fraction is the second most predominant lipid component of cabbage and sauerkraut. It has the same pattern of fatty acid distribution as other fractions, as shown in Table 9.21

TABLE 9.19
Fatty Acid Composition (%) of Total Lipids in Cabbage and Sauerkraut

		Sauerkraut (Months)				
Fatty Acid	**Cabbage**	**0**	**2**	**4**	**8**	**12**
12:0	1.6	1.6	1.9	2.0	tr	tr
12:1	—	—	2.5	1.7	0.6	0.7
13:0	0.6	2.1	1.5	2.0	tr	0.7
13:1	2.0	3.1	1.7	1.7	0.5	0.5
14:0	2.9	3.3	1.8	1.9	0.6	0.5
14:1	1.2	1.2	0.6	0.8	tr	tr
15:0	2.3	1.7	1.0	1.1	1.0	0.9
15:1	1.4	1.4	0.6	0.6	0.6	0.6
16:0	7.5	8.8	10.5	13.1	11.3	12.5
16:1	2.6	2.6	1.0	1.0	2.3	2.7
17:0	0.6	0.8	0.5	tr	tr	0.6
17:1	1.0	1.6	0.7	tr	0.6	0.9
18:0	5.4	5.3	2.2	2.2	5.8	5.2
18:1	10.8	10.8	5.2	5.8	13.4	12.3
18:2	14.0	16.1	12.6	14.6	21.5	21.5
20:0	1.1	1.3	0.5	0.9	1.0	1.0
18:3	16.9	17.3	17.5	20.7	23.9	27.9
21:0	0.8	tr	—	—	0.5	tr
21:1	0.9	2.1	1.9	1.9	2.1	1.9
22:0	0.5	2.5	1.0	1.5	0.9	1.0
22:1	2.1	1.2	1.3	1.3	1.8	1.6
23:0	0.4	—	—	—	—	—
24:0	21.1?	3.2	23.8?	13.6?	1.9	1.7
24:1	1.9	8.5?	10.6?	11.0?	—	—
U/S[a]	3.2	3.1	2.8	2.7	3.4	3.5

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1975). *J. Sci. Food Agric. 26*: 1325–1332.

(Peng, 1975). Fermentation and subsequent processing cause a considerable decrease of most fatty acids; only linolenic acid is increased markedly with storage. The unsaturation ratio also increases with storage. Phospholipids constitute the smallest lipid fraction in cabbage and sauerkraut (Table 9.22). In contrast to the other classes, most fatty acid concentrations increase during fermentation and processing; however, palmitic, linoleic, and linolenic acids exhibit significant losses. When storage time is increased, the palmitic acid concentration increases steadily, whereas levels of stearic, oleic, linoleic, and linolenic acids decrease noticeably. The phospholipids contain a higher proportion of saturated fatty acids. The unsaturated fatty acids decrease with storage.

B. Cauliflower (*Brassica oleracea* cv. Botrytis)

The total lipid content of cauliflower is quite low, 0.18% (Haytowitz and Matthews, 1984). The fatty acid composition of mitochondrial lipid classes isolated from cauliflower buds shows large amounts of short-chain fatty acids, linolenic, palmitic, linoleic acids (Schwertner and Biale, 1973), and hexadecatrienoic acid (16:3) in glycolipids, as illustrated in Tables 9.23 and 9.24.

TABLE 9.20
Fatty Acid Composition (%) of Neutral Lipids in Cabbage and Sauerkraut

		Sauerkraut (Months)				
Fatty Acid	**Cabbage**	**0**	**2**	**4**	**8**	**12**
12:0	5.1	1.8	2.0	1.5	0.5	0.7
12:1	—	—	1.1	—	tr	1.6
13:0	2.1	1.0	1.7	—	tr	1.1
13:1	3.7	0.9	1.0	—	1.0	1.5
14:0	5.4	1.8	2.3	1.2	0.6	1.2
14:1	2.5	0.6	0.9	1.1	tr	0.9
15:0	3.7	1.3	1.9	1.6	1.1	2.1
15:1	1.5	0.7	1.1	1.0	0.6	1.3
16:0	13.3	14.3	11.0	13.9	13.5	13.1
16:1	2.3	1.8	2.6	2.1	2.0	3.2
17:0	1.1	0.5	0.8	0.6	tr	1.0
17:1	1.0	0.5	1.2	1.2	tr	1.1
18:0	3.6	4.6	5.7	5.9	4.9	6.4
18:1	7.0	11.1	11.1	10.7	12.6	10.6
18:2	6.7	21.7	19.3	18.3	23.4	16.5
20:0	—	tr	1.2	0.7	0.7	1.6
18:3	24.0	24.6	22.7	28.3	26.0	18.8
21:0	—	tr	0.8	tr	1.1	1.1
21:1	4.9	1.9	1.3	1.1	1.1	1.3
22:0	1.0	0.6	4.7	0.8	1.7	0.7
22:1	—	0.6	2.3	1.9	1.7	1.5
23:0	9.9	0.5	0.6	1.9	—	0.5
24:0	0.3	—	—	—	—	9.0
24:1	—	—	—	—	—	1.2
U/S[a]	2.2	3.0	3.2	2.9	3.4	2.4

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1975). *J. Sci. Food Agric. 26*: 1325–1332.

C. Turnip (*Brassica rapa*)

The turnip is an herbaceous dicotyledonous biennial plant. Its total lipid content is 0.10% (Haytowitz and Matthews, 1984). Different cultivars may have different percentage distributions of fatty acids, but the pattern of distribution is similar (Lima et al., 1965; Lepage, 1967). The main fatty acids are palmitic, oleic, linoleic, and linolenic acids, of which linolenic acid is the most abundant (Table 9.25). Turnip lipids have higher unsaturated fatty acids. The fatty acid composition of turnip, broccoli, Brussel sprout, Kohlrab, and Mustard Greens in various preparations are shown in Tables 9.26 through 9.30, respectively.

IV. CUCURBITS

All vegetable crops in this group belong to the Cucurbitaceae family.

A. Cucumber (*Cucumis sativus*)

The cucumber is an herbaceous annual vine trailing plant. The total lipid content is 0.10%–0.14% (Kinsella, 1971; Peng, 1976a,b; Peng and Geisman, 1976a; Haytowitz and Matthews, 1984; Geduspan

TABLE 9.21
Fatty Acid Composition (%) of Glycolipids in Cabbage and Sauerkraut

		Sauerkraut (Months)				
Fatty Acid	**Cabbage**	**0**	**2**	**4**	**8**	**12**
12:0	7.9	5.4	4.4	3.2	2.0	—
12:1	—	—	2.2	—	—	3.3
13:0	6.0	2.9	3.3	2.5	1.4	1.7
13:1	7.1	2.2	3.8	—	2.7	3.8
14:0	7.1	3.5	7.3	5.4	—	1.5
14:1	2.6	3.7	3.0	1.2	0.6	2.0
15:0	2.4	2.0	3.0	2.7	0.5	2.2
15:1	0.7	0.6	1.1	2.9	0.5	1.2
16:0	17.1	20.2	18.9	11.1	8.2	12.5
16:1	1.1	0.5	1.6	1.5	0.7	1.5
17:0	1.2	0.6	1.2	0.8	0.5	0.7
17:1	1.5	tr	0.5	1.2	0.8	0.7
18:0	3.5	4.6	7.3	4.1	1.8	3.6
18:1	4.2	3.0	5.0	3.1	1.7	3.1
18:2	4.1	5.1	5.0	3.2	2.5	4.9
20:0	—	2.0	1.0	1.0	2.7	1.6
18:3	26.4	26.0	16.8	39.5	58.3	45.2
21:0	—	5.2	—	1.1	2.7	2.3
21:1	—	—	2.0	1.3	4.1	3.0
22:0	2.9	—	3.1	2.7	1.9	1.1
22:1	—	—	1.3	—	2.8	—
23:0	3.7	2.5	1.2	—	—	1.1
24:0	—	—	—	—	1.9	—
U/S[a]	1.7	1.4	1.1	3.0	6.3	3.3

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1975). *J. Sci. Food Agric. 26*: 1325–1332.

and Peng, 1987). The distribution of cucumber lipids is neutral lipids 29.6%, glycolipids 60.0%, and phospholipids 10.4% (Peng and Geisman, 1976a). This is in contrast to Fishwick et al. (1977), who reported that phospholipids accounted for nearly half the weight of total lipids. Linolenic acid is recorded as the major fatty acid in total lipids (Draper, 1969; Galliard et al., 1976; Peng and Geismann, 1976a) and in neutral lipids, glycolipids, and phospholipids (Peng and Geisman, 1976a; Fishwick et al., 1977; Keppler and Novacky, 1989), which is in contrast to the lower concentration reported by Kinsella (1971). Chilling induced an increase in linolenic acid, and intermittent warming increased fatty acid unsaturation (Wang and Baker, 1979; Parkin and Kuo, 1989). The major fatty acids of fresh cucumber are listed in Table 9.31 (Peng, 1976a). The predominant fatty acids are palmitic, stearic, linoleic, and linolenic acids. Polar lipids have lower unsaturated fatty acids.

Mature green European cucumber fruits, grown in the greenhouse, were treated with butylated hydroxytoluene (BHT) and calcium chloride, stored at 4°C for 7 days, and transferred to 20°C for another 7 days (Geduspan and Peng, 1987). The total lipid content is not affected by chilling or chemical treatment, but changes in the lipid classes are observed. Neutral lipids are increased, and phospholipids are decreased. The predominant fatty acids are palmitic, stearic, oleic, linoleic, and linolenic acids (Table 9.32). Lauric (12:0) and myristic (14:0) acids are decreased by chilling and

TABLE 9.22
Fatty Acid Composition (%) of Phospholipids in Cabbage and Sauerkraut

		Sauerkraut (Months)				
Fatty Acid	**Cabbage**	**0**	**2**	**4**	**8**	**12**
?	4.5	—	—	—	1.4	5.7
12:0	2.5	5.6	4.5	1.2	1.8	3.0
12:1	2.0	3.6	3.7	tr	2.3	3.1
13:0	1.5	3.3	2.2	tr	1.0	3.3
13:1	3.9	3.0	2.9	1.7	6.7	2.3
14:0	2.2	3.1	3.2	0.6	1.3	7.3
14:1	1.2	3.5	3.2	tr	1.9	1.7
15:0	1.6	5.1	3.6	1.8	1.9	3.8
15:1	tr	5.8	1.0	0.8	tr	—
16:0	26.2	13.7	13.3	18.4	23.3	24.9
16:1	1.4	3.6	2.8	1.1	0.8	1.6
17:0	tr	1.0	0.6	1.1	0.6	0.5
17:1	tr	0.6	0.9	1.9	1.4	1.8
18:0	3.4	6.5	9.2	9.4	4.4	4.5
18:1	10.1	10.4	10.2	9.0	3.3	0.9
18:2	14.2	12.8	11.1	9.3	1.7	5.6
20:0	2.0	1.3	tr	2.8	2.0	0.6
18:3	16.3	11.5	9.7	9.7	7.9	11.9
21:0	—	2.0	—	3.4	1.5	3.6
21:1	2.5	0.8	1.9	3.4	1.0	2.0
22:0	3.2	2.6	1.4	4.5	4.5	3.5
22:1	2.5	0.8	1.9	3.4	1.0	2.0
23:0	—	5.5	—	—	—	—
U/S[a]	1.4	1.7	1.4	1.0	0.5	0.6

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1975). *J. Sci. Food Agric. 26*: 1325–1332.

TABLE 9.23
Fatty Acid Composition (%) of Cauliflower Mitochondria from Different Lipid Classes

Fatty Acid	**Total Lipids**	**Neutral Lipids**	**Monogalactosyl Diglyceride**	**Digalactosyl Diglyceride**	**Phospholipids**
<16:0	1.6	33.7	23.9	13.3	16.4
16:0	14.7	25.8	10.9	11.4	24.4
16:1	0.7	9.4	2.7	3.0	1.0
16:2	0.1	0.2	1.5	1.2	—
16:3	1.0	—	5.0	2.6	—
17:0	0.1	2.2	4.1	1.7	—
18:0	1.3	0.5	3.3	3.9	1.0
18:1	8.2	5.8	7.5	7.0	6.5
18:2	14.2	5.8	5.1	5.6	17.4
18:3	58.0	16.7	36.1	50.3	33.4
U/S[a]	5.0	1.1	3.4	4.1	2.3

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Schwertner, H.A., and Biale, J.B. (1973). *J. Lipid Res. 14*: 235–242. With permission. Courtesy of Lipid Research, Inc.

TABLE 9.24
Cauliflower Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	16:0	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated	Phytosterols
Cooked without salt	0.062	0.008	0.032	0.050	0.107	0.217	0.070	0.032	
Raw	0.028	0.004	0.014	0.023	0.076	0.099	0.032	0.014	18
Frozen	0.036	0.005	0.019	0.029	0.098	0.128	0.041	0.019	

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.25
Fatty Acid Composition (%) of Turnip Lipids

Fatty Acid	Total Lipids	Neutral Lipids	Polar Lipids
16:0	13.3	10.9	14.2
16:1	1.1	1.2	1.3
18:0	0.8	1.2	0.9
18:1	9.1	8.8	9.3
18:2	19.1	17.0	19.6
18:3	56.6	60.9	54.7
U/S[a]	6.7	7.2	5.5

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Lepage, M. (1967). *Lipids 2*: 244–250. With permission. Courtesy of the American Oil Chemists' Society.

TABLE 9.26
Turnip Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	12:0	14:0	16:0	18:0	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked without salt	0.001	0.001	0.008	0.001	0.009	0.032	0.042	0.008	0.005
Frozen			0.015	0.001	0.019	0.064	0.085	0.017	0.010
Raw			0.010	0.001	0.012	0.040	0.053	0.011	0.006

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.27
Broccoli Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked without salt	0.056	0.009	0.013	0.029	0.051	0.119	0.17	0.079	0.04
Frozen	0.039		0.005	0.020	0.031	0.105	0.136	0.044	0.020
Raw	0.029		0.006	0.010	0.017	0.021	0.038	0.039	0.011

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.28
Brussel Sprouts Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated	Phytosterols
Cooked without salt	0.001	0.090	0.004	0.005	0.033	0.077	0.168	0.26	0.105	0.039	
Frozen	0.001	0.072	0.003	0.004	0.025	0.061	0.134	0.207	0.084	0.031	
Raw		0.053	0.002	0.003	0.019	0.045	0.099	0.153	0.062	0.023	24

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.29
Kohlrabi Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked without salt	0.001	0.012	0.001	0.008	0.022	0.028	0.053	0.014	0.008
Raw		0.011	0.001	0.007	0.020	0.026	0.048	0.013	0.007

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.30
Mustard Greens Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	16:0	16:1	18:0	18:1	18:2	18:3	22:1	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked	0.006	0.001	0.002	0.018	0.024	0.022	0.069	0.046	0.012	0.110
Frozen	0.006	0.001	0.002	0.02	0.027	0.024	0.077	0.051	0.014	0.124
Raw	0.005		0.002	0.015	0.020	0.018	0.058	0.038	0.010	0.092

Source: USDA National Nutrient Database for Standard Reference.

chemical treatment. Palmitic acid is the major fatty acid and increases in each lipid class, especially in glycolipids, which increase to more than half the total acids after chemical treatment and chilling. Linoleic acid is increased by calcium chloride treatment in each fraction. However, the linolenic acid response is varied with chilling and chemical treatment. Calcium chloride treatment improves unsaturation slightly.

B. Pickles

Pederson et al. (1964) found marked changes in all lipid fractions during fermentation from both good and bloated dill pickles. Changes in lipid composition and fatty acid distribution of raw cucumbers and fresh-packed pickles during storage were studied by Peng and Geisman (1976a). Total lipids, neutral lipids, and phospholipids increased, and glycolipids decreased throughout the

TABLE 9.31
Major Fatty Acids in Fresh Cucumber

Fatty Acid	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
12:0	1.4	1.8	7.8	3.1
13:0	1.1	1.0	—	2.0
14:0	0.6	1.2	3.5	2.3
15:0	0.9	1.2	2.7	2.9
16:0	19.8	11.5	27.9	21.5
18:0	2.9	1.9	5.2	8.6
18:1	1.9	1.2	1.8	5.8
18:2	16.4	3.9	1.6	2.5
18:3	33.0	30.2	17.1	19.7
21:0	2.8	1.7	2.8	4.4
23:0	2.8	—	6.4	—
24:0	5.7	27.6	—	—
U/S[a]	2.3	2.6	0.6	0.9

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C. (1976a). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Res. Circ. 213*: 29.

TABLE 9.32
Fatty Acid Composition (%) of European Cucumber Lipids after Chemical Extraction

Treatment	Lipid Content	Fatty Acid							
		12:0	14:0	16:0	18:0	18:1	18:2	18:3	U/S[a]
Total lipids									
Untreated, unchilled	0.11	1.4	2.0	34.2	6.7	5.9	23.7	18.4	1.2
Acetone-sprayed, chilled as BHT control	0.11	0.5	1.9	34.2	3.0	3.5	30.5	23.5	1.7
1% BHT, chilled	0.11	0.6	0.9	27.3	3.6	4.6	33.9	22.9	2.0
Water-dipped, chilled as $CaCl_2$ control	0.09	0.6	1.7	38.3	3.3	3.6	25.1	21.5	1.2
6% $CaCl_2$, chilled	0.09	0.1	0.2	34.0	1.7	3.8	29.7	30.6	1.8
Neutral lipids									
Untreated, unchilled	17.7	4.1	4.1	39.6	4.5	4.1	11.4	13.9	0.7
Acetone-sprayed, chilled as BHT control	25.6	0.6	3.3	50.5	6.0	10.6	7.9	3.4	0.4
1% BHT, chilled	26.1	0.7	1.9	42.7	5.0	7.6	14.6	7.1	0.6
Water-dipped, chilled as $CaCl_2$ control	24.1	0.6	1.4	49.1	4.3	4.7	12.4	8.0	0.5
6% $CaCl_2$, chilled	30.5	1.1	1.5	34.4	3.9	10.6	24.0	8.0	1.1
Glycolipids									
Untreated, unchilled	37.8	2.3	3.8	48.9	9.7	4.8	11.0	16.1	0.5
Acetone-sprayed, chilled as BHT control	53.6	1.4	0.6	64.9	13.3	2.8	3.3	3.6	0.1
1% BHT, chilled	36.8	0.2	0.3	53.7	7.2	2.0	12.1	12.5	0.4
Water-dipped, chilled as $CaCl_2$ control	42.2	0.3	0.4	64.1	7.6	5.3	14.0	5.5	0.3
6% $CaCl_2$, chilled	40.5	0.4	0.4	64.9	4.3	4.9	20.6	3.6	0.4
Phospholipids									
Untreated, unchilled	44.5	1.3	1.4	47.1	5.8	4.0	20.3	15.7	0.8
Acetone-sprayed, chilled as BHT control	20.8	0.4	1.5	52.5	3.6	4.1	16.4	15.1	0.6
1% BHT, chilled	37.1	0.7	1.5	53.9	3.5	3.9	21.7	14.2	0.7
Water-dipped, chilled as $CaCl_2$ control	33.7	0.1	0.3	52.1	2.9	3.2	20.6	11.4	0.6
6% $CaCl_2$, chilled	29.0	0.1	1.0	42.2	3.6	4.7	25.8	20.8	1.1

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Geduspan, H.S., and Peng, A.C. (1987). *J. Sci. Food Agric. 40*: 333–339. Courtesy of Society of Chemical Industry.

TABLE 9.33
Lipid Content (%) of Cucumber and Pickles

Fatty Acid	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
Cucumber	0.14	29.6	60.0	10.4
0	0.19	24.7	53.7	21.6
2	0.19	25.2	56.4	18.4
4	0.19	27.5	53.2	19.3
8	0.19	34.6	52.7	12.7
10	0.20	39.0	51.1	9.9

Source: From Peng, A.C. (1976b). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Ser. 437*: 59.

entire experiment. Table 9.33 compares the distribution of three lipid classes of cucumber and dill pickles (Peng, 1976b). The distribution of fatty acids from the total lipids (Table 9.34) is representative of fresh-packed pickle composition. Alternation may occur through the use of different spice oils. Considerable differences in fatty acids are observed in their quantitative distribution at different storage times among different lipid classes. During storage, total unsaturated fatty acids change more than the saturated acids. The gross changes of fatty acids in the neutral lipid fraction (Table 9.35) illustrate that the major fatty acids in fresh cucumber are palmitic, linoleic, linolenic, and lignoceric (24:0) acids. After packing, most of the fatty acids increased except linoleic and lignoceric acids. After 12 months of storage, all major fatty acids generally increased in quantity. An increase in glycolipid content is typical of a photosynthesizing green plant. The fatty acid distribution of the glycolipid fraction (Table 9.36) is dominated by palmitic, linolenic, and tricosanoic (23:0) acids, followed by lauric, tridecenoic (13:1), stearic, and tricosenoic (23:1) acids. Packing and storage cause a considerable decrease of most fatty acids; however, unsaturation increases with packing and storage. The concentration of all fatty acids in the phospholipid fraction is increased after packing (Table 9.37) except linolenic acid. During storage, the concentration of palmitic, oleic, and linoleic acids decrease consistently, whereas that of lauric, myristic, stearic, and linolenic acids vary. Unsaturation decreases consistently.

Throughout the entire period of storage, palmitic and linolenic acids are the two predominant fatty acids distributed among all four lipid fractions and found in higher concentrations with random alterations. It is also interesting to note that the shorter-chain fatty acids (8:0–10:0) do not appear in the raw cucumber except for a small amount of capric (10:0) acid in the neutral lipids (Table 9.35). The presence of these shorter-chain fatty acids in pickles may indicate degradation of long-chain fatty acids and could influence the flavor of the product during storage.

To better understand brine changes, the fatty acid composition of fresh and recycled brine was analyzed (Peng and Geisman, 1976b). Table 9.38 shows that the predominant fatty acid is linolenic acid followed by caprylic (8:0) and palmitic acids. These three acids are in highest concentration in the fresh brine, then decrease during the first and second recycles, and increase in the third recycle. Lauric acid concentration increases as the brine is recycled. The fatty acids, especially the short-chain fatty acids, could be responsible for the characteristic odor associated with recycled brine.

C. Pumpkin (*Cucurbita* spp.)

Pumpkin is an annual plant. It contains 0.10% total lipids (Haytowitz and Matthews, 1984). The main fatty acids of pumpkin's glycolipids are linolenic and linoleic acids, followed by palmitic acid (Ito et al., 1974). The fatty acid composition of pumpkin and squash/zuccini in various preparations is shown in Tables 9.39 and 9.40, respectively.

TABLE 9.34
Fatty Acid Composition (mg/100 g Sample) of Total Lipids in Cucumber and Pickles during Storage

Fatty Acid	Cucumber	Pickles (Months)				
		0	2	4	8	12
8:0	—	—	—	3.6	3.0	0.2
9:0	—	—	—	5.9	7.1	4.5
10:0	—	—	13.4	1.9	1.5	1.0
11:0	—	0.6	—	—	0.8	0.8
11:1	0.6	2.1	0.8	—	—	—
12:0	2.1	1.7	1.2	2.1	1.9	1.0
12:1	2.5	2.1	2.3	—	—	0.6
13:0	1.7	1.7	2.5	2.1	2.1	1.2
?	—	—	0.9	—	—	—
13:1	1.5	2.6	2.3	2.3	2.3	—
14:0	0.8	1.5	1.5	1.7	1.3	2.9
14:1	0.7	0.7	0.6	1.5	0.9	—
?	0.7	—	—	—	—	—
15:0	1.2	1.3	0.8	1.1	0.9	1.4
15:1	0.7	0.6	0.4	1.1	0.4	0.8
16:0	27.5	32.2	38.7	28.3	35.3	20.6
16:1	0.7	1.3	1.2	2.1	2.7	0.8
17:0	0.7	1.1	1.7	1.3	1.3	1.4
17:1	0.9	0.9	1.5	0.8	0.8	0.8
18:0	4.2	10.1	5.0	8.6	9.0	9.6
18:1	2.8	7.6	5.0	8.3	6.3	8.4
18:2	22.7	34.8	29.1	27.7	23.3	20.6
20:0	1.9	2.1	0.2	—	—	—
18:3	45.8	69.5	61.0	50.5	55.1	38.6
21:0	3.9	4.1	0.4	4.6	3.4	7.8
21:1	—	—	—	11.1	8.2	17.6
22:0	3.3	2.8	—	5.7	4.2	11.0
22:1	—	—	0.6	—	—	17.4
23:0	3.9	4.1	18.4	10.3	9.5	8.0
23:1	—	0.9	2.3	2.9	1.3	12.7
24:0	7.9	—	—	4.6	8.0	9.2
24:1	—	—	—	1.0	—	5.3
U/S[a]	2.2	2.6	2.2	2.3	1.9	2.2

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C., and Geisman, J.R. (1976a). *J. Food Sci. 41*: 859–862.

V. PULSE CROPS

All vegetable crops in this group belong to the family Leguminosae. They are also referred to as legumes or pulse vegetables, and most of them are consumed in the immature stages of pod and/or seed.

A. Peas (*Pisum sativum*)

Peas are herbaceous annual plants. The total lipid content ranges from 1.2% to 6.0% depending on cultivar (Wagenknecht, 1957; Lee and Mattick, 1961; Haydar and Hadziyev, 1973; Reichert

TABLE 9.35
Fatty Acid Composition (mg/100 g Sample) of Neutral Lipids in Cucumber and Pickles during Storage

		Pickles (Months)				
Fatty Acid	**Cucumber**	**0**	**2**	**4**	**8**	**12**
9:0	—	—	0.3	1.0	1.2	0.6
10:0	0.7	7.7	2.8	3.2	4.5	3.3
11:0	—	—	0.6	0.8	1.3	0.3
11:1	—	—	1.0	—	—	—
12:0	0.7	1.2	0.9	1.0	0.9	0.6
12:1	0.7	0.7	0.8	—	—	—
13:0	0.4	0.9	0.5	1.1	1.2	0.7
?	0.4	—	—	—	—	—
13:1	0.6	0.6	1.4	0.9	1.1	—
14:0	0.5	1.5	1.0	0.8	0.9	1.5
14:1	0.5	0.5	0.8	0.7	0.6	—
?	0.5	0.3	—	—	—	—
15:0	0.5	0.7	0.8	0.5	0.5	1.0
15:1	0.6	0.5	0.8	1.0	0.9	1.0
16:0	4.7	7.0	9.1	8.7	10.9	8.9
16:1	0.3	0.2	0.6	0.7	1.1	—
17:0	0.2	0.2	0.9	0.5	0.8	0.9
16:2	—	0.1	0.2	0.5	—	—
17:1	0.2	0.3	0.8	0.8	1.1	1.4
18:0	0.8	0.9	2.5	2.5	3.6	3.6
18:1	0.5	0.6	1.6	1.7	1.6	2.3
19:0	0.2	—	—	—	—	—
18:2	1.6	0.6	5.0	4.7	3.4	3.6
20:0	—	0.3	0.1	0.5	0.6	—
18:3	12.5	16.7	9.7	11.0	13.1	11.0
21:0	0.7	1.3	0.2	1.2	1.0	—
21:1	0.4	0.4	0.1	0.6	1.3	1.4
22:0	0.5	1.7	0.3	0.8	0.8	1.6
22:1	0.6	—	—	—	0.3	2.6
23:0	—	0.8	0.3	0.3	0.5	1.2
23:1	0.5	—	0.1	0.7	0.5	1.5
?	—		0.2	—	0.3	—
24:0	11.3	0.3	3.7	6.3	11.9	14.4
24:1	—	—	1.2	—	—	14.0
?	—	—	—	—	—	2.2
U/S[a]	2.6	2.3	1.4	1.5	1.2	1.3

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C., and Geisman, J.R. (1976a). *J. Food Sci. 41*: 859–862.

and Mackenzie, 1982; Carnovale et al., 1983; Welch and Griffiths, 1984). Fatty acids are primarily palmitic, stearic, oleic, linoleic, and linolenic acids and vary with cultivar (Haydar and Hadziyev, 1973; Welch and Griffiths, 1984; Coxon and Wright, 1985; Sasaki et al., 1989). Welch and Griffiths (1984) analyzed the lipid distribution of pea cotyledons, embryo, and testa and found that the lipid content of the embryo is considerably higher than that of the cotyledons and testa and that the testa

TABLE 9.36
Fatty Acid Composition (mg/100 g Sample) of Glycolipids in Cucumber and Pickles during Storage

		Pickles (Months)				
Fatty Acid	**Cucumber**	**0**	**2**	**4**	**8**	**12**
8:0	—	—	—	4.1	3.1	2.3
10:0	—	—	8.6	15.7	22.3	14.2
?	—	—	—	—	—	1.1
12:0	6.1	3.5	3.6	3.2	3.7	2.3
12:1	—	1.8	1.5	—	—	2.7
13:0	—	2.2	1.4	3.8	3.6	—
13:1	4.4	4.2	4.1	2.4	2.8	—
14:0	2.8	1.5	2.5	1.3	2.0	2.3
14:1	3.5	3.2	3.5	2.5	3.1	—
?	3.2	—	—	—	—	—
15:0	2.2	2.1	0.5	—	—	2.9
15:1	—	0.9	0.3	—	—	1.6
16:0	22.1	21.4	16.0	9.1	10.1	12.2
16:1	1.6	0.8	1.3	1.3	0.9	1.8
17:0	1.0	0.3	0.8	0.9	0.8	1.9
17:1	0.8	1.3	1.6	0.9	1.2	1.9
18:0	4.1	3.0	3.5	2.9	3.0	4.8
18:1	1.6	1.6	2.6	2.3	2.0	2.8
18:2	1.3	0.9	1.1	2.5	1.5	2.2
20:0	—	—	0.4	0.3	—	—
18:3	13.6	45.5	47.0	44.0	38.2	34.9
21:0	2.3	1.0	2.4	0.5	—	4.4
21:1	—	—	—	1.1	1.6	—
22:0	—	2.7	2.2	1.4	—	0.9
22:1	—	—	0.4	—	—	—
23:0	9.3	—	—	0.4	—	2.3
23:1	3.3	2.2	2.9	1.1	0.6	4.9
U/S[a]	0.6	2.0	2.6	4.1	3.2	2.3

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C., and Geisman, J.R. (1976a). *J. Food Sci. 41*: 859–862.

is high in saturated fatty acids, whereas the cotyledons and embryo contain higher unsaturated acids (Tables 9.41 and 9.42).

B. Soybeans (*Glycine max*)

Immature soybeans (*mao tou*) are soybeans picked green when the pods are greenish yellow and the beans are large and tender. The green soybeans are usually cooked in the pod with some salt and then served. The pod is removed before eating, and the beans are plump and tender. They are considered a delicate hors d'oeuvre (Wang et al., 1979).

The total lipid content of soybeans increases with maturity (Rackis, 1981). There is 1.2% total lipids 9 days after flowering (DAF), which increases to 20.8% at 97 DAF. Neutral lipids increase fivefold at the expense of polar lipids (Privett et al., 1973). Changes in fatty acids are observed in the early stages of seed development. The palmitic, stearic, and linolenic acid concentrations are

TABLE 9.37
Fatty Acid Composition (mg/100 g Sample) of Phospholipids in Cucumber and Pickles during Storage

		Pickles (Months)				
Fatty Acid	**Cucumber**	**0**	**2**	**4**	**8**	**12**
8:0	—	—	—	1.6	—	—
9:0	—	0.6	3.1	4.1	3.0	1.3
10:0	—	1.7	0.8	1.8	1.6	0.4
11:0	0.2	0.4	0.4	—	—	0.2
11:1	—	1.4	0.5	—	—	0.1
12:0	0.5	0.7	0.5	1.2	0.8	0.3
12:1	0.3	0.8	0.3	—	—	0.7
13:0	0.3	0.5	0.3	1.5	1.0	—
13:1	0.6	1.8	0.7	1.0	0.7	—
14:0	0.3	0.6	0.5	0.7	0.5	0.5
?	0.2	—	—	—	—	—
14:1	0.3	0.7	0.5	0.7	0.7	—
15:0	0.4	0.7	0.7	0.5	0.7	0.3
15:1	0.1	0.6	0.2	0.5	—	0.3
16:0	3.1	14.3	13.2	13.2	8.2	5.1
16:1	0.3	0.6	0.5	0.5	0.9	0.6
17:0	0.2	0.4	0.1	0.4	0.6	0.4
16:2	0.2	0.5	0.1	0.4	—	—
17:1	0.3	1.1	1.1	1.2	0.7	1.0
18:0	1.2	2.7	4.0	3.0	1.2	1.7
18:1	0.8	1.2	0.7	0.5	0.5	0.4
19:0	—	—	—	—	—	0.2
18:2	0.4	1.8	1.2	0.3	—	0.2
20:0	0.6	0.7	0.5	0.2	—	—
18:3	2.9	1.4	2.5	2.4	1.7	2.2
21:0	0.6	1.3	0.8	0.5	—	1.0
21:1	—	—	—	0.3	—	—
22:0	0.4	1.3	1.0	0.3	1.4	0.4
23:0	—	0.6	1.1	0.1	—	0.8
23:1	0.2	1.9	—	—	—	—
24:0	—	—	—	—	—	1.3
24:1	—	—	—	—	—	0.8
U/S[a]	0.9	0.3	0.3	0.2	0.2	0.4

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C., and Geisman, J.R. (1976a). *J. Food Sci. 41*: 859–862.

reduced, whereas oleic and linoleic acids increase (Table 9.43) from 24 DAF to 72 DAF (Rubel et al., 1972). The unsaturation ratio indicates that the percentage of saturated fatty acids decreases rapidly in the early stages.

VI. SPINACH (*Spinacia oleracea*)

Spinach is an herbaceous annual plant. It belongs to the family Chenopodiaceae (goosefoot) and has 0.35% total lipids (Haytowitz and Matthews, 1984). Linolenic acid is 54.0%–60.0%, palmitic acid

TABLE 9.38
Fatty Acid Composition of Fresh and Recycled Brine

Fatty Acid	Fresh	1 Recycle	2 Recycles	3 Recycles
8:0	20.9	20.1	17.4	18.3
10:0	5.2	5.2	6.7	4.9
12:0	1.9	2.2	2.7	3.1
12:1	1.2	1.7	6.0	4.9
13:0	2.5	2.4	—	—
13:1	2.9	3.3	3.9	3.5
14:0	1.7	2.2	2.7	2.6
14:1	1.5	1.7	2.9	2.0
15:0	2.3	2.2	2.7	2.7
16:0	11.7	9.2	6.7	9.3
16:1	2.9	3.0	3.3	2.9
17:0	—	1.1	—	1.5
17:1	1.4	1.7	2.0	1.8
18:0	2.7	3.5	2.9	4.0
18:1	6.9	4.6	5.2	5.7
18:2	2.9	3.6	2.7	3.1
20:0	2.1	2.2	2.5	1.6
18:3	27.6	27.4	25.3	26.0
?	1.3	1.6	2.8	1.6
20:0	0.4	0.8	1.6	0.4
U/S[a]	2.6	2.8	3.5	2.6

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Peng, A.C., and Geisman, J.R. (1976b). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Res. Circ. 213*: 30–31.

TABLE 9.39
Pumpkin Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Poly-unsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Mono-unsaturated	Phytos-terols
Canned	0.003	0.018	0.103		0.008		0.007	0.008	0.015	0.146	0.037	
Cooked without salt	0.001	0.005	0.026	0.005	0.002	0.005	0.002	0.002	0.004	0.037	0.009	
Raw	0.001	0.006	0.037	0.006	0.003	0.006	0.002	0.003	0.005	0.052	0.013	12

Source: USDA National Nutrient Database for Standard Reference.

12.9%–15.0%, and linoleic acid 9.4%–16.3%. Hexadecatrienoic (16:3) (4.6%–10.6%) and *trans*-3-hexadecenoic (1.8%–3.0%) acids are also present (Allen et al., 1966; Rouser et al., 1967; Kates, 1970; Hitchcock and Nichols, 1971; Kinsella, 1988). Mattick and Lee (1961) observed a 17-carbon fatty acid, *n*-heptadecanoic acid (17:0). Spinach fatty acid composition in various preparations is shown in Table 9.44.

TABLE 9.40
Squash/Zuccini Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	12:0	14:0	16:0	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked without salt	0.001	0.001	0.056	0.006	0.021	0.049	0.082	0.131	0.064	0.023
Raw	0.001	0.001	0.038	0.004	0.014	0.033	0.056	0.089	0.044	0.016
Frozen	0.001		0.024	0.003	0.009	0.021	0.035	0.076	0.027	0.014

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.41
Fatty Acid Composition (%) of Peas

			Fatty Acid					
Cultivar	**Fraction**	**Lipid**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**U/S[a]**
Scout	Cotyledons	3.15	13.5	3.3	22.6	53.6	7.1	5.0
	Embryo	8.80	14.8	3.5	17.8	53.4	10.7	4.5
	Testa	0.12	36.7	25.4	9.4	24.2	4.4	0.6
Vineta	Cotyledons	1.85	12.5	3.6	28.3	47.3	8.5	5.2
	Embryo	7.00	15.1	4.0	29.2	43.6	8.2	4.2
	Testa	0.24	34.8	16.4	15.8	22.1	11.1	1.0

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Welch, R.W., and Griffiths, D.W. (1984). *J. Sci. Food Agric. 35*: 1282–1289. With permission. Courtesy of Society of Chemical Industry.

VII. SWEET POTATOES (*Ipomoea batatas*)

The sweet potato is an herbaceous dicotyledonous perennial plant that is grown in tropical and subtropical zones. The total lipid content ranges from 0.6% to 2.7% and varies with cultivar, curing, and storage (Boggess et al., 1967, 1970; Walter et al., 1971; Alexandridis and Lopez, 1979; Charoenpong and Peng, 1990). The Centennial cultivar is composed of 42.1%–48.1% neutral lipids, 30.8%–31.2% glycolipids, and 20.7%–27.1% phospholipids (Walter et al., 1971; Alexandridis and Lopez, 1979); however, processing and storage may alter their distribution. Charoenpong and Peng (1990) found changes in the Georgia Jet cultivar lipid composition under atmospheric and low oxygen (7%) storage at 15.5°C and 85% relative humidity, as shown in Table 9.45. The total lipid content of fresh samples is 0.6%, lower than that of Centennial sweet potatoes when expressed on a dry weight basis (Boggess et al., 1967, 1970; Walter et al., 1971; Alexandridis and Lopez, 1979). Total lipids from both treatments are significantly increased after the first month of storage and then decrease thereafter; this is, probably due to lipid compositional changes during storage or synthesis from nonlipid components (Boggess et al., 1967). Neutral lipids in stored roots are markedly increased under two storage conditions at the expense of polar lipids. Although treatment does not affect the levels of total lipids and lipid fractions, storage time does affect their distributions.

The major fatty acids are palmitic, stearic, oleic, linoleic, and linolenic acids in all lipid fractions, but there are cultivar differences (Boggess et al., 1970) and storage influences (Boggess et al., 1967;

TABLE 9.42
Beans and Peas Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:1	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Pinto beans											
Frozen		0.054		0.007	0.037	0.105	0.186		0.276	0.061	0.035
Cooked without salt		0.051		0.006	0.035	0.099	0.177		0.276	0.058	0.035
Raw	0.001	0.229		0.005	0.229	0.170	0.237		0.407	0.235	0.229
Navy beans											
Cooked without salt		0.053		0.012	0.047	0.089	0.117		0.324	0.065	0.094
Raw		0.087		0.015	0.075	0.215	0.345		0.905	0.109	0.157
Kidney											
Cooked		0.074		0.01	0.045	0.123	0.194	0.005	0.318	0.083	0.045
Green beans											
Raw		0.022		0.004	0.004	0.023	0.036		0.059	0.026	0.005
Lentils											
Cooked		0.047		0.005	0.095	0.166	0.035		0.201	0.053	0.095
Raw		0.133	0.003	0.015	0.180	0.404	0.109		0.516	0.156	0.189
Lima beans											
Canned	0.001	0.028	0.001	0.008	0.012	0.052	0.023	0.001	0.074	0.039	0.015
Cooked without salt	0.001	0.065	0.002	0.018	0.029	0.118	0.052	0.002	0.174	0.034	0.089
Frozen	0.001	0.071		0.009	0.021	0.116	0.056		0.172	0.021	0.081
Raw	0.002	0.118	0.004	0.032	0.052	0.215	0.095	0.004	0.309	0.161	0.062
Mung bean											
Canned		0.011		0.003	0.008	0.015	0.006		0.020	0.016	0.008
Cooked		0.018		0.005	0.012	0.023	0.009		0.032	0.025	0.012
Raw		0.250		0.071	0.161	0.357	0.027		0.384	0.348	0.161
Soybean											
Cooked	0.025	0.952	0.025	0.320	1.956	4.465	0.598		5.064	1.297	1.981
Raw	0.055	2.116	0.055	0.712	4.348	9.925	1.33		11.26	2.884	4.404
Peas											
Canned		0.056		0.006	0.031	0.132	0.031		0.163	0.062	0.031
Cooked		0.035		0.004	0.019	0.082	0.019		0.102	0.039	0.019
Frozen		0.06		0.007	0.033	0.142	0.033		0.175	0.066	0.033
Raw		0.064		0.007	0.035	0.152	0.035		0.187	0.071	0.035

Source: USDA National Nutrient Database for Standard Reference.

Charoenpong and Peng, 1990). Linoleic acid is the predominant acid, followed by palmitic acid. Storage time and conditions also affect fatty acid distribution (Table 9.46) (Charoenpong and Peng, 1990). Changes in fatty acid composition of each lipid class are shown in Tables 9.47 through 9.49. Linoleic and palmitic acids decreased significantly from the original concentration in all samples of

TABLE 9.43
Fatty Acid Composition (%) of Soybeans at Various Stages of Development

Days after Flowering	16:0	18:0	18:1	18:2	18:3	U/S[a]
24	19.0	8.2	7.5	35.0	30.0	2.7
32	14.5	3.2	24.0	46.2	12.1	4.6
43	10.6	3.1	26.2	52.0	8.6	6.3
53	10.4	2.8	25.4	53.8	6.8	6.5
63	10.4	3.1	26.1	54.1	6.3	6.4
72	10.4	2.9	26.4	54.1	5.8	6.5

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Rubel, A., et al. (1972). *Crop Sci. 12*: 739–741. With permission. Courtesy of Crop Science of America.

TABLE 9.44
Spinach Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Canned	0.011	0.059	0.006	0.005	0.006	0.031	0.165	0.209	0.081	0.014
Cooked	0.006	0.031	0.003	0.003	0.003	0.016	0.085	0.108	0.042	0.007
Frozen		0.292				0.166		0.166	0.292	
Raw	0.01	0.049	0.005	0.004	0.005	0.026	0.138	0.165	0.063	0.010

Source: USDA National Nutrient Database for Standard Reference.

TABLE 9.45
Percentage of Changes in Total Lipids and Lipid Classes of Sweet Potatoes during Storage at 15.5°C and 85% Relative Humidity at Atmospheric and Low Oxygen

	Total Lipids		Neutral Lipids		Glycolipids		Phospholipids	
Storage Time (months)	Atmospheric	Low Oxygen	Atmospheric	Low Oxygen	Atmospheric	Low Oxygen	Atmospheric	Low Oxygen
0	0.6	0.6	38.6	38.6	38.2	38.2	23.2	23.2
1	1.1	1.0	51.0	49.9	33.8	35.0	15.2	15.1
2	1.1	1.1	52.0	53.4	33.8	35.8	14.2	10.8
3	0.8	1.0	60.0	55.4	28.0	32.2	12.0	12.4

Source: From Charoenpong, C., and Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Circ. 121*: 15–20.

TABLE 9.46
Percentage of Changes in Fatty Acid Composition of Total Lipids of Sweet Potatoes during Storage at Atmospheric and Low Oxygen Conditions

		Atmospheric Conditions (months)			Low Oxygen Conditions (months)		
Fatty Acid	Fresh 0 month	1	2	3	1	2	3
12:0	1.3	3.2	1.8	2.9	2.2	4.0	2.2
16:0	22.6	22.0	23.7	25.2	22.5	25.8	29.1
16:1	—	1.4	1.6	1.3	1.1	1.7	—
17:0	1.0	tr	1.6	2.4	0.7	2.9	tr
18:0	4.2	9.9	10.2	8.5	9.1	10.5	13.0
18:1	2.1	1.2	2.8	2.7	3.8	3.1	—
18:2	55.3	44.1	40.4	41.5	39.7	34.1	45.5
20:0	tr	1.2	2.5	3.4	4.5	4.3	tr
18:3	7.5	9.7	8.3	7.1	8.4	7.3	7.4
22:0	3.6	tr	1.5	—	0.6	—	tr
24:0	tr	3.0	tr	tr	—	—	—
U/S[a]	2.4	1.7	1.5	1.5	1.6	1.2	1.2

tr = trace.

U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Charoenpong, C., and Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Circ. 121*: 15–20.

TABLE 9.47
Percentage of Changes in Fatty Acid Composition of Neutral Lipids of Sweet Potatoes during Storage at Atmospheric and Low Oxygen Conditions

		Atmospheric Conditions (months)			Low Oxygen Conditions (months)		
Fatty Acid	Fresh month 0	1	2	3	1	2	3
16:0	25.7	18.9	26.3	27.2	21.7	29.7	28.1
16:1	—	tr	1.2	1.0	1.2	1.9	tr
18:0	7.9	11.7	11.7	9.8	9.9	11.2	15.2
18:1	1.4	4.6	3.8	3.2	—	3.4	—
18:2	53.6	34.4	37.6	42.0	42.9	35.7	46.9
20:0	tr	2.3	4.3	3.7	4.4	3.9	tr
18:3	8.2	5.8	8.5	7.4	9.2	6.9	6.5
22:0	0.8	8.7	tr	0.7	3.8	—	—
U/S[a]	1.9	1.5	1.3	1.4	1.6	1.1	1.2

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Charoenpong, C., and Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Circ. 121*: 15–20.

TABLE 9.48
Percentage of Changes in Fatty Acid Composition of Glycolipids of Sweet Potatoes during Storage at Atmospheric and Low Oxygen Conditions

	Fresh (month)	Atmospheric Conditions (months)			Low Oxygen Conditions (months)		
Fatty Acid	**0**	**1**	**2**	**3**	**1**	**2**	**3**
10:0	2.3	2.1	tr	tr	2.9	3.9	0.6
12:0	3.5	3.4	7.9	7.9	2.7	9.6	7.6
13:0	—	1.7	2.7	—	0.8	—	—
14:0	tr	1.0	tr	2.2	5.0	3.0	2.0
15:0	0.6	4.3	2.6	2.3	1.8	1.8	—
16:0	18.8	15.4	15.0	15.6	14.9	16.4	18.8
16:1	—	1.8	0.6	1.0	3.4	3.2	1.8
17:0	2.1	3.6	5.8	9.3	2.4	7.5	6.9
18:0	7.3	8.3	9.4	8.6	9.4	6.3	13.1
18:1	0.9	1.3	5.2	3.1	3.6	1.6	—
18:2	54.0	37.6	34.3	34.3	37.5	33.8	43.9
18:3	8.5	11.4	9.4	6.8	9.1	8.8	5.0
U/S[a]	2.4	2.1	2.0	1.8	2.0	1.9	1.5

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Charoenpong, C., and Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Circ. 121*: 15–20.

TABLE 9.49
Percentage of Changes in Fatty Acid Composition of Phospholipids of Sweet Potatoes during Storage at Atmospheric and Low Oxygen Conditions

	Fresh (month)	Atmospheric Conditions (months)			Low Oxygen Conditions (months)		
Fatty Acid	**0**	**1**	**2**	**3**	**1**	**2**	**3**
10:0	tr	2.3	1.1	tr	—	—	—
12:0	tr	2.2	2.0	2.2	1.1	0.9	—
15:0	tr	2.0	1.2	1.3	1.9	1.3	1.1
16:0	32.0	19.2	22.9	25.7	24.5	25.0	28.9
16:1	—	4.3	2.4	2.4	1.0	3.1	—
17:0	0.8	—	0.8	—	tr	—	2.1
18:0	5.7	9.5	8.4	9.3	9.6	9.4	16.4
18:1	2.1	4.7	4.1	5.5	4.6	3.6	tr
18:2	54.4	36.0	40.5	42.4	40.1	42.5	44.7
20:0	tr	6.9	5.0	2.5	5.4	2.5	—
18:3	1.6	6.5	7.1	6.6	7.5	6.7	6.0
U/S[a]	1.5	1.6	1.6	1.6	1.5	1.5	1.1

tr = trace.

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Charoenpong, C., and Peng, A.C. (1990). *Ohio State Univ.-Ohio Agric. Res. Develop. Center Spec. Circ. 121*: 15–20.

both treatments but increased at the end of the storage period. Stearic acid increased consistently, and linolenic acid varied with storage time. The unsaturated fatty acids decrease and saturated acids increase during storage. However, phospholipids did not show any change.

Heat processing of sweet potatoes into flakes and subsequent storage alters total lipids, lipid classes, and their fatty acid composition (Walter and Purcell, 1974; Alexandridis and Lopez, 1979). Total lipids decrease significantly, and some glycolipids and phospholipids also decrease after processing and storage. All lipid classes are composed mainly of palmitic and linoleic acids and appreciable amounts of stearic, oleic, linolenic, arachidic (20:0), and heneicosanoic (21:0) acids. Lauric (12:0) acid is found in glycolipids. Although sweet potato is high in unsaturated fatty acids (more than 50%), the unsaturation ratio is decreased upon heat processing and storage. The authors speculated that this could be due to lipids progressively becoming more difficult to extract and/or to volatilizing subsequent to lipid oxidation.

VIII. SWEET CORN (*Zea mays*)

Sweet corn is an annual herbaceous monocotyledonous plant. It belongs to the family Gramineae (grass). It is consumed fresh (e.g., corn on the cob), canned, or frozen. The total lipid content varies with cultivars and strains and ranges from 0.4% to 18.4% (Weber, 1969; Flora and Wiley, 1972; Dudley et al., 1974; Weber and Alexander, 1975; Haytowitz and Matthews, 1984). During kernel development, the total lipids, neutral lipids, glycolipids, and phospholipids reach a maximum concentration 45 days after pollination (DAP), then either decrease or level off thereafter (Leng, 1967; Weber, 1969, 1970; Pascual and Wiley, 1974). The major fatty acids are typical plant lipids containing palmitic, stearic, oleic, linoleic, and linolenic acids (Weber, 1969; Flora and Wiley, 1972; Weber and Alexander, 1975; Leibovitz and Ruckenstein, 1983). The positional distribution of those fatty acids was stereochemically analyzed, and unsaturated fatty acids were found to predominantly occupy the C-2 (middle, or β) position of corn oil triglyceride molecules (Brockerhoff and Yurkowski, 1966; Weber and Alexander, 1975; Ohnishi et al., 1989); 97%–98% of them at this position accounted for unsaturation. Saturated fatty acids were found at the C-1 and C-3 (outer, or α and α′) positions. The fatty acid composition also changes during kernel development from immature to mature (Curtis et al., 1968; Weber, 1969; Jellum, 1970; Poneleit and Davis, 1972), as indicated in Tables 9.50 and 9.51. In the triglyceride fraction, there are large changes in the fatty acid composition from 10 to 30 DAP; the percentages of palmitic, stearic, linoleic, and linolenic acids decrease and oleic acid increases, and the unsaturation ratio reaches its maximum and then remains

TABLE 9.50
Fatty Acid Composition (%) of Illinois High-Oil Corn Triglyceride

Days of Flowering	16:0	18:0	18:1	18:2	18:3	U/S[a]
10	19.1	2.1	10.9	51.7	16.2	3.7
15	14.0	1.7	35.7	46.2	2.4	5.4
30	13.9	1.3	40.4	44.1	0.3	8.2
45	12.8	1.7	35.2	49.7	0.6	5.9
60	14.2	1.7	35.2	48.5	0.4	5.3
75	11.5	1.3	32.0	54.2	1.0	6.8
90	12.6	2.1	36.1	48.6	0.6	5.8

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Weber, E.J. (1969). *J. Am. Oil Chem. Soc. 46*: 485–488. With permission. Courtesy of The American Oil Chemists' Society.

TABLE 9.51
Fatty Acid Composition (%) of Illinois High-Oil Corn Lipids

Days of Flowering	16:0	18:0	18:1	18:2	18:3	U/S[a]
10	27.0	1.5	8.7	49.8	13.0	2.5
15	24.5	1.3	8.5	58.0	7.7	2.9
30	22.1	0.9	12.1	59.7	5.2	3.3
45	20.6	1.2	12.9	60.9	4.4	3.6
60	22.7	1.8	15.9	55.2	4.4	3.1
75	21.4	1.4	23.3	50.5	3.4	3.4
90	21.5	1.3	28.4	46.9	1.9	3.4

[a] U/S = unsaturation ratio = (18:1 + 18:2 + 18:3)/(16:0 + 18:0).

Source: From Weber, E.J. (1969). *J. Am. Oil Chem. Soc. 46*: 485–488. With permission. Courtesy of The American Oil Chemists' Society.

TABLE 9.52
Fatty Acid Composition (%) of Corn Chips

Fatty Acid	Corn Chips
14:0	0.1–0.8
16:0	9.0–24.8
16:1c	0.2–0.7
18:0	2.4–4.2
18:1t	0.0–16.2
18:1c	17.8–39.0
18:2tt	0.0–1.8
18:2ct	1.4–5.5
18:2ω6	20.7–51.6
18:3ω3	0.3–2.4
20:1	0.2–0.8
Saturated	12.8–28.1
Monounsaturated	18.8–55.9
Polyunsaturated	26.3–55.4
Trans-unsaturated	0.8–22.0

Source: From Smith, L.M., et al. (1985). *J. Am. Oil Chem. Soc. 62*: 996–999. With permission. Courtesy of the American Oil Chemists' Society.

nearly constant throughout. The fatty acids of the polar lipids are more saturated, and their unsaturation ratio is much lower than that of the triglycerides. Palmitic, stearic, and linolenic acids decrease, whereas oleic and linoleic acids increase from 10 to 45 DAP; then linoleic acid and the unsaturation ratio start to fall (Weber, 1970).

Corn chips are as popular as potato chips. They are deep-fat fried, and the total lipid content is greatly increased, from 0.4%–18.4% in raw corn to 32.8%–37.6% after frying (Smith et al., 1985). The fatty acid composition of frying oil may have a great influence on the fatty acid profiles of the fried products. Smith et al. (1985) analyzed seven corn snacks purchased from market; the fatty acid contents are shown in Table 9.52. Because the source of frying oil is not known, the variation

TABLE 9.53
Yam Fatty Acid Composition in Various Preparations

Fatty Acids (g/100 g)	16:0	18:0	18:1	18:2	18:3	Fatty Acids, Total Polyunsaturated	Fatty Acids, Total Saturated	Fatty Acids, Total Monounsaturated
Cooked	0.027	0.003	0.005	0.050	0.009	0.060	0.029	0.005
Raw	0.034	0.004	0.006	0.064	0.012	0.076	0.037	0.006

in the main fatty acids is noticed, especially palmitic (9.0%–24.8%), oleic (17.9%–39.0%), elaidic (0%–16.2%), and linoleic (20.7%–51.6%) acids.

IX. YAM (*Dioscorea* spp.)

Yam is a monocotyledonous plant. It belongs to the family Dioscoreaceae. The tuber contains 0.17%–1.5% total lipid content depending on the cultivar (Osagie and Opute, 1982; Coursey, 1983; Haytowitz and Matthews, 1984). Palmitic and linoleic acids are the predominant fatty acids (Opute and Osagie, 1978; Osagie and Opute, 1982; Kouassi et al., 1988). The glycolipids exhibit a relatively higher concentration of linolenic acid (Osagie and Opute, 1982). Yam fatty acid composition in various preparations is shown in Table 9.53.

X. CASSAVA (*Manihot esculenta*)

Cassava is a basic food in the tropics. It is a dicotyledonous perennial bushy shrub and belongs to the family Euphorbiaceae. The total lipid content ranges from 0.30% to 0.48% (Odigboh, 1983; Haytowitz and Matthews, 1984; Ezeala, 1985). The major fatty acids of cassava tubers are oleic (37.5%–43.7%), palmitic (28.8%–35.1%), linoleic (14.5%–23.0%), and linolenic (5.5%–11.0%) acids (Harris, 1970; Hudson and Ogunsua, 1974; Opute and Osagie, 1978; Ezeala, 1985).

XI. AMARANTH (*Amaranthus* spp.)

Edible amaranth is an annual monocotyledonous plant, also called Chinese spinach or tampala, and belongs to the family Amaranthaceae. The total lipid content is 0.33% (Haytowitz and Matthews, 1984), and linolenic, palmitic, linoleic, and oleic acids are the major fatty acids (Fernando and Bean, 1984; Lakshminarayana et al., 1984).

SUMMARY

It is important when consuming a "healthy diet" to include fresh, frozen, or canned vegetables, and as the data presented in this chapter illustrate the consumption of vegetables, in general, give a favorable polyunsaturated to saturated fatty acid ratio and in many vegetables a favorable omega 3 to omega 6 ratio.

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10 Fatty Acids in Oilseeds (Vegetable Oils)

Pamela J. White

CONTENTS

I. INTRODUCTION

Oilseeds are, by definition, those seeds or crops grown mainly for their oil. This chapter presents data on the fatty acid composition of selected vegetable oils, factors affecting fatty acid composition, general stability of the oil, factors affecting its stability, and any special features that make the oil or its production unique. Other chapters deal specifically with fatty acids in fruits and fruit products, in vegetables and vegetable products, and in grains and grain products; therefore, oils not discussed in this chapter will be covered there. There may also be some overlap among chapters. A comprehensive reference regarding all aspects of industrial and edible oil and fat products can be found elsewhere (Shahidi, 2005).

Table 10.1 presents detailed data on the typical fatty acid composition of all oils, as traditional sources, discussed in this chapter plus some animal fats and other unique fats and oils for comparison Panford and deMan (1990). Although the data are not discussed, the table also includes typical ranges for the iodine value and the saponification value for handy reference. The compositions listed are for conventional vegetable oils. As noted in this chapter and in Chapter 15, "Genetic Alteration of Food Fats and Oils," gene alternations have vastly expanded the available fatty acid compositions possible in most vegetable oil crops; thus, many additional modified crops with altered fatty acid compositions exist.

Although the fatty acid composition of many vegetable oils has been reported in the literature since the early 1900s, the accuracy of the reported data has improved dramatically since the introduction

TABLE 10.1
Typical Compositions and Chemical Constants of Common Edible Fats and Oils[a]

Butyric	Caproic	Caprylic	Caprie	Undecanoic	Lauric	Tridecanoic	Myristic	Myristoleic	Pentadecanoic	Pentadecenoic	Palmitic	Palmitoleic	Margaric	Margaroleic	Stearic	Oleic	Linoleic
4:0	6:0	8:0	10:0	11:0	12:0	13:0	14:0	14:1	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2
	0.4	5.3	5.9		44.2		15.8				8.6				2.9	15.1	1.7
3.8	2.3	1.1	2.0	0.1	3.1	0.1	11.7	0.8	1.6		26.2	1.9	0.7	0.2	12.5	28.2	2.9
											3.9	0.2			1.9	64.1	18.7
					0.2		1.3	0.2			23.2	6.5	0.3	0.1	6.4	41.6	18.9
					0.1		0.5				28.4	0.2			3.5	23.0	37.8
							0.1				25.8	0.3			34.5	35.3	2.9
	0.5	8.0	6.4		48.5		17.6				8.4				2.5	6.5	1.5
	0.3	8.7	7.2	0.1	47.3		16.2				7.7				3.2	8.3	1.0
											12.2	0.1			2.2	27.5	57.0
							0.9				24.7	0.7	0.1		2.3	17.6	53.3
			0.1		0.1		1.5		0.2		24.8	3.1	0.5	0.3	12.3	45.1	9.9
											4.8				4.7	19.9	15.9
	0.1	1.3	1.5		46.2		32.4				5.6	0.1			2.2	8.9	1.5
							0.2				17.1	0.5			1.4	33.4	44.8
											13.7	1.2			2.5	71.1	10.0
					0.3		1.1				45.1	0.1			4.7	38.8	9.4
	0.3	3.9	4.0		49.6		16.0				8.0				2.4	13.7	2.0
							0.1				11.6	0.2	0.1		3.1	46.5	31.4
							0.1				2.8	0.2			1.3	23.8	14.6
		0.1	0.1		0.4		0.5				16.4	0.3			2.1	43.8	34.0
							0.1				6.5				2.4	13.1	77.7
							0.1				5.5	0.1			2.2	79.7	12.0
											9.9	0.3			5.2	41.2	43.3
							0.1				11.0	0.1			4.0	23.4	53.2
					0.5		0.2				6.8	0.1			4.7	18.6	68.2
			0.1		0.1		3.3	0.2	1.3	0.2	25.5	3.4	1.5	0.7	21.6	38.7	2.2
			0.2		0.3		5.2	0.3	0.8	0.3	23.6	2.5	2.0	0.5	24.5	33.3	4.0
	0.2	2.9	2.3		51.8		22.0				6.8				2.3	9.3	2.4

[a] Fatty acid compositions were determined by GLC and are expressed as mean average weight percent composition on a fatty acid basis. Trace acids (less than 0.1%) are excluded.

[b] Low-erucic-acid rapeseed oil.

[c] Values from Panford and deMan (1990).

[d] Hammond et al. (1997) identified this fatty acid as *cis*-11-eicosenoate.

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of gas–liquid chromatography (GLC) techniques in the 1950s. In fact, better separation columns and new techniques continue to provide more accurate and reliable data. Other modern procedures such as high-performance liquid chromatography (HPLC) are also good methods. The fatty acid data reported in this chapter are derived from papers published after 1960 and where modern methodology was used. Even so, the data are somewhat variable, because many GLC and HPLC techniques are available and data between laboratories are not always completely comparable.

Linolenic	Nonadecanoic	Arachidic	Gadoleic	Eicosadienoic	Arachidonic	Behenic	Erucic	Docosadienoic	Lignoceric	Iodine value	Saponification value	
18:3	19:0	20:0	20:1	20:2	20:4	22:0	22:1	22:2	24:0			Carbon atoms: double bonds
		0.1								13–18	247–254	Babassu
0.5			0.2		0.1					25–42	210–240	Butterfat
9.2		0.6	1.0			0.2			0.2	110–115		Canola oil[b]
1.3										76–80	194–204	Chicken fat
5.7		0.8								99–106	192–197	Citrus seed oil
		1.1								32–40	190–200	Cocoa butter
		0.1								7–13	248–264	Coconut oil
										8–14	250–260	Cohune oil
0.9		0.1								110–128	156–196	Corn oil
0.3		0.1								99–121	189–199	Cottonseed oil
0.1		0.2	1.3	0.1	0.4					53–168	192–203	Lard
52.7												Linseed oil[c]
		0.2								8–13	237–247	Murumuru tallow
		0.2	2.4							105–110	180–198	Oat oil
0.6		0.9								76–90	188–196	Olive oil
0.3		0.2								45–56	195–205	Palm oil
		0.1								14–24	243–255	Palm kernel oil
		1.5	1.4[d]	0.1		3.0			1.0	84–102	188–196	Peanut oil
7.3		0.7	12.1	0.6		0.4	34.8	0.3	1.0	97–110	168–183	Rapeseed oil
1.1		0.5	0.4			0.2			0.1	92–109	181–195	Rice bran oil
		0.2								138–151	186–198	Safflower oil
0.2		0.2								85–93	185–195	Safflower oil (high oleic)
0.2										104–118	187–196	Sesame oil
7.8		0.3				0.1				125–138	188–195	Soybean oil
0.5		0.4								122–139	186–196	Sunflower oil
0.6	0.1	0.1			0.4					33–50	190–202	Tallow (beef)
1.3	0.8				0.4					35–46	192–198	Tallow (mutton)
										10–14	240–250	Tucum oil

In addition to variations in reported fatty acid composition that occur because of differences in measuring techniques, there are also natural variations in the fatty acids produced in an oilseed. The fatty acids produced in any one type of oilseed may vary with geographical location, soil type, climate, moisture, temperature, maturity of the seed, and agricultural practice (Chu and Sheldon, 1979). For example, sunflower oil grown in Minnesota contained about 70% linoleic acid (18:2), whereas that grown in Texas contained about 30% (18:2) (Purdy, 1986). In addition, damage by

Nezara veridula L. (a common species of stinkbug) was shown to increase the palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1) percentages and to decrease the 18:2 and linolenic acid (18:3) percentages of soybean oils (Todd et al., 1973). With soybeans, low temperatures were associated with a low oil content and increased unsaturation of oil under field conditions (Howell and Cartter, 1953; Howell and Collins, 1957) and controlled temperature conditions (Howell and Cartter, 1958). Finally, lower temperatures produced increased unsaturation in flax (linseed) oil but the oil content also increased (Sosulski and Gore, 1964; Canvin, 1965; Dybing and Zimmerman, 1965). Factors such as these, which influence the fatty acid composition of oils, are discussed.

Recent attempts to alter genetically the fatty acid composition of major vegetable oils (e.g., canola, soybean, and sunflower) have also resulted in changing patterns of fatty acids in the oilseeds. The variation in the fatty acid pattern of any one vegetable oilseed is expanding as the demand for specialty oils for specialty markets increases. A main driving force for changing fatty acid composition, either by plant breeding techniques or by transgenic means, for edible oil use of the oilseeds has been the increasing recognition of the health implications. For example, many nations around the world have banned the sales of foods containing *trans* fats arising from the process called hydrogenation, suggested a restricted amount of *trans* fats for consumption, or begun mandatory labeling of foods containing *trans* fatty acids. The process of hydrogenation results in a shift of some of the double bonds within a fatty acid from the *cis* position, which is naturally found in vegetable oils, to the *trans* position (King and White, 1999). *Trans* fatty acids are also found in fats from animal sources such as beef, milk, and cheese. Smallbone and Saharabudhe (1985) reported amounts of *trans*-isomers of 18:1 found in partially hydrogenated soybean, canola, and sunflower oils. The original oils contained no *trans*-18:1 isomers, whereas after partial hydrogenation canola oil contained 32.9%, soybean oil contained 26.8%, and sunflower oil contained 56.0% of the isomer. Strocchi (1981) reported *trans*-18:1 levels of 59.1% in hydrogenated corn oil and 26.6% in corn oil margarine. Levels of *cis–trans*-18:2 or of *trans–trans*-18:2 ranged from trace amounts to 0.2% in both products.

In March 2003, Denmark was the first country to officially suggest restricting *trans* fatty acids in the diet. They adopted legislation restricting *trans* fatty acids in oils and processed foodstuffs containing fats and oils as ingredients to <2%, but exempted naturally occurring *trans* fatty acids in animal fats. In the United Kingdom, a major retailer, Marks and Spencer, banned sales of processed foods containing hydrogenated fats on November 24, 2005. Canada began mandatory labeling of *trans* fatty acids for processed foods on December 12, 2005 (Health Canada, 2003), followed closely by the United States, who began mandatory labeling of *trans* fatty acids in foods on January 1, 2006 (Food and Drug Administration, 2003). The goals of oilseed modifications, then, have been to provide suitable fatty acid compositions in the extracted edible oils that allow for oxidative and frying stability, as well as provide good flavor without hydrogenation, the major cause of *trans* fatty acids in oils.

II. CANOLA OIL (LOW-ERUCIC-ACID RAPESEED OIL WITH LOW GLUCOSINOLATES)

Canola is the name used to identify rapeseed cultivars of *Brassica napus* and *Brassica campestris* that have been selectively bred to produce oil that contains less than 2% erucic acid (22:1) (Eskin et al., 1996). In addition, the seed must contain low levels of glucosinolates. Original rapeseed cultivars contained 25%–50% erucic acid in the oils and fairly high amounts (80 μM/g) of glucosinolates in the seed (Abraham and deMan, 1988). These cultivars have been grown in China and India for about 4000 years and, more recently, in Northern Europe. Concern about the cardiopathic potential of 22:1 in the diet has resulted in the development of new cultivars (Daun, 1986). The glucosinolates were reduced to improve the quality of both the meal and the oil. It is thought that the glucosinolates hydrolyze during the crushing of the seed, thus releasing sulfur compounds into the oil (Rutkowski et al., 1982; Abraham and deMan, 1987, 1988). The presence of sulfur in the oil

poisons hydrogenation catalysts (Klimmek, 1984; Abraham and deMan, 1986) produces unpleasant odors during heating (Moser et al., 1965; Dobbs et al., 1978), and adversely affects the processing of rapeseed (Rutkowski et al., 1982). It may also enhance the development of thyroid disease. The current level of sulfur found in refined oils processed from the new canola varieties is about 3–10 mg/kg (Abraham and deMan, 1988). Although this low level reduces the health risk and odor problems in the oil, the small amount of sulfur still can reduce the effectiveness of hydrogenation catalysts. Methods have been developed to remove sulfur compounds from canola oil (Abraham and deMan, 1988).

The adoption of canola oil for human consumption in Canada and the United States has been unique because of the health and safety effects of the original rapeseed oils just mentioned. In Canada, the characteristics of canola oil must conform to a standard established by the Canadian General Standards Board (CGSB, 1987), which makes canola oil comparable in quality to that of any other vegetable oil currently on the market. In 1985, the Food and Drug Administration of the United States granted the status of Generally Recognized as Safe (GRAS) to canola oil, and North American companies now refine to sell it. For 2005–2006, rapeseed/canola oil was projected to rank third among major oils in world production, at 16.59 million metric tons (Table 10.2) (USDA, 2006), a ranking it has held for many years.

The typical fatty acid composition of canola oil is shown in Table 10.1. Of particular interest are its high oleic acid (18:1) content, which is similar to that of olive oil, and its high level of linolenic acid (18:3). The only other major vegetable oil to have significant amounts of 18:3 is soybean oil. In addition, canola oil has the lowest level of saturated fatty acids of all the major vegetable oils. In general, canola oil is intermediate among vegetable oils in its level of polyunsaturated fatty acids (PUFAs; 30%–35%). This level is lower than soybean, sunflower, corn, and cottonseed but higher than peanut and palm oils (Sebastian, 1985; Kupranyez et al., 1986).

The odor or flavor of fully refined, deodorized canola oil is described as bland, slightly nutty, and buttery when it is fresh, but grassy, painty, or rancid off-flavors develop during storage (Warner et al., 1989). The stability problem of canola oil is attributed to its high level of 18:3. The role of 18:3 in the development of off-flavors in oils is well documented (Moser et al., 1965), and it is discussed in this chapter under soybean oil below. Some researchers have suggested that the presence of the

TABLE 10.2
World and United States' Vegetable Oil Production (USDA, 2006)

Commodity	World Production in 2004–2005 (Million Metric Tons)	World Forecast for 2005–2006 (Million Metric Tons)	U.S. Forecast for 2005–2006 (Million Pounds)
Palm oil	34.03	34.80	—[a]
Soybean oil	32.29	33.87	20,040
Rapeseed/Canola	16.59	16.59	776
Sunflower seed	10.46	10.46	588
Peanut	4.93	4.93	219
Cottonseed	4.67	4.67	990
Palm kernel	4.20	4.20	—
Coconut	3.27	3.27	—
Olive	2.28	2.28	—
Corn	—	—	2,450
Linseed			335
Safflower	—	—	56

[a]Not listed among top ten in production amount.

small amounts of erucic acid in canola oil may inhibit the enzyme lipoxygenase, which is known to enhance the oxidation of unsaturated fatty acids, especially 18:3 (Downey, 1976), thus improving the keeping quality of rapeseed oils (Moser et al., 1965). Comparison of eight different commercially processed vegetable oils after storage for 8 and 16 days at 60°C showed that canola oil had lower peroxide values (PVs) and total GLC volatiles than the other oils tested: corn, cottonseed, olive, peanut, safflower, soybean, and sunflower (Snyder et al., 1985). However, gas chromatography and mass spectrometry revealed the presence of butylated hydroxytoluene in the canola oil, thus enhancing its stability. Canola oil was also more stable in an active oxygen test than oils from sunflower, corn, soybean, and butterfat, but less stable than olive oil, peanut oil, and lard (deMan et al., 1987). In contrast, canola oil was less stable than several soybean lines, as measured by PVs and sensory tests, in a 60°C storage test in the dark (Liu and White, 1992a). Warner et al. (1989) compared the flavor stability of canola, soybean, and sunflower oils during accelerated storage at 60°C. Sensory panelists noted that sunflower and canola oils were less stable than soybean oil. In addition, oxidation induction periods, as assessed by PV, were 1 day for sunflower and canola oils and 5 days for soybean oil; thus, canola oil was much less flavor stable than the other oils. Malcolmson et al. (1994), however, noted that oils in the latter two studies were deodorized in the laboratory, where lower temperatures of 220°C–240°C were used. If higher deodorization temperatures generally associated with commercial processing of canola oil had been used, the results might have been different.

Dobbs et al. (1978) found that low-erucic-acid rapeseed oil (LEAR; the forerunner to current canola oil), when heated to 190°C and subsequently smelled, had a significantly stronger odor than did sunflower, corn, and safflower oils. Soybean oil was intermediate in strength. Canola and soybean solid and liquid fats were compared in frying studies (Stevenson et al., 1984), in which the solid fats have been hydrogenated and the liquid fats lightly hydrogenated. The properties of the canola and soybean products were quite similar. On the other hand, in a study by Liu and White (1992b), in which bread cubes were fried in the canola and soybean oils and then stored in the dark, canola was much less stable to oxidation than were the soybean oils.

The smoke point is a good indicator of the usefulness of an oil for frying. The higher the smoke point, the more stable the oil at frying temperatures. Canola solid and liquid fats have slightly lower smoke points than similar soybean fats. Sensory evaluation of French fried potatoes using canola and soybean oils at 1, 2, 4, 6, and 10 days of heating the fats indicated few differences in quality between the two oils (Stevenson et al., 1984).

Canola and other rapeseed oils have fewer natural tocopherols than oils such as corn and soybean. Ackman (1983) reported a total tocopherol content in canola oil of 660 mg/kg oil, with alpha, gamma, and delta homologues contributing 190, 430, and 40 mg/kg, respectively. Müller-Mulot (1976) reported a total tocopherol level in canola oil of 271.4 mg/kg, with alpha, beta, gamma, and delta homologues representing 70, 16, 178, and 7.4 mg/kg of oil, respectively. These relatively low levels of tocopherols may contribute to the oxidative instability of canola oil.

As with the other major vegetable oils, new types of canola oil continue to be developed with altered fatty acid patterns to improve the quality of the oil. For example, Scarth et al. (1988) reported the development of a *B. napus* L. (Stellar) summer rape line that produces seed oil with 3% 18:3 and 28% 18:2. This oil has a shorter hydrogenation time and better stability than standard canola oils. The Stellarline was created through a combination of mutation and breeding, and it is the first rapeseed cultivar having the quality standards required for canola oil (i.e., low erucic acid and low glucosinolate). In another study, two canola oils from similar genetic origin and having a high-18:3 (12.5%) or a low-18:3 (2.5%) composition were evaluated for odor acceptance (Vaisey-Genser et al., 1994). The 18:3 content of these oils was similar to that of the licensed varieties of Westar (high 18:3) and Stellar (low 18:3). The consumer-acceptance threshold was 12.5 days for the high-18:3 canola oil and 34.3 days for the low-18:3 canola oil, indicating substantially greater stability for oil from the genetically modified cultivar. Further chemical studies on oils from these canola cultivars confirmed that the low-18:3 canola oil produced significantly fewer volatiles and lower PVs than

the high-18:3 canola oil during accelerated storage at 60°C in the dark (Malcolmson et al., 1996). Earlier studies on these oils showed that the low-18:3 canola oil did not change significantly in odor intensity or odor pleasantness during 12-day storage, whereas Westar oil changed significantly during the same time (Przybylski et al., 1993).

In frying tests, canola oil obtained from seed modified by breeding to contain 1.7% 18:3 in the oil had significantly ($p < .05$) less room odor intensity after initial heating tests at 190°C than normal canola oil that contained 10.1% 18:3 (Warner and Mounts, 1993). Other chemical indices, such as free fatty acids, polar compounds, and foam heights, were significantly ($p < .05$) less in the low-18:3 oil than in the normal canola oil after 5 h of frying. The potatoes fried in normal canola oil were described by the sensory panel as being fishy. Three high-18:1 canola oils with different levels of 18:3, along with a partially hydrogenated canola, a medium-high-18:1 sunflower oil, and a commercial palm olein, were compared during frying of potato chips (Xu et al., 1999). The 18:3 concentration of the canola oils was inversely related to sensory ranking of the chips and to the oxidative stability of the oils. The low-18:3 canola and the sunflower oils ranked best among the oils tested in sensory evaluations. Partially hydrogenated canola oil received the lowest scores in sensory evaluations.

In response to the push by industry to reduce or eliminate *trans* fats in the diet to comply with *trans*-fat labeling, Dow AgroSciences LLC released specially bred NEXERA™ canola seeds that produce oil with high (>70%) oleic and low (<3%) linolenic acid concentrations (Anon., 2006). The oils were designed to replace partially hydrogenated oils in frying operations. Bunge Canada offers this oil under the brand name of Nutra-Clear®, and Canbra Foods sells it under the brand name of Canola Harvest HiLo™.

III. CORN OIL

Corn oil is a by-product of the corn wet-milling industries and is recovered from the corn germ. Corn generally is grown to be used as starch, sweeteners, alcohol, meal, and animal feed, so the amounts of corn available for oil production are derived from those markets. In general, corn oil has historically been in short supply, although increased demand for corn sweeteners (high-fructose corn syrup) and fuel alcohol (ethanol) from corn is increasing the amount of available corn oil. World production of corn oil does not generally rank among the top 10 major oils (Table 10.2) (USDA, 2006). United States' corn oil production for 2005–2006, however, was estimated to be over 2.45 billion pounds, ranking second behind soybean oil, at 20.04 billion pounds.

Crude corn oil is refined, bleached, and deodorized to produce a good-quality edible oil. The typical fatty acid composition of commercial corn oil in the United States is shown in Table 10.1. This corn oil is derived from "Corn Belt"-grown dent corn, which was shown to have a remarkably constant fatty acid distribution when 103 samples were grown over a 2.5-year period during the 1960s (Beadle et al., 1965). However, studies have shown that the oil content and composition of some corn lines are affected by the geographical locations in which the corn is grown (Genter et al., 1956; Jellum and Marion, 1966).

Thompson et al. (1973) found considerable differences in response to temperature among four corn genotypes. One inbred line, Pa36, which was high in 18:2, was quite stable for most of its fatty acids when grown at five different controlled temperatures. Inbred GE82, which was low in 18:2, was highly responsive to temperature difference for the fatty acids. In general, there was an increased unsaturation of the seed oil with decreased temperatures during seed maturation. Other researchers have also noted that corn oil from warmer regions had a higher proportion of saturated fatty acids than did corn oil from cooler areas (Leibovitz and Ruckenstein, 1983).

The application of herbicides has also been demonstrated to alter slightly the fatty acid composition of corn oil. Wilkinson and Hardcastle (1973, 1974) found that increased application rates of certain combinations of herbicides caused changes in the fatty acid composition of corn oil. However although statistically significant, the changes were not of major importance, representing alterations

of only a few percentage points. Southern corn leaf blight (Jellum, 1971) and nitrogen and boron (Jellum et al., 1973) did not influence fatty acid composition. In general, U.S. corn oil increased in polyunsaturation, mainly as 18:2, by about 5%–8% from 1974 to 1986 (White and Weber, 2003), likely because of the selection of parental lines with desirable agronomic traits. Although environmental factors have some effect on fatty acid composition, the principal cause of variation is genetic (Leibovitz and Ruckenstein, 1983).

Oils from different corn cultivars have different fatty acid compositions. Corn oils from Argentina, Russia, and Italy were reported to be more saturated than those produced in the United States (Sonntag, 1979). A wide range of fatty acid profiles was found in corn oil from elite, unadapted corn-breeding materials originating from the United States, Chile, Argentina, and Uruguay and grown in Ames, Iowa (Dunlap et al., 1995a). A total of 250 different lines (50 lines from each of five accessions) from each country were evaluated. The oil from corn of foreign origin was slightly more saturated than that of United States' origin (14.4%–15.1% vs. 13.4%). The United States' corn oil averaged 30.5% 18:1 and 53.7% 18:2. This fatty acid profile closely matched that of values obtained by Tan and Morrison (1979) for United States' corn oil. In other work, Dunlap et al. (1995b) reported fatty acid averages of 25.2% 18:1 and 59.7% 18:2 for 418 corn hybrids and 98 corn inbreds grown in Iowa. These values represent compositions typical of commercially available corn oils, since they were taken from adapted, elite materials. The compositions were similar to values reported by others for commercially refined oils from Corn Belt populations (Beadle et al., 1965; Leibovitz and Ruckenstein, 1983). Even so, Dunlap et al. (1995b) found relatively wide ranges of fatty acid profiles present in these adapted, elite corn-breeding materials with ranges for each fatty acid as follows: 16:0, 6.7%–16.5%; 16:1, 0.0%–1.2%; 18:0, 0.7%–6.6%; 18:1, 16.2%–43.8%; 18:2, 39.5%–69.5%; 18:3, 0.0%–3.1%; and arachidic acid (20:0, 0.0%–1.0%). Small amounts of myristic (14:0), margaric (17:0), and gadoleic (20:1) acids were also found. Oleic acid composition positively correlated with kernel oil concentration (Alrefai et al., 1995).

Elsewhere, Thompson et al. (1973) reported ranges in fatty acid compositions of three inbred lines and one variety of corn. The 16:0 ranged from 9.4% to 13.3%; 18:0, 1.2% to 11.5%; 18:1, 17.0% to 49.1%; 18:2, 36.4% to 70.8%; and 18:3, 0.6% to 1.5%. Jellum bred corn for fatty acid variability and achieved the following ranges for various fatty acids in corn cultivars: 16:0, 5%–24%; 18:0, 0.5%–20%; 18:1, 15%–65%; and 18:2, 15%–75%. After that, he released an inbred with 18% 18:0 (Jellum, 1984). P. J. White and E. G. Hammond (personal communication, 2006) surveyed over 500 lines of corn for variations in fatty acid composition. These composition values ranged as follows: 16:0, 7%–24%; unknown (16:1?), 0%–9%; 18:0, 0%–13%; 18:1, 15%–50%; 18:2, 5%–70%; 18:3, 0%–9%; and 20:0, 0%–11%. The results form these many studies give a starting point for future work of breeding corn designed to produce oils of specific fatty acid composition. The E. I. du Pont de Nemours & Company were issued United States' and world patents for a corn oil from seed genetically modified to contain 65% 18:1 (Leto and Ulrich, 1995, 2001). This oil was evaluated for its frying quality and oxidative stability, and was shown to have significantly ($p < .05$) lower total polar compound levels after 20 h of heating and frying at 190°C than normal corn oil (23.8% 18:1), hydrogenated corn oil, and high-oleic sunflower oils (80% and 90% 18:1) (Warner and Knowlton, 1997). The high-oleic corn oil was also significantly ($p < .05$) more stable than normal corn oil after accelerated storage at 60°C, as evaluated by sensory-panel flavor scores and PVs.

A patent was issued for a method to improve corn lines by altering fatty acid composition, as well as protein, total oil, and starch, by introgressing genes from the native corn relative, *Tripsacum dactyloides* L. through several cycles of selective breeding and evaluation (Duvick et al., 2003). Fatty acid alterations included elevated oleic acid, and/or elevated or lowered saturated fatty acid compositions. The progeny from these lines backcrossed with Corn Belt inbred lines were grown and evaluated (Duvick et al., 2006). The relative amounts of oleic acid and saturated fatty acids (palmitic and stearic acids) were greatly increased by selective breeding within the *Tripsacum* introgressed corn germplasm. The target was to create new corn oils with more healthful fatty acid compositions and products with reduced *trans* fats from these corn lines. To reduce the C:16 fatty acids

(palmitic) and make other alterations in the fatty acid profile of corn oil, genes encoding acyl–acyl carrier protein thioesterases were cloned from maize (Rubin-Wilson et al., 1998).

Corn oil generally is considered to be fairly stable to oxidation; thus, its flavor quality is very good. A study evaluating the flavor quality of oils stored in the dark at 60°C for 12 days (Shen et al., 2001) revealed that by day 6, corn oil had significantly ($p < .05$) less off-flavor than canola oil, and tended to have less off-flavor than soybean oil. There may be several reasons for this innate stability. As noted above, corn oil contains only a trace of 18:3, a fatty acid that oxidizes more than twice as quickly as 18:2 and 20 times as quickly as 18:1 (Fatemi and Hammond, 1980). Furthermore, the unsaturated fatty acids tend to occupy the 2-position of the triacylglycerol molecule, thus affording protection to some of the unsaturated acids (Weber, 1978; Strocchi, 1981; Lau et al., 1982). In addition, corn oil is the only oil containing a significant amount of ubiquinone (200 mg/kg; a benzoquinone with a side chain of 6–10 prenyl units), an excellent antioxidant that helps protect oils against oxidative rancidity (Folkers, 1974; Kamei et al., 1986). The oil contains relatively good amounts of tocopherols (also good antioxidants) with estimates ranging from 495 to 1071 mg/kg oil (Müller-Mulot, 1976; Carpenter, 1979; Slover et al., 1983; Van Niekerk and Burger, 1985; Tan, 1989; Warner and Mounts, 1990; Boki et al., 1992; Clough, 1992). Individual homologues ranged as follows: alpha, 121–281 mg/kg; beta, 0–94 mg/kg; gamma, 346–790 mg/kg; and delta, 10–42 mg/kg. Small amounts of tocotrienols, differing from tocopherols by their unsaturated side chain, were also reported. Corn oil also contains many other phenolic components, especially ferulic acid (5.79 mg/kg), a very active antioxidant, present as an ester of dihydro-beta-sitosterol, that likely contributes to the oil's excellent oxidative stability (Bernwieser and Sontag, 1992).

IV. COTTONSEED OIL

Cottonseed oil was the first edible vegetable oil produced in the United States, and its production dominated the world edible oil market until just before World War II. At that time, more sophisticated cultivation of soybeans resulted in good-quality soybean oil and its dominance in the world market. Cottonseed oil was projected to rank sixth for 2005–2006 in world production of major oils, at 4.67 million metric tons (Table 10.2) (USDA, 2006). Cottonseed oil is a by-product of cotton fiber production and is derived largely from the seeds of *Gossypium hirsutum*. A small portion (2% of the United States' crop and 8% worldwide) of cottonseed comes from the seeds of *Gossypium barbadense.*

Crude cottonseed oil is unusual in that it contains almost 2% of nonglyceride materials, most of which are removed during refining (Sonntag, 1979). These materials include gossypol, phospholipids, sterols, resins, carbohydrates, and related pigments. The most significant of these is gossypol, which is a complex phenolic substance that gives a strong red to brown color in crude cottonseed oil and that has been shown to have antioxidant properties (Sonntag, 1979). Gossypol is also toxic, so its removal from the oil is of concern. Many studies have reported the effects of gossypol on animal production and physiology (Berardi and Goldblatt, 1980). This subject will be discussed elsewhere in this book. In practical terms, all but a trace of the gossypol is removed during refining (Yabe et al., 1984), but enough is left to give cottonseed oil its characteristic yellow color.

The typical fatty acid composition of refined cottonseed oil is presented in Table 10.1. As with most vegetable oils, the actual fatty acid composition varies, depending on genetic and environmental factors. In a study by Cherry et al. (1978), the fatty acid composition and oil quantity varied significantly among cultivars and growing location within Texas. The range in contents of the major fatty acids was as follows: 16:0, 21.6%–26.2%; 16:1, 0.6%–0.8%; 18:0, 2.3%–2.9%; 18:1, 15.2%–19.9%; and 18:2, 49.1%–57.6%. Badami et al. (1978) studied the fatty acid composition of five varieties of cottonseed oils grown in India and found the following ranges: 14:0, 0.8%–1.1%; 16:0, 23.0%–23.9%; 18:0, 2.7%–4.2%; 18:1, 11.9%–22.8%; 18:2, 47.5%–58.1%; 20:0, 0.3%–0.7%; and 22:0, 0.3%–1.4%. Badami et al. (1977) reported similar fatty acid ranges for the oils from seven varieties of cottonseed grown in India. Yazicioglu and Wetherilt (1985) reported the effects of three

different growing locations in Turkey on the fatty acid compositions of 22 cottonseed varieties. The wetter the climate, the more unsaturated were the oils, but the total unsaturates varied only slightly, from 73.3% in the wettest climate to 71.7% in the driest climate. The triacylglycerol composition of cottonseed oil is unique in that almost 29% of the molecules contain two 18:2 and one saturated fatty acid (most likely, 16:0) (Bezard et al., 1990; Bland et al., 1991). The next most common type (16%–19%) consists of three 18:2 molecules.

Cottonseed oil also contains small quantities of cyclopropenoid fatty acids (CPFAs), presumably sterculic and malvalic, in amounts measured between 0.1% and 0.3% (Lawhon et al., 1977), 0.6% and 2.1% (Badami et al., 1978), or 0.6% and 1.1% (Badami et al., 1977). The removal of CPFAs is necessary, because the fatty acids have been shown to display several adverse biological effects (Carter and Frampton, 1964). In addition, as little as 25 mg/day of sterculic acid in the diet of laying hens was shown to cause pink coloration in the eggs (Masson et al., 1957). Fortunately, the deodorization step in the refining of cottonseed oil removes all but small amounts of the CPFAs. Eaves et al. (1968) reported a drop in CPFAs from 0.53% to 0.04% with conventional deodorization. Researchers continue to examine methods for the removal of CPFAs from cottonseed oil (Gilkison and Shone, 1993).

Traditionally, in the breeding and production of cottonseed, oil quality has been ignored. With increased competition from other oil crops, the cottonseed growers have recognized the need to produce better quality seed and oils. New cottonseed cultivars have been developed to improve several quality factors, including (1) quantities of oil and (2) changes in select fatty acids, in particular, a significant decrease in 16:0 and an increase in 18:1 (Cherry, 1983). One variety, Acala SJ-2, and a newer cultivar (Acala SJ-5) were shown to have increased oil contents, increased unsaturation, and reduced gossypol when compared with traditional cottonseed varieties. Some work on the effect of mutagens on fatty acids in cottonseed oil was reported by Russian scientists with decreases in 16:0 and 18:1 and increases in 18:2 (Daminov and Topvoldiev, 1974; Topvoldiev et al., 1975). The influence of herbicide applications on fatty acid composition in cottonseed oil has also been studied, with only minor changes in the fatty acids reported (Wilkinson and Hardcastle, 1972a,b). Novel gene constructs were applied to cottonseed to alter fatty acid composition of the oil (Green et al., 2001). The 18:0 was increased from ~2%–3% up to 40% by down regulating the *ghSAD-1* gene, and the 18:1 from ~15% up to 77% by silencing the *ghFAD2-1* gene (Liu et al., 2002). Furthermore, these researchers were able to stack the gene effects to achieve novel combinations of the major fatty acids in cottonseed oil, thus helping cottonseed oil enter the competitive market of nonhydrogenated, no-*trans* edible oils for use in margarines, deep-fat frying, and potentially high-value confections.

Interest in finding oils with altered fatty acid patterns has also resulted in searches of wild species within the *Gossypium* genus. Khattab et al. (1977) found that 16:0 ranged from 17.9% to 34.9% in *Gossypium sturtianum* and *G. hirsutum*, respectively, and that 18:1 ranged from 14.4% in *Gossypium somalense* to 32.5% in *Gossypium anomalum*. Earlier, Carter et al. (1966) observed a change in 16:0, from 22% for *Gossypium arboretum* L. to 35% for *Gossypium aridum* Skovsted; in 18:1, from 16% for *Gossypium aridum* to 36% for *Gossypium armourianum* Kearney; and in 18:2, from 35% for *G. armourianum* Kearney to 55% for *G. hirsutum* L. Perhaps the genes from the wild species would be valuable in the production of commercial genotypes with improved oil composition.

Starting in the early 1960s, there was considerable interest in the development of cottonseed varieties known as glandless, which contain no gossypol. It was thought that the gossypol-free seed might be used as a protein seed for human consumption. Although the protein and oil quality of some glandless varieties are comparable to or better than glanded varieties, the market for glandless seed did not catch on (Cherry, 1983). The oil produced from the glandless seed is light colored because of the absence of the gossypol pigment. Cherry (1983) showed that there was little difference in oil and fatty acid composition of seed from the glanded and glandless varieties; however, Lawhon et al. (1977) reported that glandless oil was slightly more unsaturated than glanded oil.

Cottonseed oil contains natural antioxidants in the form of tocopherols, with around 1000 mg/kg oil of total tocopherols in the crude oil (Müller-Mulot, 1976; List and Friedrich, 1989). Refined oils

generally retain about 60%–70% of the tocopherols found in the crude oil. Slover et al. (1969) recovered 320 mg/kg of alpha-tocopherol and 313 mg/kg of gamma-tocopherol from a commercially refined oil, for a total of 633 mg/kg tocopherol. Cottonseed contains high amounts of phenolic acids compared with other oilseeds, some of which would presumably be carried into the oil during extraction and could contribute to oxidative stability of the oil. The predominant phenolic acids of both glanded and glandless cottonseed include *trans*-ferulic, *trans-p*-coumeric, *p*-hydroxybenzoic, and *trans*-caffeic acids (Dabrowski and Sosulski, 1984).

Refined cottonseed oil is typically used as a salad and cooking oil and in the preparation of shortenings and margarines. The refined oil is not as stable to oxidation as is the crude oil, likely because of removal of the gossypol and part of the tocopherols. Berger et al. (1970) reported that cottonseed oil was not particularly stable when measured by a standard active oxygen test (AOM). Snyder et al. (1985) found cottonseed oil to be more resistant to peroxide development than corn, olive, peanut, safflower, sunflower, and soybean oils under storage conditions of 60°C for 16 days; however, at 8 days, cottonseed oil was only intermediate in its oxidative stability. The flavor of foods fried in pure cottonseed oil had the highest intensity of fried-food flavor (considered to be a positive attribute) when compared with foods fried in a variety of oil blends made from high-oleic sunflower and cottonseed oils (Warner et al., 1997). As might be predicted, however, the higher levels of 18:2 in the cottonseed oil resulted in reduced frying oil stability as measured by total polar compounds. In general, cottonseed is suitably stable for use in most foods, and provides desirable flavors in fried-food appearance, which is likely caused by the high proportion of triacylglycerol molecules containing two 18:2 and one saturated fatty acid, as noted earlier (Bland et al., 1991).

V. LINSEED OIL (FLAX)

Flax is grown primarily for the production of linseed oil, which, because of its high content of α-linolenic acid (18:3n-3), is an excellent drying oil that is used in paints, varnishes, and linoleum (Bhatty and Rowland, 1990). Worldwide production of linseed oil does not generally rank among the top ten major vegetable oils (USDA, 2006); however, its production as an edible oil product has increased in recent years, with the development of linseed varieties producing oils with modified fatty acid compositions. Linseed oil production in the United States was estimated to be 335 million pounds for 2005–2006 (Table 10.2) (USDA, 2006). There is only one original species of cultivated flaxseed, *Linum usitatissimum*, and it contains 18:3n-3 ranging from 40% to 63% or higher (Batta et al., 1985). A typical fatty acid distribution for linseed oil is shown in Table 10.1.

The fatty acid composition of linseed oil is highly affected by temperature and other climatic conditions (Yermanos et al., 1969; Agrawal, 1971). Rai et al. (1989) grew 35 different linseed genotypes under three different agroclimatic conditions and found highly significant differences among genotypes (and varieties) and between genotypes and environment. All the fatty acids were affected significantly except for 16:0. In general, flaxseed grown in northern, cooler climates in Canada produces linseed oil with more unsaturation than that grown in southern, warmer climates (also in Canada) (McGregor and Carson, 1961). Sosulski and Gore (1964) found 18:3 percentages of 48.1% and 51.6% for two flax types when grown at 65°F. At 75°F, the same two types produced 35.4% and 38.0% 18:3, respectively.

Recent reports on the nutritional value of n-3 fatty acids have prompted interest in the development of edible linseed oils with 18:3n-3 levels comparable to those found in soybean and canola oils (Bhatty and Rowland, 1990). With such high-18:3 content, linseed oil is not currently suited for use in foods because of its tremendous oxidative instability.

Mutation breeding of the cultivated flaxseed, *L. usitatissimum*, along with traditional methods of selective breeding, has resulted in new and improved linseed types, producing oil suitable for human consumption. Green and Marshall (1981) and Green (1986) reported some success in mutation breeding of flax, resulting in oil containing less than 2% 18:3. Australian researchers developed a low-18:3 linseed oil containing 2% 18:3 and 72% 18:2, which they call Linola™ (Haumann, 1990). Commercial production of Linola™ began in Australia during the 1993–1994 crop year,

and in Europe during 1995–1996. In Canada, solin is the generic name for flaxseed that yields oil containing less than 5% 18:3, and the Flax Council of Canada adopted the name solin oil as the common or usual name used for this oil. Under grade specifications established by the Canadian Grain Commission during 1995, solin must have a yellow seed coat. Linola™ 947, 989, and 1084 are exclusive lines developed by the United Grain Commission from the Australian variety (J. Daun, personal communication, 1998). The first Linola 947 crop was grown in Manitoba and Saskatchewan provinces in 1994. The Linola 989 variety, bred for early maturity and higher yield, was first grown in 1997. Researchers at the Crop Development Centre, University of Saskatchewan, developed solin varieties through mutagenesis of the Canadian flaxseed variety of McGregor (Rowland, 1991; G.G. Rowland, personal communication, 1998).

Malcolmson et al. (1998) evaluated the oxidative and photooxidative stability of solin oil under accelerated storage conditions. In the presence of light, solin oil was more stable to peroxide development and flavor volatiles than sunflower oil. In the absence of light, solin oil was more stable to peroxide development but was less stable to painty odor development than sunflower oil. Regardless, the high concentrations of 18:2 in Linola and solin oils make them particularly susceptible to oxidation when compared with most other vegetable oils (Łukaszewicz et al., 2004). The addition of natural antioxidants, β-carotene and quercetin, improved the oil stability (Łukaszewicz et al., 2004), as did a blend of alpha-tocopherol, ascorbyl palmitate, citric acid, ascorbic acid, and ethoxylated ethylene glycol (Rudnik et al., 2001).

VI. OAT OIL

Oat (*Avena sativa* L.) groats have the highest lipid concentration among the cereal grains, with lipid amounts ranging from 2% to 11% (Youngs, 1986). However, groats have not been considered a potential source of edible oil, because this amount is too low to make extraction profitable. The typical fatty acid composition of oat oil is listed in Table 10.1. By using a GLC equipped with a capillary column, Saastamoinen et al. (1989) detected myristic acid (14:0), palmitoleic acid (16:1), 20:0 and erucic acid (22:1) in most oat varieties and lines, and 16:0, 18:0, 18:1, 18:2, 18:3, and gadoleic (20:1) in all samples. Sahasrabudhe (1979) found 20:1–20:5 fatty acids in small amounts (0.5%–3.0% total) and traces of behenic, erucic, and lignoceric acids.

The 16:0, 18:1, and 18:2 composition makes up over 95% of the fatty acids; however, the exact percentage of each of these fatty acids varies depending on several factors. Table 10.3 lists the relative fatty acid composition of oil from different oat cultivars from five different studies, indicating considerable variation in the proportions of each fatty acid based on the cultivar. Data from several studies revealed that as the total groat lipids increase, 16:0 and 18:2 tend to decrease and 18:1 tends to increase (Youngs, 1986; Holland et al., 2001). Indeed, the greater amount of 18:1 and the lesser amounts of 16:0 and 18:2 noted in Table 10.3 for Holland et al. (2001) were after nine cycles of recurrent selection for greater groat-oil content. This shift is likely because the storage lipids (in the form of triacylglycerols), which are the ones to change as the total amount of lipid changes, contain relatively more 18:1 than do the structural lipids (glycolipids and phospholipids). Saastamoinen et al. (1989), however, found that low growth temperature increased overall lipid synthesis and synthesis of 18:1 and 18:2, with 16:0, 18:0, and 22:1 decreasing.

The relative fatty acid distribution changes during maturation of the oats. During oat development, the 18:3 percentage starts high and then drops, whereas 18:2 increases with maturation (Lindberg et al., 1964; Brown et al., 1970). As with many other plants, oats grown under cool temperatures generally have greater unsaturation than those grown in warm environments (Youngs, 1986; Saastamoinen et al., 1989).

Oats, like many other potential oil crops, have been studied for their ability to produce a better edible oil. In the case of oats, a better usable oil is based on increasing the percentage of oil from the oat and on controlling a potent lipase enzyme extracted with the oil, and that causes rapid hydrolysis and deterioration of the oil quality (Hutchinson and Martin, 1952). Frey and Hammond (1975)

TABLE 10.3
Range and Mean of Relative Fatty Acid Composition of Oat Cultivars from Five Studies

Fatty Acid	Youngs and Püskülcü (1976)[a] (%)	Frey and Hammond (1975)[b] (%)	de la Roche et al. (1977)[c] (mol. %)	Sahasrabudhe (1979)[d] (%)	Lasztity et al. (1980)[e] (%)	Holland et al. (2001)[f] (%)
Myristic	0.4–0.8 (0.6)	—	—	0.5–4.9 (2.1)	—	
Palmitic	16.2–21.8 (18.9)	14–23	17.2–23.6 (19.6)	15.6–25.8 (20.5)	12.9–25.8 (17.5)	13.8–16.0
Stearic	1.2–2.0 (1.6)	1–4	0.8–1.8 (1.4)	1.6–3.9 (2.6)	1.1–11.0 (2.5)	2.2–2.7
Oleic	28.4–40.3 (36.4)	29–53	26.5–47.5 (38.7)	25.8–41.3 (34.9)	25.4–36.9 (31.4)	44.1–50.2
Linoleic	36.6–45.8 (40.5)	24–48	33.2–46.2 (38.9)	31.3–41.0 (37.0)	38.1–46.7 (42.0)	32.4–36.7
Linolenic	1.5–2.5 (1.9)	1–5	0.9–2.4 (1.5)	1.7–3.7 (2.6)	1.5–19.4 (3.9)	0.8–1.0

[a]Data from groats of 15 oat cultivars, three replications of each, grown at three locations in the United States during two crop years.

[b]Data from groats of 64 cultivars and collections.

[c]Calculated from data from nine cultivars of oats grown in Canada.

[d]Calculated from data for 12 cultivars of oats (groats) grown in Canada. Not shown here is "other" fatty acids (range 0%–2.4%), which included C_{20} and C_{22} fatty acids.

[e]Data from oats of 12 cultivars grown in Hungary.

[f]Data from nine cycles of recurrent selection for greater groat-oil content in a genetically broad-based oat population.

Source: Youngs, V.L. (1986). Oat lipids and lipid-related enzymes, in *Oats—Chemistry and Technology* (F. Webster, ed.), American Association of Cereal Chemists, St. Paul, MN, pp. 205–226 and Holland, J.B., et al. (2001). *Euphytica 122*: 69–79.

estimated that for oats to be an economically feasible oilseed crop in Iowa, the oil percentage would have to be increased to around 16%. They reported that oil percentage was inherited polygenetically, and that there was a tendency for high oil percentage to be partially dominant. K.J. Frey and E.G. Hammond (personal communication, 1998) bred oat lines containing up to 16% oil and, for 300 oat lines bred for high oil, a mean oil content of 14.2% was reported. After many years of selection for oat quantity, and after nine cycles of recurrent selection in one population, the researchers achieved a consistent oil content of 15.8% (Holland et al., 2001). The possibility of using such an oil may depend on the ability to reduce the lipase activity in the oats. The lipase can be inactivated by heat or by 95% ethanol (Frey and Hammond, 1975), or it may be possible to breed low-lipase-containing oats. Kalbasi-Ashtari and Hammond (1977) reported that if Dal oats were extracted quickly, before the lipase could act, a relatively stable oil was produced. However, another problem encountered in oat oil production was the high (25%–30%) alkali-refining loss because of phosphatides and other gummy substances.

Kalbasi-Ashtari and Hammond (1977) compared petroleum ether-extracted oil from Dal oats that had been refined, bleached, and deodorized in the laboratory with refined soybean oil from a commercial manufacturer that had been deodorized in the laboratory. Citric acid was added to the oils. Storage at both 25°C and 55°C resulted in oat oil being significantly more stable than soybean oil, as measured by PV.

Oats are known to contain natural antioxidants in the form of ferulic and caffeic acids (Daniels and Martin, 1961; Daniels et al., 1963; Xing and White, 1997). Kalbasi-Ashtari and Hammond (1977) were unable to detect these compounds in their crude solvent-extracted oat oil; likely because the acids are not soluble in petroleum ether. They did detect alpha-tocopherol with 38 mg/kg oil present in the bleached oil. Other researchers have reported higher tocopherol levels in oat lipids, with Chow et al. (1969) reporting 175 mg/kg oil of total tocopherol in oat oil, including 122.5, 31.5, 12.3, and 8.7 mg/kg of alpha, beta, gamma, and delta homologues, respectively. They also reported

minor amounts of alpha-, beta-, gamma-, and delta-tocotrienols. Earlier, Green et al. (1955) measured a total of 610 mg/kg tocols in oat oil, including alpha-, gamma-, and delta-tocopherols and alpha- and beta-tocotrienols. White and Armstrong (1986), Duve and White (1991), and Tian and White (1994a) demonstrated the presence of a unique sterol (delta-5 avenasterol) in oat oil that protects against deterioration at frying temperatures. They removed the sterol from oats by methanol extraction and added it to soybean oil as a high-temperature polymerization inhibitor. Further work also demonstrated the usefulness of the methanolic extract of oats as a room-temperature antioxidant when added to soybean and cottonseed oils and their emulsions (Tian and White, 1994b) and in bread cubes stored after frying in oils containing the extracts (Tian and White, 1994a). The total sterol content in seven oat cultivars grown at three Swedish locations varied between 350 and 491 μg/g dry weight, with the most abundant being beta-sitosterol (237–321 μg/g) followed by campesterol (32–36 μg/g), delta-5-avenasterol (15–47 μg/g), and stigmasterol (11–21 μg/g) (Määtä et al., 1999). Many phenolic compounds were identified in the extracts, including ferulic, *p*-coumaric, caffeic, vanillic, *p*-hydroxybenzoic, 4-hydroxyphenylacetic, *o*-coumaric, sinapic, and salicylic acids, and vanillin and catechol (Xing and White, 1997). In particular, bran-rich fractions had greater phenolic acid concentrations and antioxidant activity than the starch-rich fractions (Gray et al., 2000). The polar lipid portion of oat oil has good emulsifying properties, which makes oat oil desirable in both food and cosmetic applications (Peterson, 2002).

VII. OLIVE OIL

Olive oil is briefly discussed here because of its significance as a major oil, but it is also discussed in the chapter on fruits and fruit products (see Chapter 11). Olive oil, which has been used since ancient times, is obtained from the fruit of an evergreen tree, *Olea europaea* (Rana and Ahmed, 1981). The olive tree grows best in the subtropical climates of the countries bordering the Mediterranean Sea and North Africa.

Olive oil is mechanically or physically pressed from the fruit pulp to obtain "virgin" olive oil, which is the highest grade (Firestone et al., 1996). Successive pressings yield lower grades of oil. Olive-pomace oil is obtained by solvent extraction of olive-pomace, yielding several categories of edible and inedible oils. Solvent extraction applied to the residue from the last pressing can be used to produce an inedible oil called "olive foots" or "sulfur olive oil" that can be used in the manufacture of soap and for industrial purposes (Sonntag, 1979). The world production of olive oil in 2005–2006 was projected to rank ninth among major vegetable oils, at 2.28 million metric tons, a ranking it has held for over a decade until world palm kernel oil production surpassed that of olive oil (Table 10.2) (USDA, 2006).

A typical fatty acid composition of olive oil is shown in Table 10.1, and it is characterized by its high (>70%) content of 18:1. In fact, olive oil is currently considered to be highly nutritionally desirable because of this amount of monounsaturated fatty acids (Grundy et al., 1988). Like many other vegetable oils, olive oil tends to become more unsaturated as the climate becomes colder and as the fruit matures, resulting in oils containing between 65% and 85% of 18:1, depending on the growing conditions (Sonntag, 1979). In partial agreement with this data, two Italian olive cultivars grown in Andalusia at two different altitudes, revealed greater 16:0 and 18:2 percentages, and a lesser 18:1 percentage when grown at the lower altitude (Aguilera et al., 2005). The International Olive Oil Council (IOOC) reported 18:1 ranges of 55%–83% (IOOC, 1993), but Libyan-grown olive oil contained only around 45% 18:1 and about 30% 18:2 and 18% 16:0 (Rana and Ahmed, 1981). Higher 16:0 and 18:2 contents were typical in the oils containing lower amounts of 18:1. Differences were noted in 18:1 content of olives grown in Tunisia (62.5%), Turkey (71.3%), and Europe (78%) (Firestone et al., 1996). Carpenter et al. (1976) analyzed 14 different consumer-available liquid vegetable oils, including one olive oil that contained 10.5% 16:0, 78.6% 18:1, and 6.2% 18:2. Eleven olive oils representing most of the native Tuscan olive varieties were surveyed for their fatty acid composition (Pinelli et al., 2003). The 18:1 ranged from 72.2% to 77.2% in 10 out of 11 varieties,

with one variety having 59%. The 16:0 ranged from 16.4% in the latter variety to 9.8%. In this case, the low-18:1 variety had a typical percentage of 18:2. The Cornicabra olive, as well as the Picual olive, both from Andalusia, have especially high concentrations of 18:1 and low concentrations of 18:2. The Cornicabra olive oil, evaluated from five crop seasons 1995/1996 to 1999/2000 with $n = 224$, ranged in fatty acid composition as follows: 7.0%–11.1% 16:0; 2.6%–4.4% 18:0; 76.5%–82.5% 18:1; and 3.1%–6.6% 18:2 with small amounts of other fatty acids (Aranda et al., 2004). The four most important olive varieties, Arbequina, Manzanilla, Nevadillo, and Ascolana, cultivated in the semiarid region of Traslasierra Valley, Córdoba, Argentina (Torres and Maestri, 2006) had somewhat lower 18:1 values than those from Andalusia and Tuscany. Fatty acid compositions ranged in 18:1 from 61.3% for Arbequina to 74.6% for Nevadillo. Correspondingly, the 16:0 was 17.8% and 18:2 was 15.8% in Arbequina and the 16:0 was 13.7% and 18:2 was 7.7% in Nevadillo.

Olive oil tends to be quite stable to oxidation because of its high content of 18:1, low (usually) content of 18:2, and lack of 18:3. It has only small amounts of tocopherols, with reported values in milligrams per kilograms of oil as follows: alpha, 145–154 and gamma, 21–25 (Slover et al., 1983); alpha, 93 and gamma, 7.3 (Müller-Mulot, 1976); and alpha, 70 and gamma, 20 (Carpenter, 1979). Carpenter et al. (1976) found total tocopherols to be 140 mg/kg oil in their consumer-available olive oil. Several phenolic compounds have been identified in virgin olive oil, including *p*-coumeric, syringic, vanillic, ferulic, caffeic, *p*-hydroxybenzoic, and protocatechuic acids and 4-hydroxyphenylethanol, 3,4-dihydroxyphenylethanol, and others (Nergiz and Unal, 1991; Montedoro et al., 1992; Tsimidou et al., 1992). One study showed that the antioxidant activity of the native olive oil phenols, caffeic acid, and 3,4-dihydroxyphenylethanol was much greater than that of alpha-tocopherol (Blekas et al., 1995). Elsewhere, a significant positive correlation was found between oxidative stability and total phenol content ($r = .52$) of the oil from the four most important olive varieties, Arbequina, Manzanilla, Nevadillo, and Ascolana, cultivated at Traslasierra Valley, Córdoba, Argentina (Torres and Maestri, 2006).

VIII. PALM, PALM KERNEL, AND COCONUT OILS

The oil palm, *Elaeis guineensis*, originated in West Africa and has spread to most parts of the tropical and subtropical zones of the world, with Southeast Asia, including Malaysia, being one of the main growing regions in the world (Jalani et al., 1997). The species, *Elaeis oleifera*, originated in Central and Latin America, with much less planted for commercial use. The oil palm produces a palm fruit that is quite unusual, because it yields two distinct oils, palm oil and palm kernel oil. Palm oil is obtained from the fruit itself, whereas the palm kernel oil is extracted from the seed inside the fruit. Oil from another palm fruit, the coconut (*Cocos nucifera*), is derived from the dried meat called copra. Palm oil is economically of much greater importance than palm kernel or coconut oils. For 2005–2006, palm oil was projected to rank first in world oil production among the major oils, at 34.80 million metric tons, whereas palm kernel and coconut oils ranked seventh and eighth, respectively (Table 10.2) (USDA, 2006). Palm oil overtook soybean oil in world production amount for the first time in 2004–2005. The majority of commercial palm and palm kernel and coconut oils come from trees grown in Malaysia, West and Central Africa, Indonesia, and Brazil and other Central and South American countries (Sonntag, 1979).

Palm kernel and coconut oils are very similar to each other in fatty acid composition. The other oil, palm oil (*E. guineensis*), has a much less saturated fatty acid profile. Typical fatty acid patterns for all three oils are shown in Table 10.1, although recent efforts to increase the unsaturated fatty acid composition of palm oil, and its nutritional quality, are providing oils with more 18:1 and 18:2. The *E. oleifera* palm tends to have more 18:1, with contents as high as 55%–56% (Rajanaidu et al., 1985), so it has been crossbred with *E. guineensis* used to diversify the genetic base. The fatty acid composition of the palm oils can vary with origin and variety. For example, the Nigerian population of *E. guineenis* had wide variance in fatty acids, such as 27%–55% 16:0, 28%–56% 18:1, and

6.5%–18% 18:2 (Rajanaidu et al., 2000). Palm fruits from the northeastern region of Brazil had 37% 16:0, 4.7% 18:0, 45% 18:1, and 10.7% 18:2 (Bora et al., 2003). Other reports revealed fatty acid variance also based on region, for the Congo (Kinkela and Vizard, 1993), Cameroon (Kapseu and Parmenther, 1997), and other Nigerian locations (Epka and Epke, 1996a,b; Akpanabiatu et al., 2001). The palm kernel oil from the Brazilian study (Bora et al., 2003) was more saturated than that listed in Table 10.1, with 53% 12:0, 19% 14:0, 10% 16:0, and 5.5% 18:1. Palm fruits have high amounts of enzymes that can hydrolyze the fatty acids, causing some palm (including palm, palm kernel, and coconut) oils to contain as much as 50% free fatty acids, thus proper processing techniques are necessary (Sonntag, 1979). Even good-quality palm oils have a higher free fatty acid content than do most oils. If the oil has been carefully processed, it is very stable to oxidative deterioration (Fritsch et al., 1971; Clegg, 1973).

Palm oil has about 500–800 mg/kg oil of total tocopherols, with the distribution being 288, 280, 80, and 80 mg/kg for alpha, gamma, delta, and other homologues, respectively (Clegg, 1973). Maclellan (1983) also reported 800 mg/kg of tocopherols in palm oil, with 360 mg/kg of alpha, 360 mg/kg of gamma, and 80 mg/kg of delta homologues. Other researchers measured lower total amounts of tocopherols, but also reported the presence of tocotrienols, with gamma-tocotrienol at 132 mg/kg (Van Niekerk and Burger, 1985). Chase et al. (1994) reported finding good amounts of gamma- and delta-tocotrienols (325 and 88.9 mg/kg, respectively) in refined, bleached, and deodorized palm oil, and Jalani et al. (1997) reported totals of 600–1000 mg/kg tocopherols and tocotrienols in crude *E. guineesis* palm oil, with gamma-tocotrienol making up 45% of the total. Coconut oil was reported to contain only very low levels of tocopherols, with 2.4 and 20.0 mg/kg oil, respectively, of delta- and alpha-homologues (Sonntag, 1979) and minor amounts of tocotrienols (Tan, 1989). Young (1983) stated that palm kernel and coconut oils contain total tocopherol contents of about 40–100 mg/kg oil. Even these low levels are important for protection against oxidation.

Palm oil is also a nutritionally important source of alpha- and beta-carotenes, which may improve its oxidative stability (Tan, 1989). Carotenoids in palm oil total about 400–1000 mg/kg and, in addition to the *trans*-alpha- and *trans*-beta-carotenes that comprise over 90% of the carotenoids, include *cis*-alpha- and *cis*-beta-carotenes; phytoene; phytofluene; gamma-, delta-, and zeta-carotenes; neurosporene; alpha- and beta-zeacarotenes; and lycopene (Yap et al., 1991; Jalani et al., 1997).

Although the source of most palm and palm kernel oils is the *E. guineensis* palm and of coconut oil, the *C. nucifera* palm, the entire family Palmae, is estimated to consist of about 217 genera and about 2500 species (Idiem' Opute, 1979). Researchers have analyzed the compositions of some of these unusual palms and have found considerable variation in their fatty acid patterns compared with the more common palm oil just described (Idiem' Opute, 1979; Cole et al., 1980). Among these are babassu (*Orbignya oleifera*) and murumuru (*Astrocaryum murumuru*) (Litchfield, 1970), tucum (*Astrocaryum vulgare* or *tucuma*), and cohune (*Attalea cohune*) (Sonntag, 1979). Their typical fatty acid compositions are listed in Table 10.1. These oils have fatty acid patterns similar to those of coconut and palm kernel oils.

IX. PEANUT OIL

Peanut (*Archis hypogaea* L.) seed is an important oil crop throughout the world and is grown in large quantities in Africa, India, and China. The oil is used in many edible products, including shortening, margarine, and mayonnaise, as a cooking and frying oil, and as a salad oil. In the United States, the main product for consumption is peanut butter. The amount of peanut oil produced worldwide increased from 2.47 million metric tons in 1961 to a projected 4.93 million metric tons in 2005–2006, but the relative rank in production dropped from second to sixth during that time (Table 10.2) (USDA, 2006). U.S. production for 2005–2006 was forecast to be 219 million pounds for a ranking of 9.

The typical fatty acid composition of peanut oil is presented in Table 10.1. Peanut oil differs from most other vegetable oils in that it can contain up to 6% of long-chain saturated fatty acids, including arachidic (20:0), behenic (22:0), and sometimes lignoceric (24:0) acids. If proper GLC

separation techniques are not used, 18:3, 20:0, and 20:1 fatty acids will elute at the same time, causing considerable confusion and improper reporting of the fatty acids present in peanut oil (Worthington, 1977). In fact, several papers have reported 18:3 contents in peanut oil of up to 1.4% (Koman and Kotuc, 1976). Worthington and Holley (1967) examined oils from seven genetically diverse peanut cultivars and reported less than 0.1% (0.02%–0.04%) of 18:3. Later, Worthington (1977) evaluated eight peanut cultivars grown in four locations and found 18:3 values ranging between 0.03% and 0.13%. To unambiguously identify and measure 18:3 in oils that also contain 20:0 and 20:1, GLC liquid phases must have polarity characteristics that permit elution of the C18 series of fatty acid methyl esters prior to elution of the C20 series.

The peanut cultivar, Florunner, which was released in 1969, has become the most popular runner-type peanut cultivar in the United States, and it accounted for over 50% of the total production area in 1998; however, newer cultivars with better oil stability and quality characteristics have been released (Branch et al., 1990). The fatty acid composition was determined among seven United States' runner-type peanut cultivars that had been released since 1982: Florunner, Sunrunner, GK-7, Southern Runner, Sunbelt Runner, Okrun, and Langley (Branch et al., 1990). Significant cultivar differences were found within these fatty acid profiles, with 3-year averages among cultivars ranging as follows: 16:0, 10.2%–10.9%; 18:0, 1.4%–1.9%; 18:1, 49.2%–56.3%; 18:2, 24.1%–30.6%; 20:0, 1.0%–1.4%; 20:1, 1.3%–1.4% (not significant); 22.0, 2.6%–3.1%; and 24:0, 1.5%–1.9%. Southern Runner had the best 18:1 to 18:2 ratio and iodine values, whereas Florunner, Sunrunner, and Langley were the highest in unsaturated and lowest in saturated and long-chain fatty acids. In addition to the genotypic differences, years were also found to have a significant effect on some of the fatty acids, including 16:0, 18:0, 18:2, and 22:0. Other researchers reported yearly effects on fatty acid composition of peanut oils (Young et al., 1974; Norden et al., 1987). A survey of 732 lines of peanuts grown during 1995 in Griffin, Georgia, revealed a great deal of native variation in fatty acid composition of the oils (Hammond et al., 1997). The 16:0 varied from 8.2% to 15.2%; 18:0, 1.1% to 7.2%; 18:1, 31.5% to 60.2%; 18:2, 19.9% to 45.4%; 20:0, 0.8% to 3.2%; 20:1, 0.6% to 2.6%; 22:0, 1.8% to 5.4%, and 24:0, 0.5% to 2.5%. The 20:1 was found to be *cis*-11-eicosenoate rather than the *cis*-9 isomer reported or assumed by other researchers. In addition, epoxy fatty acids were found in many of the lines, ranging as high as 2.5%. Several correlations among the fatty acid percentages were noted, including a positive correlation of 16:0 with 18:2 and a negative correlation with 18:1, 20:1, and 24:0.

Young et al. (1974) examined nine varieties or strains of Spanish-type peanuts for variations in fatty acid composition based on a number of different factors. In general, they found higher 16:0 and 18:1 contents and lower 18:2 contents in nonirrigated peanuts and those grown in Georgia as compared with those grown in Oklahoma. El-Hinnaway et al. (1978) grew one variety, Baladi-107, in three field experiments and found no influence of nitrogen and phosphorus fertilizers, together or individually, on the fatty acid composition of the peanuts. Maturity of the peanuts has also been shown to influence the total oil content as well as the fatty acid composition of peanuts (Young et al., 1972). The 18:1 increased with maturity, whereas 18:2 decreased. Iverson et al. (1963) were interested in the effects of roasting (a process used in the production of peanut butter) on the fatty acid composition of peanut oil. They determined that there was little effect on the fatty acids of commercial peanut oils during roasting.

In general, the stability of peanut oil is quite good. The oleic/linoleic acid ratio (O/L) has been used as an indicator of peanut oil stability and is often the value reported when estimating stability of peanut oils (Sonntag, 1979). Branch et al. (1990) predicted the best stability, among seven cultivars studied, from Southern Runner followed by Langley on the basis of their high O/L values. New peanut cultivars with at least 75% 18:1 have been developed and were shown to have much greater ($p < .01$) oxidative stability than peanuts with normal (56%) 18:1 compositions (O'Keefe et al., 1993). These experimental lines were used in breeding work to develop cultivars with high O/L ratios, including linoleic acid contents of 4% or less (Moore and Knauft, 1989; Pattee et al., 2002a; Branch, 2003, 2005). In follow-up work, two high-oleic (>80%) peanut varieties, F1250 and

BC93Q10, had much better flavor quality and stability than peanuts with 53% 18:1 during storage after roasting (Mugendi et al., 1998) or at –20°C (Pattee et al., 2002b).

Peanut oil antioxidants consist mainly of alpha- and gamma-tocopherols. Müller-Mulot (1976) reported tocopherol levels in peanut oil as follows: alpha, 169 mg/kg; beta, 5.4 mg/kg; gamma, 144 mg/kg; and delta, 13 mg/kg; whereas, Chase et al. (1994) found values of alpha, 77.4 mg/kg; beta, 16.2; gamma, 241 mg/kg; and delta, 40.5 mg/kg. These levels are relatively low when compared with the amounts found in other common vegetable oils such as corn, soybean, and sunflower. Van Neikerk and Burger (1985) also noted the presence of minor amounts of tocotrienols in peanut oil. High-oleic acid peanut cultivars, whose parent lines were Sunoleic and Tamrun 96, were analyzed for tocopherol, phytosterol, and phospholipid compositions (Jonnala et al., 2006). The breeding lines were rich in alpha-tocopherol and a line derived from Tamrun had the highest total phytosterol content.

Peanut oil was shown to be unexpectedly atherogenic in the diets of experimental animals (Tso et al., 1984). However, when the fatty acids were rearranged (randomized) on the glycerol backbone, the oil became less atherogenic. Therefore, it was suggested that the structure of the component triacylglycerols may be as important to atherogenicity as the fatty acids themselves. Native peanut oils contain more triacylglycerols with 18:2 in the 2-position and saturated fatty acids in the 1- and 3-positions than do randomized oils (Myher et al., 1977). The dietary significance of this finding in humans was questioned by Alderson et al. (1986), and, more recently it was suggested that the reduced atherogenic response of randomized peanut oil is likely a result of the chemical effects of randomization (Kritchevsky et al., 1997). During the randomization process, the oil is washed with strong alkali, a process that removes native lectins from the peanut oil. Lectins are proteins believed to enhance atherogenicity in humans. The topic of dietary influence of fats and oils on health and disease is discussed elsewhere in this book.

X. RICE BRAN OIL

Rice (*Oryza sativa*) bran, an industrial by-product composed primarily of the seed coat and associated layers and representing 5%–8% of the grain (da Silva et al., 2005), recently has been recognized as a source of edible oil. Indeed, rice bran oil is recognized as a premium health food in Japan (Arumughan et al., 2004). The rice grain contains 2%–3% fat, most of which is concentrated in the embryo or germ and in the outer seed layers. Milling of rice separates the germ and bran layers from the endosperm and concentrates the fat in the residue that is known as "bran," which contains 10%–26% oil (Prabhakar and Venkatesh, 1996; da Silva et al., 2005). Rice is grown in large quantities throughout the world, especially in Asia and India. In certain countries, among them Japan and Burma, rice bran oil already contributes significantly to the edible oil supply (Kahlon et al., 1992). The production of rice bran oil has been limited by several problems that influence the quality of the edible oil. The high-PUFA composition suggests that it could be used as a salad or cooking oil if properly handled; however, its stability problems need more attention before it could be widely accepted as an edible oil of consistent quality. If the lipase activity were controlled, rice bran oil has the potential to be a good-quality oil. The high-lipase activity in the bran quickly hydrolyzes the oil into fatty acids, causing severe flavor problems in the oil (Aoyagi et al., 1985; Orthoefer, 1996). Stabilization of the bran and oil has been studied by several groups (Prabhakar and Venkatesh, 1986; da Silva et al., 2005).

Limited research exists on the fatty acid composition of bran lipids; however, a few well-designed studies have been reported. The typical fatty acid composition of rice bran oil is listed in Table 10.1. Hemavathy and Prabhakar (1987) reported the total lipid composition of bran from three varieties of rice grown in India. The 16:0 ranged from 21.2% to 25.5%; 18:0, 09.3% to 3.0%; 18:1, 37.4% to 41.0%; and 18:2, 31.1% to 33.2%. In particular, the 16:0 content was somewhat different from the 16.4% listed in Table 10.1. Othoefer (1996) reported fatty acid values in rice bran oil that were similar to those reported by Hemavathy and Prabhakar (1987). Gaydou and Raonizafinimanana (1980) reported only small variability in fatty acid composition among six rice bran oils that are

widely grown in Madagaskar, including 16:0 (16%–20%), 18:1 (41%–44%), and 18:2 (31%–37%). Perhaps, as with other vegetable oils, there is some variability of fatty acid composition based on environmental conditions during growing or among different genotypes. The variability reported here is quite minor compared with that of oils such as sunflower oil. Efforts to increase the alpha-linolenic acid in rice by genetic transformation to enhance its nutritional value have been successful. A *chimeric* gene, constructed from a maize *Ubil-P-int* and a soybean *GmFAD3* cDNA, was introduced into rice plants by *Agrobacterium*-mediated transformation, resulting in up to a tenfold increase in the alpha-linolenic acid concentration in rice seed oil (Anai et al., 2003). Such a transgenic rice is an example of a novel change in the fatty acid composition of rice oil, to serve both commercial food and industrial interests.

Both microwave heating (Ramezanzadeh et al., 1999) and parboiling (da Silva et al., 2005) helped prevent hydrolytic rancidity in rice bran, treatments that could be used to stabilize the bran prior to oil extraction. Proctor and Bowen (1996) determined that rice bran oil extracted with isopropanol was significantly more stable to heat-induced oxidation than was hexane-extracted oil. They attributed the difference to increased levels of antioxidants in the oil extracted with isopropanol. Even hexane-extracted rice bran oil contains many natural antioxidants, including total tocopherols of about 400 mg/kg oil (322 alpha, 61 gamma, and a trace of delta) (Sonntag, 1979) and ferulic acid (Tanaka et al., 1971). One phenolic component, oryzanol, has gained attention because of its possible hypocholesterolemic activity (Sharma and Rukmini, 1986). Oryzanol is a compound composed of ferulic acid esters of sitosterol and cycloartenol (Orthoefer, 1996). Rice bran oil also contains a fair amount of waxes (Yoon and Rhee, 1982), but these could be removed by proper extraction procedures. The fatty acid steryl esters (FASE) and wax esters (WE) in rice bran oil can enhance its value in cosmetic, nutraceutical, and pharmaceutical formulations, accounting for about 4.0% of the weight of rice bran oil (Gunawan et al., 2006). Oleic and linoleic acids were the major fatty acids in the FASE fraction, and 4-desmethyl, 4-monomethyl, and 4,4-dimethyl sterols predominated. Phytosterol esters have been shown to lower plasma cholesterol concentration by inhibiting the absorption of cholesterol from the small intestine (Sierksma et al., 1999). The major constituents of WE were saturated esters of C_{22} and C_{24} fatty acids and C_{24}–C_{40} aliphatic alcohols (Gunawan et al., 2006).

XI. SAFFLOWER OIL

Safflower oil comes form the seed of the plant *Carthamus tinctorius* L. (Fedeli, 1983). It is a very old crop that was originally used in textile dyeing. With a reduction in hull content and an increase in the amount of seed oil, it has become commercially important as a vegetable oil (Kneeland, 1966). The amount of safflower oil produced worldwide does not rank among the top 10; however, U.S. 2005–2006 production of safflower oil was forecast to rank tenth among edible fat and oil sources, at 56 million pounds (Table 10.2) (USDA, 2006).

A typical fatty acid profile for safflower oil is shown in Table 10.1. With 77.7% 18:2, safflower oil has the highest 18:2 percentage of any known native oil, not considering modern genetic fatty acid modifications of certain oils. A recent study reported slightly greater amounts of 18:2 in a Korean safflower oil, with the fatty acid distribution as 16:0, 5.53%; 18:0, 1.62%; 18:1, 11.00%; 18:2, 81.5%; and 18:3, 0.40% (Lee et al., 2004). For this reason, most safflower oil is used by health-conscious individuals as a highly polyunsaturated oil even though it is expensive (Smith, 1985). High-oleic safflower oils have been developed as experimental lines, but commercial safflower oil is still primarily the "high-linoleic" type (Sonntag, 1979). The high-18:2 percentage makes safflower oil highly suitable as a drying oil for use in the paint and coating industry (Rheineck and Cummings, 1966).

The fatty acid composition of commercial safflower oil appears to be fairly consistent under most environmental conditions, with the 18:2 level ranging between 75% and 80% (Applewhite, 1966; Knowles, 1972). For example, seed from Arizona or Montana varied only 2%–3% in 18:2

content (Smith, 1985). Experiment lines have been developed that produce oils with different fatty acid compositions, including medium-linoleic lines with 56%–63% 18:2 (Knowles, 1972), high-oleic lines with 74%–79% 18:1 (Craig, 1953; Fuller et al., 1967; Knowles, 1972), and a high-stearic line with 5%–10% 18:0 (Ladd and Knowles, 1970). Bartholomew (1971) examined the effect of growing temperature on the fatty acid composition of safflower oils differing in levels of 18:2. The high-linoleic and high-oleic lines were relatively stable, but the medium-18:2 line showed a strong response, with low temperatures producing an oil with almost 75% 18:2 and high temperatures producing an oil more like the high-oleic oil. This response is similar to that of sunflower oil (Ermakov and Megorskaya, 1972). Low temperatures also reduced the levels of 18:0 in a high-stearic acid line.

Knowles (1972) also examined 12 wild species of safflower as a source of genes for modifying the fatty acid composition of safflower oil; however, they found limited variability in the fatty acids of these species, with most being remarkably similar to commercially grown high-linoleic types. Indeed Işiğigür et al. (1995) evaluated the fatty acid composition of 17 varieties of safflower seed of Turkish origin. One of these varieties was a high-oleic line containing 73.9% 18:1; however, the remaining 16 lines had relatively similar fatty acid profiles, with values ranging as follows: 16:0, 6.6%–9.6%; 18:0, 0.8%–3.6%; 18:1, 8.7%–13.7%; and 18:2, 73.2%–83.0%.

Müller-Mulot (1976) measured the tocopherols in safflower oil and found the following homologue distributions; alpha, 223 mg/kg; beta, 7 mg/kg; gamma, 33 mg/kg; and delta, 3.9 mg/kg for a total of 266.9 mg/kg. This level is somewhat lower than the amounts he found for other major vegetable oils such as cottonseed, corn, soybean, and sunflower oils. In safflower oil extracted by mechanical press in a laboratory setting, Lee et al. (2004) measured slightly greater total amounts of the tocopherol homologues, with distributions of alpha, 386 mg/kg; beta, 8.9 mg/kg; and gamma, 2.4 mg/kg. They also found 5.2 mg/kg gamma-tocotrienol and 8.4 mg/kg delta-tocotrienol. Lee et al. (2004) also reported four phospholipid classes, phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylcholine (PC), for a total of 19.4 mg/kg phosphorus, with PE providing 60% of the phospholipids, and PI providing 32.6%. Safflower oil, with its high-18:2 percentage and low levels of natural antioxidants, is not particularly stable to oxidation; however, the high-oleic types have improved stability. Fuller et al. (1967) examined the polymer formation at 185°C as a measure of stability, of a high-oleic safflower oil (UC-1), commercial safflower oil, soybean, and cotton-seed oils. The UC-1 oil was the most stable and the commercial safflower oil was the least stable of the oils tested. Purdy (1985) reported the oxidative stabilities of "normal" safflower and sunflower oils compared with high-oleic types of each oil. Normal safflower and northern-grown sunflower oils had similar active oxygen method (AOM) values, whereas the high-oleic types of each oil had improved AOM values of about 3.5 times. The oxidative stability was related directly to the 18:2 level. When Korus and Mousetis (1984) compared thermal stabilities of safflower and rapeseed oils, they also found a direct correlation between 18:2 percentage and polymerization. Snyder et al. (1985) compared PVs and total GLC volatiles of most major vegetable oils and found safflower oil to be the least stable by these measures.

It is difficult to predict the future of safflower oil production as an industry. Because the oil is not very stable and fairly high priced, it offers little advantage over other oils except for its possible health benefits as an oil high in PUFAs. The market for the more stable high-oleic safflower oil may increase, especially if the current nutritional interest in high monounsaturates continues.

XII. SESAME OIL

Sesame oil (*Sesamum indicum* L.) has been used as an edible oil since ancient history. Its seed has an oil content of around 45%, which can be removed fairly easily by pressing. Sesame oil is generally sold as a gourmet oil, with high natural oxidative stability and a unique flavor (Toro-Vazquez and Gallegos-Infante, 1996). Mexico is one of the main producers of the oil. World production of sesame oil does not usually rank among the top ten, as also forecast for 2005–2006 (USDA, 2006).

The typical fatty acid composition of sesame oil is listed in Table 10.1. The 18:1 and 18:2 percentages are generally almost equal, and 18:3 represents only a minor portion of the total fatty acids. Little variation in fatty acid composition occurred among sesame seed oils grown in different locations, although oils grown in India tended to have a slightly higher 16:0 composition (12%–17% vs. 10%) than those grown elsewhere (Kaufmann and Mankel, 1963; Sreenivasan, 1968; El Tinay et al., 1976). The fatty acid composition of oil from sesame seed grown in Taiwan was within 2%–3% of each fatty acid listed for same oil in Table 10.1 (Yen, 1990). The fatty acid composition of four collections of the cultivated *S. indicum* L. species grown in the Sudan also had very similar profiles to those just listed (Kamal-Eldin and Appelqvist, 1994). Three related wild species of sesame contained less total oil (approximately 30%) and somewhat greater variations in fatty acid composition than the cultivated species. For example, one species had approximately 35% 18:1 and 45% 18:2. East African sesame accessions evaluated over the 3 years of 2002–2004 showed significant variations in oil and fatty acid content among the years (Were et al., 2006). Oil content correlated negatively with 16:0 and 18:2, and positively with 18:0 and 18:1. The sesame crop from this geographic area is generally used in the confectionary industry, rather than as an oilseed, and has not been selected for oil content or quality.

The tocopherol level of sesame oil is relatively low, with the following reported distribution in milligrams per kilogram of pure refined sesame oil (unroasted): alpha, 13; beta, 5.6; gamma, 244; and delta, 32 (Müller-Mulot, 1976). Shahidi et al. (1997) reported finding only gamma-tocopherol in hexane-extracted sesame oil at a level of 330–387 mg/kg. Slover et al. (1983) found the following tocopherol levels in cold-pressed sesame oil: alpha, 82–88 mg/kg; gamma, 155–167 mg/kg; and delta, 0 mg/kg. They did not report levels of beta-tocopherol.

Although sesame oil contains low level of tocopherols, it has good amounts of other phenols that seem to be unique to sesame oil and that contribute to its superior oxidative stability. These compounds include sesamin, sesamolin, and sesamol (Budowski, 1964; Suja et al., 2004). The compound sesamol forms from sesamolin during processing and was originally thought to be responsible for the high stability of sesame oil (Budowski, 1964). Since then, however, products including a sesamol dimer (a positive intermediate of the oxidative degradation of sesamol) have been thought to be the antioxidative agents in sesame oil (Kikugawa et al., 1983). Fukuda et al. (1986) suggested that the conversion of sesamolin to sesaminol was responsible for the stability of sesame oil. The sesamin content in oils extracted from dehulled and coated seeds ranged from 5800 to 6490 mg/kg; whereas, the sesamolin content ranged from 1830 to 3490 mg/kg (Shahidi et al., 1997).

Sesame oils from both unroasted and roasted seeds are widely used (Fukuda et al., 1986). Both oils are stable to oxidation but the oil prepared from the roasted seed has a distinctive flavor and longer shelf life (Manley et al., 1974; Kikugawa et al., 1983). Yen (1990) determined that, among oil from unroasted seed and oils from seed roasted at different temperatures (180°C–260°C), the highest levels of sesamol and gamma-tocopherol were found in oil roasted at 200°C–220°C. He found 480 mg/kg gamma-tocopherol in oil from seed roasted at 200°C compared with 289 mg/kg in oil from unroasted seed. He attributed the remarkable stability of oil from roasted sesame seed to the high levels of these compounds. Abou-Gharbia et al. (1997) noted that sesame oil extracted from coated seeds was more stable to oxidation during storage than oil extracted from dehulled seeds after several different preextraction treatments. The difference was likely caused by the release of natural antioxidants from sesame seed hull into the oil.

XIII. SOYBEAN OIL

Soybean oil is obtained from the seeds of the legume *Soja max*. Total production of soybean oil has ranked number one in the United States for many years, with the 2005–2006 production forecast to be 20.04 billion pounds (Table 10.2) (USDA, 2006). The 2005–2006 world production of soybean oil was estimated to be 33.87 million metric tons, ranking second behind palm oil (Table 10.2) (USDA, 2006). In 2004–2005, palm oil overtook soybean oil for the first time in worldwide

production amount. Soybean oil, similar to most major oils, is refined, bleached, and deodorized before consumption.

The fatty acid composition of soybean oils varies depending on many environmental and genetic factors that were mentioned at the beginning of this chapter. The typical fatty acid composition of soybean oil is shown in Table 10.1. Although there is an environmental effect on fatty acid composition (e.g., soybeans grown in warmer climates produce more saturated oils and those grown in cooler climates produce more unsaturated oils), Hammond and Fehr (1984) demonstrated that the ranking of many soybean lines for 18:3 percentage remained the same when grown in Iowa (cooler climate) as compared with Puerto Rico (warmer climate). Thus "heritability" of the genetic influence on fatty acid production is high. A multilocational field trial with seven Indian soybean cultivars at four growing locations determined significant genotypic, locational, and geotypic × locational interactions for oil amount as well as for all the unsaturated fatty acids (Kumar et al., 2006). Average daily mean temperature during bean development was negatively associated with linolenic acid percentage, as mentioned previously. Nearly 100% of the 18:3 in soybean oil is reported to be in the form 18:3 (n-3) (Jaselskis et al., 1982; Manku, 1983). These n-3 fatty acids may have some nutritional advantages in reducing heart disease, a topic that is discussed elsewhere in this book.

A current objective of plant breeders is to improve the nutritional and/or functional properties of soybean oil by modifying the fatty acid composition (Durham, 2003). New genetically altered soybean lines have been developed, targeting different end uses. Reducing the PUFA composition and/or increasing oleic acid concentration improves oxidative stability of the oil without hydrogenation. In contrast, increasing the saturated fatty acid concentration can provide soybean oils with enough solids for use in margarine and shortening production, with no or minimal hydrogenation. The interest in lowering PUFA composition, and particularly the 18:3 content of soybean oil originally arose from the association of 18:3 with flavor instability. The relative oxidation rates of 18:1, 18:2, and 18:3 as fatty acid methyl esters, when present as mixtures, was reported to be in the ratio of 1:10.3:21.6, respectively (Fatemi and Hammond, 1980). Elevating 16:0 to 10.6% and/or lowering 18:3 to <2.6% increased the oxidative and flavor stability (Shen et al., 1997a). Earlier, White and Miller (1988) and Liu and White (1992a) reported soybean oil with elevated 18:0 percentage to have significantly greater oxidative stability than normal soybean oil, and soybean oils with low-18:3 percentage oxidized at significantly lower rates than did oils with normal 18:3 levels. Reducing the 18:3 and/or increasing the 18:0 percentage also resulted in enhanced frying stability of the oil (Miller and White, 1988; Liu and White, 1992b; Warner and Mounts, 1993; Mounts et al., 1994; Gerde et al., 2006). Lines have been developed containing oil with <3% 18:3 (Hammond and Fehr, 1983; Fehr et al., 1992), up to 28% 18:0 (Graef et al., 1985; Lundeen et al., 1987), with reduced palmitic, total saturated linolenic acids (Streit et al., 2001), and with high- (~80%) and mid- (~55%) oleic acid (Kinney, 1997; Liu, 1999; Wilson, 1999). Other soybean lines produce oils with even lower (<2%) 18:3 and/or higher 16:0 and/or 18:0 concentrations. A study comparing soybean oils with ~1% 18:3, ~3% 18:3, and typical fatty acid compositions showed a stability advantage to the oil with ~1% 18:3 (Gerde et al., 2006).

Oil from soybean cultivars with reduced 18:3 (3%) was first marketed in 1994 by Kraft Food Ingredients (Anon., 1994). Another experimental cultivar was developed to have low total saturated fatty acid composition to compete with new varieties of canola marketed as having less than 6% total saturated fatty acids (Fehr and Hammond, 1996a,b). Most recently, the push by industry to reduce or eliminate *trans* fats in the diet to comply with *trans*-fat labeling resulted in the release of newer low (<3%) and ultra-low (~1%) linolenic acid soybeans. The company, Asoyia, LLC, grows a soybean line developed by Iowa State University and markets the ultra-low linolenic acid oil by this name, with hexane processing and refining done by Cargill. Innovative Growers, LLC, uses expeller processing and physical refining to process ultra-low linolenic acid soybean oil. Similarly, Monsanto markets a low-linolenic acid bean, which they developed under the name, VISTIVE™, whereas a Bunge–Dupont alliance introduced a low-linolenic soybean oil from a soybean variety developed by Pioneer Hi-bred International, Inc., marketed under the NUTRIUM™ brand. Monsanto and Iowa

State University have goals to release both mid-oleic/low- or ultra-low linolenic beans and mid-oleic/low- or ultra-low linolenic acid/low (<7%) saturated fatty acid beans within the next few years.

The practice of partially hydrogenating oils to harden them for the production of margarines and shortenings is declining rapidly, because of nutritional concerns about *trans* fats. However, a small amount of fully hydrogenated, thus fully saturated and containing no *trans*-fatty acids, can be blended with oils to create the functions needed for use in manufacturing margarines and shortenings. Margarines and shortenings vary in fatty acid composition, depending on the manufacturer and the type of product produced. For example, the composite fatty acid values of 17 stick or brick margarines made from coconut oil, corn oil, cottonseed oil, palm oil, peanut oil, safflower oil, and/or soybean oil were reported as follows, listed in g/100 g margarine: 16:0, 9.2 ± 1.9; 18:0, 5.4 ± 0; 18:1, 37.5 ± 8.2; 18:2, 21.8 ± 7.5; and 18:3, 0.8 ± 1.0 (Weihrauch et al., 1977). Soft tub margarines (13) had fatty acid values as follows, listed in g/100 g margarine: 16:0, 9.1 ± 1.2; 18:0, 5.1 ± 1.1; 18:1, 31.2 ± 5.6; 18:2, 28.5 ± 6.2; and 18:3, 1.7 ± 1.1. The authors of the study summarized that margarines (and shortenings) formed relatively uniform groups of products regardless of the oil source. No distinction was made between *cis*- and *trans*-isomers in the study by Weihrauch et al. (1977). Enig et al. (1990), however, estimated that margarines produced at that time contained an average of 23% *trans*-fatty acids, whereas shortenings contained an average of 25.3%. Since 1990, however, many companies have traded *trans*-fatty acids for saturated fatty acids to create the desired functions. The new soybean oils high in 16:0 or 18:0 obviously are more solid, requiring less hydrogenation than normal oils. In addition, oils with at least 15% 16:0 product smooth margarines because of the interference of the 16-carbon fatty acid with the crystalline structure of a "solid" fat that is made up mainly of 18-carbon units.

Another factor besides the 18:3 content thought to affect the flavor stability of soybean oil is the presence of lipoxygenase enzymes that rapidly oxidize 18:2 and 18:3 to generate the "beany" undesirable flavor in soybean oils. Soybean lines lacking one or more of the lipoxygenase enzymes have been developed (Kitamura et al., 1983; Davies and Nielsen, 1986; Davies et al., 1987; Shen et al., 1996; King et al., 1998). The general findings, however, revealed no improvement in soybean oil quality with the removal of two or three of the enzymes from the beans, when optimal conditions were used (Shen et al., 1996, 1997b; King et al., 1998). It is possible that an improvement in oil quality of the oils from beans lacking the enzymes over that of oil from normal beans might be noted if soybean storage and/or oil processing conditions were unfavorable.

The general stability of soybean oil is aided by the presence of naturally occurring tocopherols and other minor constituents. Müller-Mulot (1976) reported tocopherol levels in refined soybean oil as follows: alpha, 116 mg/kg; beta, 34 mg/kg; gamma, 737 mg/kg; and delta, 275 mg/kg. Slover et al. (1983) found tocopherol levels similar to these amounts in two soybean oil samples that they measured. The following values in milligrams per kilogram were listed: alpha, 82–84; gamma, 662–758; and delta, 276–287. The beta homologue was not measured. Chase et al. (1994) compared tocopherol levels in crude and refined soybean oils, reporting only minor losses of the tocopherols during the refining, bleaching, and deodorizing process. The refined oil contained 1360 mg/kg, whereas the crude oil had a total of 1490 mg/kg tocopherols. Individual tocopherol homologues for refined oil followed by crude oil are listed: alpha, 69.2–72.2 mg/kg; beta, 32.9–33.9 mg/kg; gamma, 818–933 mg/kg; and delta, 436–453 mg/kg. Warner (2005) reported typical amounts of tocopherol homologues in soybean oil in milligrams per kilogram as follows: alpha, 120; gamma, 610; and delta, 260. These high levels of tocopherols, especially the gamma homologue, are thought to help protect soybean oil against oxidation and flavor instability (Warner and Mounts, 1990; Warner et al., 1990). A study, in which tocopherols native to soybean oil were replaced with tocopherols native to sunflower oil and vice versa, revealed greater oxidative and flavor stability of either oil aged in the dark when the tocopherol profile native to soybean oil was present, and greater stability of either oils aged in the light when the tocopherol profile native to sunflower oil was present (Warner, 2005). A comparison of soybean oil stability with that of other major vegetable oils is discussed under canola oil above. Expeller pressed and physically refined soybean oil tested favorably during frying

of French fries, even though it had less total tocopherols and phytosterols than hexane-extracted, refined, bleached, and deodorized soybean oil (Warner and Dunlap, 2006). The authors speculated that Maillard reaction products produced during the thermal processing step used for expeller oil contributed antioxidant products that enhanced lipid oxidation stability.

Vlahakis and Hazebroek (1998) reported significant correlations between total tocopherol level, PUFA composition, and decreasing temperature during seed development. Researchers previously have noted a positive link between tocopherol and PUFA composition, speculating that the plant produced more tocopherol as a protective measure against oxidation of the polyunsaturates. Vlahkis and Hazebroek (1998), however, explain that both these biochemically unlinked traits are controlled by temperature.

XIV. SUNFLOWER OIL

Sunflower oil is obtained from the seed of the plant *Helianthus annuus* L., which is native to North America (Campbell, 1983). The plant is grown in large quantities in Russia, Argentina, China, the European Economic Community (ECC), and in the United States (Anon., 1986). Worldwide, the production of sunflower oil is the fourth largest of edible oils, being surpassed by soybean, palm, and rapeseed (including canola) oils, with the 2005–2006 forecast at 10.46 million metric tons (Table 10.2) (USDA, 2006). U.S. production of sunflower oil for 2005–2006 was forecast to rank seventh, at 588 million pounds (Table 10.2) (USDA, 2006). Generally, sunflower oil is used for edible purposes as a cooking and salad oil and in the manufacture of shortenings and margarines (Sonntag, 1979). High-oil seed sunflowers (40% oil) are generally grown for oil production, whereas low-oil seeds (about 30% oil) are grown for confectionery, nut, and birdseed markets.

Sunflower oil is refined, bleached, and deodorized to produce a bland-flavored edible oil. The typical fatty acid composition of sunflower oil consumed in the United States is shown in Table 10.1. The fatty acid composition of sunflower oil is naturally variable, depending on climate, temperature, genetic factors, and position of seed location in the flower head (Howell and Collins, 1957; Campbell, 1983). In fact, few vegetable oils reflect the influence of these factors as significantly as does sunflower oil. The 18:2 and 18:1 averages of northern sunflower oil grown above 39° latitude in the United States compared with southern-grown sunflower oil varied, with 18:2 ranging from 44% to 68% and 18:1 from 19% to 47% (Campbell, 1983). The warmer the temperature during maturation of the seed, the lower the 18:2 and the higher the 18:1 levels became.

Nagao and Yamazaki (1983) found fatty acid compositions of four varieties of sunflowers grown under seven environmental conditions (planting location and date) in Japan to vary considerably in fatty acid composition. The 16:0 ranged from 4.2% to 6.2%, 18:0 from 2.4% to 6.0%, 18:1 from 13.6% to 49.9%, and 18:2 from 43.8% to 75.4%. There were significant differences in the percentage of 18:2 among the four varieties; however, the greatest differences were due to environmental conditions. The 18:2 percentage was negatively correlated with the temperature and the 18:1 percentage was positively correlated. The combined percentage of 18:1 and 18:2 was constant, at about 90%, and was not influenced by temperature. Therefore, it is likely that the temperature during maturation of the seed influenced the desaturation of 18:1–18:2. Other researchers have noted the close reverse dependence between 18:1 and 18:2 in sunflower oil (Ermakov and Megorskaya, 1972; Mancha et al., 1994). Similarly, two high-18:1 hybrids, Platon and Vyp70, developed by the Italian seed company, AGRA, experienced a decrease in 18:0 and 18:1 and an increase in 16:0 and 18:2 with irrigation (Flagella et al., 2002). Thus, stress conditions of drought actually increased the 18:1 concentration. Because of the variability in the 18:1 and 18:2 production, it is difficult to give an accurate fatty acid content of sunflower oil without actually measuring it. In general, however, most standard sunflower oil produced in the United States is grown in northern climates, so the typical fatty acid composition shown in Table 10.1 is fairly accurate for standard sunflower oil used in the United States.

Despite the great influence of temperature and other environmental conditions on fatty acid composition, sunflowers have been developed that consistently produce sunflower oil high in 18:1 even when grown in northern climates. Treatment of normal varieties of sunflower seed with chemical mutagens and development of their progenies have resulted in hybrids bearing oil with 18:1 contents greater than 80%, and 18:2 contents of less than 10% (Purdy, 1986). The fatty acid composition of the varieties seems to be unaffected by climatic conditions. Patents for these unique sunflower oils were issued to the Lubrizol Corporation (Fick, 1984, 1985). In 1984, high-oleic seed was grown commercially in the United States for the first time in North Dakota, California, and Texas, and very little difference in either 18:1 or 18:2 levels was noted among locations. High-18:1 sunflower oil is currently grown around the world in areas including North and South America, Europe, Asia, and Australia. Mid-18:1 NuSun® sunflower hybrids with 18:1 levels of 50%–70%, and high-18:1 hybrids, with 18:1 levels of >80% are available from Mycogen® (Dow AgroSciences, LLC). In Australia, Sunola is the registered trademark name given to high-18:1 sunflower oil sold by Meadow Lea Foods.

Since 1988, Mancha et al. (1994) have led an extensive mutagenic program to induce further variability in the fatty acid composition of sunflower seed oil. They have obtained seeds with high-16:0 (26%–34%) or high-18:0 (8%–9%) compositions. In Spain, by chemical mutagenesis, sunflower seeds with oil having very high-18:0 concentrations of ~35% have been developed (Fernández-Moya et al., 2000, 2002, 2005). In addition, a line with an 18:0 of 10.0% and an 18:1 of 38.3% was reported (Fernández-Moya et al., 2005). These lines have not yet been evaluated for agronomic performance, but a temperature impact was noted. The maximum 18:0 concentration was obtained when the plants were grown at 39°/24°C, and temperatures greater than 30°C/20°C were needed for good expression of the phenotype (Fernández-Moya et al., 2002).

The total tocopherol concentration in sunflower oil has been reported to be similar to that of soybean, corn, and cottonseed oils at about 700 mg/kg of oil weight (McLaughlin and Weihbrauch, 1979; Landers and Rathmann, 1981). For sunflower oil, the total included 500 mg/kg of the alpha homologue and 100 mg/kg of the gamma homologue, whereas soybean oil had 100 mg/kg of alpha and 600 mg/kg of gamma homologues. Warner et al. (1990) reported tocopherol levels in soybean oil of 120, 10, 610, and 260 mg/kg for alpha, beta, gamma, and delta homologues, respectively, whereas for sunflower oil, they reported levels of 610, 10, 30, and 10 mg/kg for alpha, beta, gamma, and delta homologues, respectively. The alpha form has the highest vitamin E activity; however, gamma-tocopherol has the highest antioxidant activity. In studies by Warner et al. (1990) and Warner and Mounts (1990), sunflower tocopherols were added to stripped soybean oil and vice versa. The stability pattern of sunflower oil, which is generally less stable than soybean oil, mimicked that of soybean oil. With sunflower tocopherols added, soybean oil had a stability pattern similar to that of sunflower oil. More specific follow-up work with the same experimental research design showed greater oxidative and flavor stability of both soybean and sunflower oils aged in the dark when the tocopherol profile native to soybean oil was present, and greater stability of either oils aged in the light when the tocopherol profile native to sunflower oil was present (Warner, 2005). Demurin et al. (1996) noted that oil from near-isogenic lines of wild sunflower species differed in their proportions of alpha- and gamma-tocopherols. They compared the oxidative stability of these oils, and confirmed that oils having the greatest amount of gamma-tocopherol were the most stable. These researchers suggested that this type of genetic variability in sunflowers be used to produce a more stable sunflower oil containing mainly gamma- rather than alpha-tocopherols. Other researchers, however, still suggest that alpha-tocopherols have the better activity.

The high-PUFA content of sunflower oil makes it susceptible to oxidative deterioration and, thus, reduced flavor stability. In general, sunflower oil tends to be less stable than many oils, including soybean oil (Snyder et al., 1985; deMan et al., 1987; Warner et al., 1989). Sunflower oil of normal 18:2 content was particularly susceptible to autoxidation in the presence of sunlight (Topallar et al., 1997). High-oleic sunflower oil, however, dramatically improved oxidative stability, as measured by the AOM when compared with normal sunflower oil (Purdy, 1985). The improved stability was

attributed entirely to the shift in fatty acids from 18:2 to 18:1. The alpha-tocopherol levels in the high-oleic sunflower oil were similar to those found in regular sunflower oil (Purdy, 1986). Other researchers demonstrated that polyunsaturated vegetable oils, including canola, corn, and soybean oils, blended with high-oleic sunflower oils had greatly improved oxidative stability (Frankel and Huang, 1994). Additionally, high-oleic sunflower oil had greater frying stability and oxidative stability of potato chips fried in the oil during subsequent storage than cottonseed oil or than cottonseed/sunflower oil blends (Warner et al., 1997). Foods fried in the high-oleic oil, however, lacked some of the good flavors normally associated with fried foods, presumably because of the formation of fewer volatiles arising from 18:2. Mid-oleic levels (65%) of sunflower oils have been developed to address this problem (Warner and Kleingartner, 1998). Dobarganes et al. (1993) also noted enhanced thermal stability and frying performance of high-oleic sunflower oil over normal sunflower oil. No difference in stability was noted between olive and high-oleic sunflower oil over normal sunflower oil. No difference in stability was noted between olive and high-oleic sunflower oils, each containing similar amounts of 18:1.

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11 Fatty Acids in Fruits and Fruit Products

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CONTENTS

I. INTRODUCTION

The search for new sources of fats and oils is an ongoing process. By far, plant seeds are the most promising sources of oils for nutritional, industrial, and pharmaceutical purposes. The application of an oil for a particular purpose, however, is determined by its fatty acid and triglyceride compositions. No oil from a single source has been found to be suitable for all purposes, because oils from different sources have different fatty acid compositions. It is for this reason that the search for new sources for novel oils is so important. So far, a large number of plants have been analyzed, and some of these have been cultivated as new crops (Hirsinger, 1989). Another approach to develop novel oils is to produce new cultivars from already established oil crops. The development of low-erucic acid cultivars of *Brassica* is one good example (Downey and Robbelen, 1989). Other novel crops that are finding greater utilization include hemp (Parker et al., 2003; Illingworth, 2004), crambe (Yaniv et al., 1991), sea buckthorn (Yang and Kallio, 2001), and cuphea (Isbell and Behle, 2003). In addition to the nutritional, industrial, and pharmaceutical uses of oil, the variation in fatty acid composition in plants has proven to be a useful tool in taxonomic and phylogenetic studies (Vickery, 1971).

The potential supply of lipid material from fruits and fruit by-products may be enormous and should be investigated. Palm oil, palm kernel oil, and coconut oil are good examples of commercially successful oils extracted from palm fruit. Future edible oil supplies may depend on the discovery and development of similar types of plants. A listing of the lipid content and fatty acid composition of oils extracted from well-established sources and from new plant sources would be an important first step in this search.

A relatively untapped source of lipid and protein raw material is the by-product of fruit-processing plants. Many millions of pounds of fruit seeds are discarded yearly and in some cases resulting in disposal problems. Proper utilization of these waste products could lead to important new sources of oil and meal.

Many developing countries that are unable to fulfill their edible oil requirements for lack of currency to purchase commercial oils may benefit from indigenous oil-yielding plant sources.

New sources of oil and fat could supplement conventional supplies or even replace imports. An adequate supply of fats and oils could help reduce hunger and improve the nutritional status of the population by furnishing essential fatty acids in the diet.

Fatty acid composition is an important consideration with many oils, especially in affluent countries, where the need for calories is not as important as health concerns. Information on the relationships between total fat consumption, saturated fats in the diet, blood cholesterol levels, and cardiovascular disease is contradictory and confusing. Nevertheless, the National Heart, Lung and Blood Institute has recommended a diet with no more than 30% of total calories coming from fats and with saturated fats providing no more than 10% of total calories. In an article published in the *New England Journal of Medicine* (August 17, 1989), it was shown that a mixed diet rich in monounsaturated fatty acids (12.9% saturates, 15.1% monounsaturates, and 7.9% polyunsaturates) was as effective in lowering serum low-density lipoprotein (LDL) cholesterol as a diet rich in polyunsaturated fatty acids (PUFAs) (12.6% saturates, 10.8% monounsaturates, and 12.7% polyunsaturates). Another class of PUFAs that has received a great deal of attention is the ω-3 fatty acids. These fatty acids are found in fish oils and in some vegetable oils. The two most common ω-3 fatty acids in fish oils are eicosapentaenoic acid (EPA, 20:5) and decosahexaenoic acid (DHA, 22:6). There is substantial evidence indicating that ω-3 unsaturated fatty acids may provide health benefits in preventing heart diseases, cancers, hypertension, and autoimmune disorders (Connor, 2000; Tapiero et al., 2002). Interest in the ω-3 fatty acids began with the observations that Greenland Eskimos who consume a high-fat, high-cholesterol diet have a very low incidence of cardiovascular disease. This was attributed to their consumption of fish oils high in the ω-3 fatty acids. Soybean and canola oils contain substantial levels of these types of fatty acids in the form of α-linolenic acid.

World production of fats and oil was about 137 million metric tons (MMTs) in 2004–2005 (Tables 11.1 and 11.2). At the same time, the consumption of oil is forecast to be 138 MMTs. This deficiency is of little concern to the developed countries that have resources to purchase these scarce oil supplies. The developing countries, on the other hand, with very little foreign exchange cannot compete and must depend on domestic production if available. For example, Pakistan produces 1.62 MMTs, and imports 1.83 MMTs of oil yearly, whereas Bangladesh imports 1.13 MMTs, and consumes 1.21 MMTs of oil and has only limited production capabilities. As the economy of these developing countries improves, greater and greater demands on the world's oil supplies will ensue as they utilize their new purchasing powers. China produced 18.4 MMTs of oil in 2004/2005 but consumed 25.3 MMTs and as a result had to import 7.02 MMT to meet its needs. The development of new sources of fats and oils is one way to help mitigate this growing problem.

The information presented in this chapter is by no means a comprehensive listing of all possible fruits and fruit by-products. In some tables, only a representative sample of the types and variety of plants is given. Also, because the fatty acid composition is so dependent on factors such as maturity, soil conditions, climate, and variety, the compositions stated in some cases are averages from a

TABLE 11.1
World Production of Fat and Oil by Region (2004/2005)

Regions	Million Metric Tons
Europe and former USSR	24.08
Africa	4.27
North and South America	39.09
Asia	61.36
Australia, New Zealand, and others	3.62

Source: Oil World Annual 2006.

TABLE 11.2
World Production of Fats and Oils by Commodity (2004/2005)

Commodity	Million Metric Tons
Soybean	32.85
Cottonseed	4.99
Groundnut	4.47
Sunflower	9.39
Rapeseed	15.73
Sesame	0.84
Corn	2.08
Olive	3.02
Palm	33.32
Palm kernel	3.94
Coconut	3.16
Butter	6.62
Lard	7.49
Fish oil	1.03
Linseed	0.61
Tallow/grease	8.23

Source: Oil World Annual 2006.

number of studies or varieties, and in other cases a single representative composition is given. Where data are available, the toxicological properties of the oil are mentioned.

Each table presents the common name, scientific name, % lipid, fatty acid composition, % saturated, %unsaturated, and references. The fatty acid compositions are given for the fruit mesocarp, fruit kernel, tree nuts, palm fruits, vegetable butters, miscellaneous plants, spices, and condiments.

II. FATTY ACID COMPOSITION OF FRUIT MESOCARP

The mesocarp, or pulp, of fruits generally contains very low levels of lipid material (0.1%–1.0%) and as such does not constitute an important source of edible or industrial fats and oils. A few notable exceptions are avocado, palm, and olive. The avocado is a cultivated fruit in which the oil is a main component (on a dry weight basis). The tree grows in a number of countries, including India, Egypt, Mexico, and the United States. The high oil content (15%–30%) and fatty acid composition depend on many factors such as variety, geographical location, climate conditions, and stage of fruit development. Avocado oil is obtained by either solvent extraction or centrifugal separation. The oil is highly unsaturated, with 75%–80% oleic acid, 7%–10% linoleic acid, and only trace levels of stearic acid (Werman and Neeman, 1987). The values given in Table 11.3 for avocado mesocarp fatty acid composition are the averages from four cultivars grown in the Mediterranean region. *cis*-Vaccenic acid (1%–2%) has been identified and is reported as the sum of vaccenic and oleic acids, because it coelutes with oleic acid. The fatty acid composition has been shown to change with maturation. Thirty-six weeks after flowering, the oleic + vaccenic acid levels increased steadily, whereas all other major fatty acids decreased. The three minor fatty acids—palmitoleic, stearic, and linolenic acids—either decreased or remained constant (Ratovohery et al., 1988).

Olive oil has been used since ancient times. The tree grows in the subtropical climates of the lands surrounding the Mediterranean Sea and in North Africa. The olive fruit pulp may contain

TABLE 11.3
Fatty Acid Composition of Fruit Pulp Fatty Acids (%)

Common Name	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Saturation	Unsaturation	References
Apple[a]	*Malus sylvestris*	0.4	1.2	26.7	0.6	3.9	7.9	48.9	10.1	32.4	67.5	USDA (1984)
Apricot	*Prunus armeniaes*	0.4		8.8		1.1	62.0	28.1		9.9	90.1	USDA (1984)
Avocado	*Persea americana*	6.5–25.5		9.0	1.8	0.5	77.7	8.5	0.9	9.5	88.9	Ratovohery et al. (1988); Werman and Neeman (1987)
Banana	*Musa paradisiaca*	0.2		41.9	2.1	3.8	14.4	16.1	21.7	45.7	54.3	Goldstein and Wick (1969)
Blueberry	*Vaccinium corymbosum*	0.43–0.55	2.1	7.38		2.8	24.0	38.7	27.1	12.2	89.8	Wang et al. (1990)
Cherry (sour)	*Prunus cerasus*	0.3	0.8	20.0	0.4	6.7	33.8	19.2	18.3	27.5	71.7	USDA (1984)
Cherry (sweet)	*Pranus avium*	1.0	0.7	19.8	0.4	6.8	33.8	19.2	18.5	27.3	71.9	USDA (1984)
Chironji fruit	*Buchanania lanzan*			34.4		2.0	62.3	1.2	0.2	36.4	63.7	Hemavathy and Prabhakar (1989)
Currant	*Ribes nigrum*	0.4		7.4	0.4	2.6	20.7	39.5	26.6	10.0	87.2	USDA (1984)
Durian	*Durio zibethinus*	4–5	0.5	39.8	8.5	0.8	45.8	1.8	2.7	41.1	58.8	Berry (1981)
Fig	*Citrus ficus carica*		0.8	17.0		4.4	24.4	53.3		22.2	77.7	USDA (1984)
Gac	*Momordica cochinchinensis spreng*	22 dw	0.5	29.2	0.3	7.7	32.3	28.1	0.5	30.0	70	Ishida et al. (2004)
Goldenberry[b]	*Physali peruviana*	0.2	0.5	9.6	1.1	2.9	20.1	44.4	1.1	16.1	83.8	Ramadan and Morsel (2003a)
Gooseberry	*Ribes* spp.	0.6		4.7		3.2	12.6	66.7	11.3	7.9	90.6	USDA (1984)
Grapefruit	*Citrus paradis*	0.1		23.5	2.0	1.9	23.5	37.3	9.8	25.4	72.6	USDA (1984)
Grape	*Vitis vinifera*	0.6	3.4	42.5		5.8	6.0	34.1	10.2	51.7	50.3	USDA (1984)
Guava	*Psidium guajava*	0.6	2.5	30	0.6	3.3	10.8	37.9	14.8	35.8	64.1	USDA (1984)
Lemon	*Citrus limon*	0.3	0.7	25.2	0.7	1.4	7.2	45.3	18.7	27.3	71.9	USDA (1984)
Loquats[c]	*Eriobotrya japonica*	0.2	0.7	23.0		2.8	5.7	55.4	9.3	27.2	70.4	USDA (1984)
Madeira[d] laurel	*Laurus* spp.		1.0	19.2	0.4	1.1	34.8	21.5	1.2	39.2	60.8	Castilho et al. (2004, 2005)
Mango	*Mangiferaindica*	0.1–0.4	2.4	23.6	27.4	1.3	26.4	1.8	16.7	27.3	72.3	Bandyopadhyay and Gholap 1973)

Nabk[c]	*Ziphus jujuba*	4		11.3	1.1	6.7	53.8	20.6		19.9	79.1	Al-Khatib et al. (1987)
Olive[d]	*Olea europea*	50.7		9.5	0.5	3.0	81.0	0.5	0.5	13.0	87.0	Mousa et al. (1996)
		35–70[d]		12.0	0.4	4.0	72.0	10.0	0.5	16.4	83.0	Fedeli (1983); Rana and Ahmed (1981)
Orange	*Citrus sinensis*	0.3		20.6	4.7		31.7	28.6	11.1	20.6	76.1	USDA (1984); Nicolosi-Asmundo et al. (1987)
Papaya[c]	*Carica papaya*	0.14	6.3	28.6	17.8	1.8	16.1	5.3	22.3	37.6	61.5	USDA (1984)
Peach	*Prunus persica*	0.9		10.1	1.1	1.1	38.0	49.4	1.1	11.2	89.6	USDA (1984)
Pear[f]	*Pyrus communis*	0.4		8.5	1.0	1.5	40.5	46.5	0.5	10.0	89.0	USDA (1984)
Pineapple	*Ananas comosus*	0.43		8.4	1.3	4.8	19.9	37.2	27.4	13.2	85.8	USDA (1984)
Plum	*Prunus* spp.	0.62		7.0	0.84	1.5	67.9	22.7		8.5	91.4	USDA (1984)
Quince[g]	*Cydonia oblonga*	0.1		7.29		2.1	37.5	51.1		9.4	89.6	USDA (1984)
Raspberry	*Rubus* spp.	0.55		3.4		0.8	12.7	54.0	27.3	4.2	94.0	USDA (1984)
Strawberry	*Fragaria xananassa*	0.37		5.4	0.4	1.5	19.8	41.9	30.2	6.9	92.3	USDA (1984)
Tangerine	*Citrus reticulata*	0.2	1.1	21.5	4.4	1.1	32.3	29.0	10.7	23.7	76.4	USDA (1984)
Tamarind	*Tamarindus indica*	0.6	1.3	32.8		11.7	35.3	11.5		45.8	46.8	Andriamanantena et al. (1983)
Sea[j] Buckthorn	*Hippophae rhamnoides* L.	2.8		27.8	32.8	0.8	17.3	19.1	3.2	28.6	71.4	Yang and Kallio (2001)

[a] 0.6% 12:0.
[b] 8.7% 18:3n-6, 2.0% 20:3n-6, 2.7% 22:1n-9, 2.6% 24:0, 3.7% 24:1n-9.
[c] 0.7% 12:0.
[d] 17.3% 12:0, 0.5% 20:1.
[e] 1.9% 20:0, 1.1% 20:1, 1.4% 20:2, 1.1% 20:4.
[f] 0.4% 20:0, 0.2% 20:1.
[g] 0.9% 12:0.
[h] 0.5% 20:1.
[i] 1.0% 20:1.
[j] 9.1% 18:1n-7.

as high as 75% oil on a dry weight basis. Olive oil is characterized by a high percentage of oleic acid—up to 93% in some cases (Rana and Ahmed, 1981). Olive oil is unusual among vegetable oils in that it has a low iodine number yet remains liquid at low temperatures (down to about 0°C). This oil is more stable to oxidation than most other liquid vegetable oils owing to its low-linoleic acid content and higher phenolic content. Three types of oil are produced from olive pulp: virgin olive oil, refined olive oil, and residue-refined olive oil. The first is used mainly for salad dressings, the second for cooking and salad oils, and the third for cooking (Fedeli, 1983).

The durian is a highly prized and delicious fruit that grows throughout Southeast Asia. Its delicate flavor has been attributed to low molecular weight thiols/thioethers and esters. The strong odor that emanates from the edible portion (aril) is not appreciated by all people. The aril surrounds the seeds and contains 4%–5% lipid (Berry, 1981). The oil from the aril is yellow and is free of cyclopropene fatty acids, which are commonly found in plants belonging to the order Malvales to which durian belongs. The oil is primarily composed of palmitic and oleic acids. Unsaturated fatty acids constitute 62%–70% of the total fatty acids. Linolenic acid is found in levels ranging from 2% to 7% (Berry, 1980). It is of interest to note that the palmitic/palmitoleic acid ratio shows a relationship to taste panel scores: The lower the ratio, the higher the organoleptic acceptance score (Berry, 1981). This relationship needs more investigation with respect to maturity; degree of ripeness; content of carbohydrates, proteins, and water; and total concentration of flavor constituents. The fruit of *Visnea mocanera*, the so-called mocan fruit, contains 1.5% fat. These fruits are small, red blackish berries that are the size of a cherry when fully ripe. They are consumed fresh and the seed and peel are spat out by the natives of the Canary Archipelago (Perez et al., 1994).

The sea buckthorn is a deciduous shrub that grows wild in Central Asia. It produces yellow to orange-red berries that have high nutritional and medicinal values. The fruit pulp oil (Table 11.3) contains a high level of palmitoleic acid (16:1n-7, 27%–39%), which is unusual in the plant kingdom. The pulp oil is also a good source of carotenoids, tocopherols, and phytosterols. The seed oil (Table 11.4) is rich in two essential fatty acids, linoleic acid (37%–43.6%) and linolenic acid (25.4%–36%). This new oil is gaining greater popularity as a food supplement and ingredient in Japan, Europe, and North America (Yang and Kallio, 2001).

Gac is a fruit indigenous throughout Asia and is used for food and medicine. The aril, the red fleshy pulp that surrounds the seeds, has a bland and nutty taste. The oil content of the aril is approximately 22% (dw) with 69% unsaturated fatty acids of which 35% are polyunsaturated. The oil contains significant amounts of vitamin E, lycopene (2227 μg/mL), and β-carotene (718 μg/mL), making this oil a valuable source of natural antioxidants (Ishida et al., 2004).

III. FATTY ACID COMPOSITION OF FRUIT KERNELS AND SEEDS

The processing of many fruits results in the accumulation of large quantities of seeds and kernels. Proper utilization of these by-products could reduce waste disposal problems and serve as a potential new source of fats and proteins for use in food and feed. The fatty acid compositions of a select group of fruit seeds and kernels are given in Table 11.4. The lipid contents are high, ranging in most cases between 20% and 60%. A few seeds such as litchi and longan (Matthaus et al., 2003), avocado, durian, jackfruit, and tamarind seeds have lipid levels below 10%. As a potential source of new oils, this group has as its major fatty acids palmitic, oleic, and linoleic acids. A comparison of four *Prunus* species (apricot, cherry, peach, and prune) revealed a very high oleic (>60%) and linoleic (30%) acid content. These two fatty acids give these fruit kernels over 90% unsaturation. It is interesting to note that apricot and peach kernel oils have fatty acid compositions very similar to that of almond oil (Javed et al., 1984).

The argan fruit has an outer fleshy coat that surrounds a hard shell that contains the seeds. The seeds of the organ nut, contain a rich and tasty hazelnut-like oil. The oil is high in mono- and diunsaturated fatty acids, phytosterols, and tocopherols and is widely traded on the dietetic, nutraceutic,

TABLE 11.4
Fatty Acid Content (%) of Fruit Seeds and Kernels

Common Name	Scientific Name	Lipid (%)	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0	Saturation	Unsaturation	References
Apple seed	*Malus sylvestrris*	25.7					9.0		2.3	41.3	45.6	0.7	1.1			12.4	87.6	Kamel (1982); Kamel et al. (1982)
Apricot kernel	*Prunus armeniaea*	49.50					4.8	0.42	0.23	69.2	25.4					5.0	95.0	Filsoof et al. (1976); Javed et al., (1984)
Argan nut	*Argania spinosa*					0.1	12.4		6.0	47.7	32.1	0.07		0.43	0.16	19.1	80.2	Hilali et al. (2005)
Cantaloupe seed	*Cucumis melo*	36[a]				34	27.4		6.4	12.9	17.7	1.6				67.8	32.2	El-Magoli et al. (1979)
		34[b]					26.7		6.7	16.7	46.6	3.3				33.4	66.6	
Cherry kernel	*Prunes avium*	30–38					7.7		2.4	60.8	30.8	tr				10.0	91.6	Weckel and Lee (1960)
Coffee[c] seed	*Coffea arabica*	11				tr	36.6	tr	8.1	10.2	40.7	4.1	0.3			45.0	55.0	Al-Kanhal (1997)
		11.8				2.0	28.8		4.5	18.9	44.0		1.6			36.9	62.9	Clarke and Macrae (1985); Sivetz (1963); Subrahmanyam and Achaya (1959)
Cranberry[d]	*Vaccinium oxycoccus*						7.8		2.0	22.7	44.3	22.3				9.7	90.3	Parker et al. (2003)
Currant seed (red)[e]	*Ribes rubrum*	25.2					4.5		1.5	15	42	29				6.0	95	Traitler et al. (1988)
Currant seed (black)[f]	*Ribes nigrum*	30.5					6.4		1.6	10.3	46.5	13.6				7.7	92.3	Traitler et al. (1988)
Egusi seed	*Colocynthis citrullus*	52				0.02	10.4	0.14	9.8	15.9	62.8	0.41				22.4	77.6	Akobundu et al. (1982); Sawaya et al. (1983)
Gac[g]	*Momordica cochinchine*	15.7–36.6					5.6	0.1	60.5	9.0	20.3	0.5				66	34	Ishida et al. (2004)
Goldenberry	*Physalis peruviana*	10.5			0.35	1.0	7.3	0.5	2.5	11.7	76.1	0.02				11.3	88.7	Ramadan and Morsel (2003a)
Gooseberry[h] seed	*Ribes uva crispa*	18.3					7		1	17	40	19.5				8.0	92	Traitler et al. (1988)

Continued

TABLE 11.4
(Continued)

Common Name	Scientific Name	Lipid (%)	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0	Saturation	Unsaturation	References
Grape seed	*Vitis vinifera*	13–18.4				0.05	7.0		3.9	15.6	72.2	0.24				10.9	88.1	Kamel et al. (1985); Mattick and Rice (1976)
Grapefruit seed	*Citrus*[i] *paradisi*	41.4	8.4				42.6		0.83	12.2	34.5	1.5				51.8	48.2	Habib et al. (1986)
Lime seed	*Citrusaurantifolia*	42.7	0.8		0.99		19.1	0.28	0.84	17.1	18.0	42.3				21	78.5	Habib et al. (1986)
Mandarin[j] seed	*Citrus reticulata*	40.2	3.1	4.5	4.4	4.9	25.7	2.9	11.1	23.9	19.2					53.7	46.0	Habib et al. (1986)
Melon seed	*Citrullis*[k] *vulgaris*	23.1				0.55	12.2	0.13	11.2	11.1	54.7	0.18				23.9	66.1	Oyenuga and Fetuga (1975); Salem et al. (1983); Kamel et al. (1985)
	Colocynthis vulgaris					1.5	20.0	0.3	7.1	21.6	24.5	15.9				28.6	67.4	Omogbai (1990)
Orange[l] seed	*Citrus sinensis*	45.5		5.4			28.8	0.65	2.6	23.7	30.9	6.5				36.8	61.7	Habib et al. (1986)
Peach kernel	*Prunus persica*	45.1–54.5				0.88	13.4	0.16	6.4	63.8	15.4	tr				20.7	79.3	Rahma and El-Aal (1988); Javed et al. (1984); Filsoof et al. (1976)
Prune kernel	*Prunus domestica*	28.6					8.0		1.7	61.2	29.1					9.7	90.3	Javed et al. (1984)
Pumpkin seed	*Curcurbita mixta*	36				tr	16.4		7.7	33.8	42.0					24.7	75.8	Kamel (1982); Kamel et al. (1982); Salem et al. (1983)
African Pear	*Dacryodes edulis*	12					61.9			18.3	19.0					61.9	37.3	Obasi and Okolie (1993)
Tomato[m] seed	*Lysopersicon esculentum*	22.4				0.4	14.4	0.5	3.9	21.7	54.8	3.2	0.5			19.8	80.2	Lazos and Kalathenos (1988)
	Calodendram capense	60					23.6		4.5	33.7	35.6	1.4				29.1	70.7	Munavu (1983)
Avocado seed	*Persea Americana*	1–1.5				0.8	22	3.2	0.8	25	41.5	4.5	tr	tr		23.6	74.2	Nagy and Shaw (1980)
Date seed	*Phoneix dactylifera*	10.4		2.8	17.3	11.4	10.3	0.17	2.8	44.2	8.4	0.6	0.6	0.3	0.2	45.8	53.4	Sawaya et al. (1984)

Durian[n] seed	*Durio zibethinus*	0.5			0.12	12.2	1.1	1.4	8.4	6.5	11.3		1.2	14.9	81.5	Berry (1981)
Fig seed	*Ficus carica*	30				7.2		2.6	14.9	30.6	44.7			9.8	90.2	Yazicioglu and Karaali (1983)
Guava seed	*Psidium guajava* L.	16			0.1	6.6		4.6	10.8	76.4	0.1	0.3		11.6	87.3	Prasad and Azeemoddin (1994)
		10.1			1.2	8.9		4.8	53.9	29.2	1.1			16.9	84.2	Subrahmanyam and Achaya (1959)
Jack fruit[o]	*Artocarpus integrifolia*	6.1			3.3	30.2		3.3	6.4	40.2	9.4			38.8	63.2	Daulatabad and Mirajkar (1989)
	Artocarpus heterophyllus	9.9[p]	2.4	2.8	2.7	27.7		17.3	19.3	5.7		21.9		74.8	25.0	Chowdhury (1997)
Mango kernel	*Mangifera indica*	6–14				10.5		27	48.3	14.2				37.5	62.5	Arogba (1997); Ali et al. (1985); Baliga and Shitole (1981); Char et al. (1977); Dhingra and Kapoor (1985); Lakshminarayana et al. (1983); Rukmini and Vijayaraghavan (1984)
Papaya kernel	*Carica papaya*	50.1			0.2	17.2	1.3	3.6	77.3	0.4				21.0	79.0	Subrahmanyam and Achaya (1959)
Passion fruit seed	*Passiflora edulis* f. *flavicarpa*	20				8		2.2	12.6	77.2				10.2	89.8	Assuncao et al. (1984)
Pomegranate	*Punica graaatum*	5.1–15.2			tr	20.4	1.2	9.3	28.1	34.5	3.7	1.7		32.0	67.5	Melgarejo et al. (1995)
Soursop seed	*Annona muricata*	24			0.34	17.0		5.8	61.0	12.1				23.1	73.1	Subrahmanyam and Achaya (1957)
Sea buckthorn	*Hippophae*[q] *rhamnoides* L.	11.3				7.4		3.0	17.1	39.1	30.6			10.4	89.6	Yang and Kallio (2001)
Tamarind Seed (India)	*Tamarindus indica*	7.4		tr	tr	14.8		5.9	27.0	7.5	5.6	4.5	12.2	22.3	59.7	40.1	Pitke et al. (1977)

Continued

TABLE 11.4
(Continued)

Common Name	Scientific Name	Lipid (%)	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0	Saturation	Unsaturation	References
Tamarind[r] seed (Madagascar)	*Tamarindus indica* L.	6–8				0.2	17.4	0.3	6.7	19.5	42.4	0.1	2.8	4.0	5.5	36.8	63.2	Andriamanantena et al. (1983)

[a] Variety Malti Bahtim, 3.7% 18:4.
[b] Variety King Bahtim.
[c] 0.2% bahenic, 4.5% 18:4.
[d] 0.98% 20:2.
[e] 4% γ-18:3.
[f] 18.2% γ-18:3.
[g] 1.1% 20:1n-9, 3.0% 20:1n-7.
[h] 11% γ-18:3.
[i] Six citrus species of the *Rutaceae* family have also been analyzed by Ajewole and Adeyeye (1993).
[j] 2.4% 15:0, 3.2% 15:1.
[k] 5% 21:0.
[l] 0.43% 15:1.
[m] 0.6% 17:0.
[n] 15.7% malvalic acid, 38.5% sterulic acid.
[o] 7.2% ricinoleic acid.
[p] Perianths containing seed.
[q] 2.8% 18:1n-7.
[r] 0.9% 20:1.

and cosmetology markets. The status of argan oil has changed from an exotic tourist curiosity into a prized oil over the past 15 years. It is now an important commodity of the Moroccan economy (Hilali et al., 2005).

Short-chain fatty acids (C_8–C_{12}) are found in the citrus seed oils of grapefruit, lime, mandarin, and orange. Mandarin seed oil contains the largest number of fatty acids (11 identified, including 15:0 and 15:1 and 6 unknown peaks), whereas grapefruit seed oil contains only six. The fatty acid pattern for orange oil is similar to that of cottonseed oil, whereas lime seed oil is similar in unsaturation to soybean oil. Lime seed oil also contains a high amount of linolenic acid (42%). Other oils in this group with high-linolenic acid are cranberry (22.3%), red currant seed (29%), gooseberry seed (19.5%), melon seed (15.9%), and fig seed (44.7%). It should also be noted that these oils have a more favorable n-6 to n-3 fatty acid ratio than many other oils with high-PUFA content.

Egusi belongs to the melon family (Cucurbitaceae) and is grown in Nigeria only for its seed, because the pulp is very bitter. Egusi kernels contain 52% oil composed mainly of linoleic (62.8%) and oleic (15.9%) acids. The meal from the seeds is made into patties and served as a meat substitute. Undefatted meal and whole seeds are also consumed (Akobundu et al., 1982). The increased use of this important raw material will depend on the knowledge of its chemical, functional, and nutritional properties. Other Cucurbitaceae seeds include watermelon (*Citrullus vulgaris*), sweet melon (*Cucumis melo*), and pumpkin (*Cucurbita mixta*). These seeds are consumed after frying in many parts of the world, and their high levels (42.0%–64.8%) of linoleic acid make them a very nutritious snack.

Linoleic acid and its homologs are the precursors of eicosanoids. These compounds regulate a number of physiological functions such as arterial pressure, contraction of smooth muscles, platelet aggregation, and contraction and dilation of vascular glands. It has been suggested that certain diseases are caused by deficiency not of linoleic acid (LA, *cis*-9, 12) but of its homologs such as γ-linolenic acid (GLA, all-*cis*-6,9,12-octadecatrienoic), dihomo-γ-linolenic (all-*cis*-8,11,14-eicosatrienoic), and archidonic (all-*cis*-5,8,11,14-eicosatetraenoic) acids. This deficiency may occur as a result of low 6-desaturase activity, which is responsible for the transformation of LA to GLA. It is possible to alleviate essential fatty acid deficient status by direct intake of GLA. The sources of natural GLA are few, and at present only borage (21%–25%), evening primrose (7%–10%), hemp (3%–6%), and hopseed oils (3%–4%) are well known. The fruit seeds belonging to the Ribes family have recently been shown to contain significant levels of GLA: black currant oil (15%–18%), red currant oil (4%–6%), and gooseberry oil (10%–12%). The Ribes family seeds appear to be one of the richest sources of GLA investigated until now. Black currant oil also contains 13.5% α-linolenic acid (α-LN, 18:3,9,12,15) and 3.5% of the unusual stearidonic acid (α-SA, 18:4,6,9,12,15). Both α-LN and α-SA belong to the ω-3 PUFA series, which are precursors of eicosapentaenoic and docosahexaenoic acids (Traitler et al., 1984, 1988).

A compilation of 23 wild berry species, and their nutritional composition, used for food by North American native people has been documented by Kuhnlein (1989). This is the first comprehensive report on the nutrient content in the edible portion of these berries that range in fat content from 0.4% to 5.6%.

Approximately 10 million tons of mango fruits are produced in India and 500,000 tons in Bangladesh. The seeds in most cases are a waste product and are thrown away after being separated from the pulp. The seed represents up to 15%–24% of the weight of the fruits and contains 25% water, 5.5% protein, and 7%–11% fat depending on the variety (Char et al., 1977; Ali et al., 1985). This represents a potential source of oils and fats for human and animal consumption. Mango fat has received attention in recent years as a cocoa butter substitute. The proportion of disaturated to monosaturated glycerides however is very low (0.4:1) compared to that of cocoa butter (3.7:1), making it unsuitable as a cocoa butter substitute. The fat has been fractionated from acetone at low temperatures to isolate suitable hard fractions that have physical properties closer to that of cocoa butter, and some of the acetone fractions can serve as a partial substitute for cocoa butter (Baliga and Shitole, 1981). The oil is rich in stearic (39%) and oleic (41%) acids and low in linoleic acid (7.6%).

Traces of 2% of 17:0 fatty acid are present. Some of the large variations in fatty acid composition observed between investigators could be due to variety, climatic conditions, soil type, and maturity differences in the plant (Lakshminarayana et al., 1983). The fat melts at 34.5°C and has a cream color (Rukmini and Vijayaraghavan, 1984). It is bland, has a good plastic range, and is free of toxic substances, which makes it a potential edible fat for pastries and other products for which a saturated fat is required. It has also been used in the preparation of quality soaps and as a deep-fat-frying oil for potato chips (Char et al., 1977).

Tamarindus indica is cultivated in many parts of the world: India, Florida, Egypt, Sudan, Formosa, Southeast Asian countries, and Madagascar. Large differences have been observed in the fatty acid composition of the oils from the various countries. The tamarind tree bears flat pods that on ripening yield an edible pulp and a dark brown hard seed. The kernel oil from India is unique in having a large proportion (22.3%) of the saturated fatty acid, lignoceric acid. Significant levels of arachidic and behenic acids were also detected. The only other oil that has been reported to contain substantial quantities of lignoceric acid comes from the seed of coral wood (*Adenanthera pavonia* L.). The Malagasy tamarind kernel oil is very different from the Indian oil. The major fatty acids are linoleic (36%–48%), oleic (15%–26%), and palmitic (15%–19%) acids. Stearic, arachidic, behenic, and lignoceric acids were detected in smaller amounts. Odd-numbered fatty acids such as pentadecanoic, heptadecanoic, heptadecenoic, and tricosanoic acids were also found in small amounts (Andriamanantena et al., 1983).

Guava fruit is one of the richest sources of vitamin C and is produced in large quantities in Brazil, United States, India, and Egypt. At the present time, large amounts of guava fruit seeds are discarded during processing, and this aggravates a serious disposal problem. The seeds represent 6%–12% of the whole fruit weight and constitute a potential source of edible oil. The oil contains 76.4% linoleic acid and resembles safflower oil in composition (Prasad and Azeemoddin, 1994). This extremely high linoleic acid content is in direct contrast to the lower values (29%) reported by Subrahmanyam and Achaya (1959).

IV. FRUIT NUTS

Tree nuts are one of the oldest sources of food for humans and animals. At present, the world production is in excess of 1 million tons. Nuts are concentrated stores of aroma, flavor, fat, and protein and supply approximately 5–6 calories per gram.

The fat content of most nuts is high, ranging from 70% to 78% in macadamia nuts, pecans, and hickory nuts to 1.7% in ginkgo nuts to 3.1% in chestnuts (Table 11.5). The majority contains more than 50% fat, and all are highly unsaturated. A few species (butternut, black walnut, English walnut, filbert, and pecan) contain less than 10% saturated fatty acids. Brazil nuts are characterized by a very high linolenic acid content of 47%. Black walnut, English walnut, and faveleira nuts all contain over 50% linoleic acid, and acorn, cashew, filbert, pecan, and pistachio nuts contain over 60% oleic acid.

A mixture of phenolic olefins (cardanol) has been isolated and identified in cashew seed and germ oils. The levels range from 20 to 40 mg/100 mL of oil. It appears that these compounds are derived from the cashew nut liquid during the processing of the nuts. Other potentially toxic compounds (cyclopropene fatty acids) have been identified in Chinese chestnuts. Their levels are 5.4% dehydrosterculic, 0.39% malvalic, and 19.1% sterculic acids. These acids produce adverse physiological effects on experimental animals and may be carcinogenic and atherosclerotic (Berry, 1982). Apparently, cooking the nuts does not decrease the levels of these fatty acids.

The tropical tree known as mafura that grows in Tanzania and other West African countries produces a seed that is high in oil and protein. Commercial utilization of mafura seed has been prevented by the presence of a strong, bitter, and nauseating taste and a brown coloration in the oil. These problems have been reduced by the use of oil-refining procedures to produce an oil of acceptable flavor and color. In contrast, the oil from the husk is pale yellow and has no bitterness or emetic properties. The husk contains 35%–45% oil (Fupi and Mork, 1982).

TABLE 11.5
Fatty Acid Composition (%) of Fruit Nuts

Common Name	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	Satur-ation	Unsatur-ation	References
Acorn	*Quercus* spp.	23.8		12.5		1.1	66.2	20.1				13.6	86.3	USDA (1984)
Almond	*Prunus amygdalus*	50–65	0.1	12.2	1.5	0.1	44.5	40.5	0.6			12.8	87.2	72, 18, 101, 35
Beechnut	*Sylvatica fagus* spp.	50	0.13	7.6	0.6	2.5	40.3	39.1	3.6		5.9	10.2	89.5	USDA (1984)
Black walnut	*Juglan nigra*	56–59		2.8		2.8	32.7	57.6	3.9			5.6	94.2	99, 18, 81
Brazilian nut	*Berthrolletia myrtaceae*	68.7		14.1	0.3	8.6	29.0	46.6	tr	0.3		22.7	76.2	Beuchat and Worthington (1978)
Butternut	*Juglans cinerea*	60		1.6		0.8	19.0	61.9	16.0			2.4	96.9	Beuchat and Worthington (1978)
Cambodia[a] nut	*Irvinga malayana*	70	42.1	7.3		1.6	2.3	1.5				3.8	96.2	Bandelier (2002)
Cashew	*Anacardium occidentale*	46.5		10.9	0.3	9.1	63.3	15.6	tr	0.5		20.6	79.2	Toschi et al. (1993); Beuchat and Worthington (1978); Ojeh (1981)
Chestnuts	*Castanea* species[b]	5.2		12.5		0.9	52.3	31.3	3.0			13.4	86.6	Senter et al. (1994)
China[c] chestnuts	*Sterculia monosperma*	3.1	0.15	23.4	1.3	2.5	24.9	18.2				26.1	74.4	Berry (1982)
English walnut	*Juglan regia*	67.4		7.3	0.2	2.3	19.1	57.4	13.1			9.6	89.8	Beuchat and Worthington (1978)
European chestnuts	*Castanea mollisima*	2.5		14.6	0.7	1.1	54.0	24.9	2.7			15.7	82.3	Beuchat and Worthington (1978)
Faveleira[d] nut	*Cnidoscolus phyllacanthus*	32		17.4		9.4	15.1	55.4	1.0	0.4	0.2	27.6	71.7	Daun et al. (1987)
Filbert/hazelnuts	*Corylus avellana*	62		4.7	0.2	1.6	76.4	16.3	0.15	0.1		6.4	93.0	Beuchat and Worthington (1978); Bonvehi and Coll (1993)
Ginko	*Ginko biloba*	1.68	0.4	19.1	5.2	1.0	33.9	38.3	1.4		0.7	20.5	79.5	USDA (1984)
		2.2[e]		6.9	3.5	1.0	37.0	43.9	1.6			7.9	92.0	Hierro et al. (1996)

Continued

TABLE 11.5
(Continued)

Common Name	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	Saturation	Unsaturation	References
Hickory	*Caryaovata ovata*	70.4		8.8	0.5	2.3	52.0	33.5	1.7	0.2		11.3	87.7	Beuchat and Worthington (1978)
Macadamia	*Macadamia tetraphylla*	73.2–78	0.7	10.1	20.1	4.3	57.4	2.5		2.8	1.9	17.8	81.5	Fourie and Basson (1990); Beuchat and Worthington (1978)
Mafura	*Trichilia emetica*	55–56		38.6		2.2	48.0	10.2	1.0			40.8	59.2	Fupi and Mark (1982)
Pecan	*Carya illioensis*	70.3	0.05	5.7	0.11	2.2	66.9	22.1	1.1	0.21	0.4	8.1	90.6	Fourie and Basson (1990); Beuchat and Worthington (1978)
Pili nut	*Canariium ovatum*	70		33.3	0.3	10.9	44.7	10.1	0.5	0.24		44.5	55.5	Kakuda et al. (2000); Pham et al. (1998)
Pine nut	*Pinus pinea*	50.7		8.0	0.45	3.6	39.2	45.2	1.4		2.1	11.6	88.3	USDA (1984)
Pistachio	*Pistachia vera*	54–57		8.6	0.7	2.3	68.8	17.8	0.3	0.3	0.6	11.2	87.7	Beuchat and Worthington (1978); Kashani and Valadon (1983)

[a] 2.7% 10:0, 42.5% 12:0, 42:1% 14:0.
[b] 6.0% of 20:3.
[c] 3.2% 18:3 + 20:0.
[d] 0.4% 20:4.
[e] Includes *Castanea sativa*, *C. pumila* (American chinkapin), and *C. dentata*.

Macadamia nut oil contains predominantly oleic acid (57.4%) and palmitoleic acid (20.1%). Behenic, docosenoic, and lignoceric acids have also been reported (Beuchat and Worthington, 1978). This oil has a low-PUFA content and as such should have good oxidative stability. The high-linolenic acid content of Brazil nuts (46.6%), butternuts (16%), and English walnuts (13.1%) should make these kernels the least stable if the degree of unsaturation is a predictor of oxidative rancidity. It should be remembered that storage conditions, moisture levels, oil content, and stage of maturity also have a large influence on stability.

Almond oil is used in cosmetics, pharmaceuticals, and food products. In the food area, almonds are distributed to confectioners, salters, bakeries, ice cream manufacturers, nonsalted nut retail packagers, and exporters. The main fatty acids are oleic (44.5%) and linoleic (40.5%). Differences in fatty acid composition have been noted by various researchers. Fourie and Basson (1990) reported traces of myristoleic acid but no linolenic, arachidic, or eicosenoic acid. These discrepancies may be due to differences in the analytical techniques used for quantification, but more likely they are due to differences in the maturity of the kernels. It has been shown that oleic acid increases with kernel maturity, whereas linolenic and arachidic acids are greatly reduced. Overall the unsaturated fatty acids increase constantly, whereas the total saturated fatty acids decrease (Munshi and Sukhija, 1984; Soler et al., 1988).

The main fatty acids in pecan oil are oleic and linoleic acids, which account for 90% of the total. A total of 23 fatty acids have been positively identified in pecan oil; 13 of which account for less than 0.2%. Small quantities of linolenic, eicosenoic, and palmitoleic acids were positively identified (Fourie and Basson, 1990).

Walnuts are used in a wide variety of food products, including cookies, cakes, fudges, granola bars, candy, ice cream, cereal-based products, and especially light foods. Walnuts are a good source of vitamins, minerals, and proteins. They contain no cholesterol and are low in saturated fat, sodium, and sugar and high in dietary fiber. Oleic and linoleic acids represent 85% of the lipids in walnut kernels. The major difference between English walnuts and black walnuts is the higher content of linolenic acid and lower content of oleic acid in the English walnut samples. Twenty-one fatty acids ranging in chain length from C_{12} to C_{22} were identified in each of the five cultivars (Senter et al., 1982).

Chestnuts are atypical of tree nuts in that they contain very little fat and are very high in starch. The lipid content for Chinese chestnut and European chestnut are 3.1% and 2.5%, respectively. The only other low-fat nut is the ginkgo nut (1.7% lipid).

The Cambodian nut, also known as "chambak or krabok," is collected in large quantities in the forest areas of Cambodia. The oil content of the nut is 70%. Most of the fatty acids are saturated and include 45.2% lauric acid and 42.1% myristic acid. These high-myristic acid levels are normally not found in common vegetable oils and to have both short-chain fatty acids in the same oil is completely unique. Because of its high fusion range (around 39°C), short-chain fatty acid, sterol and tocohpherol content, the Cambodian nut oil has very promising applications in the margarine, cosmetic, and pharmacology industries.

The pili nut (*Canarium ovatum*) that is native to the Philippines has a fruit kernel with 70% fat. The oil is light yellowish in color with 33.3% palmitic and 44.7% oleic acids. Fractional crystallization of the oil produces a high melting fraction with properties similar to cocoa butter and may find uses as a cocoa butter substitute in confectionery products (Kakuda et al., 2000).

Some minor nuts that are available but not in substantial amounts include chinquapins, or bushy chestnuts (*Castinea pumila*); heartnut, or Japanese walnut (*Juglans sieboldiana*); shellbark hickory (*Caryaovata laciniosa*), mocknut hickory (*C. tomentosa* and *C. alba*), and pignut hickory (*C. glabra*). Overall the fruit nuts are low in saturated fatty acids and high in unsaturates. They are a good source of the essential fatty acid linoleic acid and, in the case of Brazil nuts, butternuts, and English walnuts, are also a good source of the ω-3 fatty acid, α-linolenic acid.

V. PALM OILS

The palm family is estimated to consist of about 217 genera and over 2500 species. They are indigenous to the tropical and subtropical regions. They supply approximately 14% of the fats and oils in the world (Bandyopadhyay and Gholap, 1973). The majority of the palms contain neutral lipids, with lauric and myristic acids as the major components. This is reflected in the highly saturated fatty acid composition of these oils, that range from 80 to 95% (Table 11.6).

Coconut oil is obtained from copra that contains about 65%–68% oil and 4%–7% moisture. Approximately 3 MMTs of coconut oil are utilized annually for food and nonfood purposes. This oil contains a high amount of short- and medium-chain fatty acids and tends to be solid at room temperatures. It is characterized by having a sharp melting curve or short plastic range and a high resistance to rancidity (Swern, 1964).

Palm kernel oil is extracted from the kernel of the palm fruit. It is high in lauric and myristic acids and is very similar to coconut oil except for a slightly higher iodine value. Palm kernel oil can be separated into a stearin fraction for use as a cocoa butter substitute and a liquid fraction for use in baked goods and in soap manufacture (Swern, 1964). The oil extracted from the mesocarp of the same palm fruit has properties very different from those of the kernel oil. Palm oil is high in palmitic (40.1%) and oleic (42.7%) acids. The levels of lauric and myristic acids are less than 2%. Palm oil is produced in greater amounts than palm kernel oil and is used in making vegetable shortening, margarine, and soap. The main sources of palm oil are in Malaysia, West and Central Africa, and Indonesia. Other sources include Brazil and other South and Central American countries (Swern, 1964).

The *Arecastrum romanozoffianum* palm (queen's palm, plummy coconut, or pindo palm) is found both in the tropical and subtropical forests. The oval fruits grown in Nigeria consist of a slimy-sweet and fibrous pulp covering a hard shell. The kernel inside the shell yields 33% of a pale yellow fat with a nutty aroma. The cake from the defatted kernels is richer in carbohydrates and minerals and lower in fiber than *E. guineensis* kernel cake, making it a potentially better additive in livestock feed. Pindo palm oil has lauric acid (55%) as its major fatty acid and in general is similar to palm kernel oil, but it differs from coconut oil in having smaller amounts of C_{10} and C_8 and greater amounts of C_{12} and C_{14} fatty acids.

The fruit from the palm *Jubaea spectabilis* (subclass Cocoideae) weighs about 7.5 g and looks like a miniature coconut. This palm is native to certain parts of Chile. The oil from the kernel has a low melting point and remains fluid at low temperatures. This property is unique for an oil from the family Palmae and is due to a high content of short-chain fatty acids (C_8–C_{12}). Table 11.6 shows the much higher levels of these short-chain C_8 and C_{10} fatty acids (Cole et al., 1980).

The palm known as tucum or awarra (*Astrocaryum vulgare*) grows in Brazil, Peru, Venezuela, the Guianas, and Nigeria. The fruit yields a pulp oil used in soapmaking and a kernel fat that is consumed by the local population. The two oils are distinctly different; lauric acid is the predominant fatty acid in the kernel fat, whereas, as is generally seen in other pulp oils such as palm oil and olive oil, the pulp fat consists mainly of oleic and palmitic acids (Oboh and Oderinde, 1988, 1989).

Other palm kernel oils from Central and South American palms include the babassu, murumuru, ouricuri, and cohune. They are similar to the palm kernel oil from *E. guineensis* in fatty acid composition, with 42%–46% lauric acid and 9%–16% myristic acid. Murumuru oil is different, with 36% myristic acid and only 1.6% capric acid (Swern, 1964).

The Amazonian palms of the *Oenocarpus jessenia* complex produce an oil with a composition and taste very similar to those of olive oil. The most important species appears to be *Jessenia bataua*, which grows throughout the Amazon valley on either dry or swampy land. The oil is extracted from the mesocarp by pressing or boiling and is consumed as is or eaten with raw cane sugar or toasted cassava granules (Pryde et al., 1981). The major fatty acid is oleic acid (77.7%).

The kernel oil from the *Raphia* palm is characterized by high levels of polar lipids (phospholipids and glycolipids) and comparatively smaller amounts of triglycerides. The neutral lipids from

TABLE 11.6
Fatty Acid Composition (%) of Palm Family

Common Name (Family)	Scientific Name	Lipid (%)	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	Saturation	Unsaturation	References
Coconut	*Cocos nucifera*	63–74	0.7	8.3	8.2	44.6	16.8	8.2	0.4	2.8	7.4	2.6			89.6	10.4	Idiem-Opute (1979); Oderinde and Oboh (1988)
Pindo	*Arecastrum romanozoffianum*	32–65		1.7	2.0	55.5	20.4	6.6		1.7	10.2	1.7			87.9	11.9	Oderinde and Oboh (1988)
Palm pulp	*Elaeis guineensis*[a,b]	50				0.04	0.9	46.7	0.1	5.6	35.3	10.6	0.36	0.38	53.6	46.3	Ekpa et al. (1994)
		50				0.1	1.4	40.1	0.3	5.5	42.7	10.3	0.2		53.5	46.2	Pryde et al. (1981);
		50	0.2	3.3	3.7	47.0	16.4	8.1		2.8	11.4	1.6			81.5	13.0	Swern (1964); Idiem-Opute (1979); Oderinde and Oboh (1988)
Palm kernel	*Jubaea spectabilis*	51–54	1.9	17.5	19.0	43.5	4.9	2.8		1.6	7.4	1.4			91.2	8.8	Cole et al. (1980)
Amazonian palm[c]	*Jessenia bataua*	8–10						13.2	0.6	3.6	77.7	2.7	0.6		16.8	81.6	Pryde et al. (1981)
Tucum or awarra	*Astrocaryum vulgare*[d]																Oboh and Oderinde (1988, 1989)
Pulp		22.0						30.4	2.2	59.9	2.9			4.6	94.9	5.1	
Kernel		41.1				45.4	20.2	5.6	5.6	12.7	6.0				88.4	11.6	
Babassu	*Orbygnia martiana*		0.2	4.4	7.1	44.6	16.0	7.2		3.8	14.0	2.1		0.45	82.5	17.5	Swern (1964)
Murumuru	*Astrocaryum murumuru*			1.1	1.6	42.5	36.9	4.6		2.1	10.8	0.4			88.8	11.2	Swern (1964)
Ouricuri	*Syagrus coronata*		1.8	9.8	8.2	45.8	9.0	7.7		2.3	13.1	2.2		0.1	84.7	15.3	Swern (1964)
Cohune	*Attalea cohune*			7.5	6.6	46.4	16.1	9.3		3.3	9.9	0.9			89.2	10.8	Swern (1964)
Cuphea seed[e]	*Cuphea wrightii*	33.6			34.7	54.0	3.2	1.3			1.9	4.2			93.2	6.1	Graham and Kleiman (1985); Thompson and Kleiman (1988); Wolf et al. (1983)

[a] Palm pulp contains 0.1% of 20:1.
[b] Two varieties: red and yellow fruit of *Dura* and *Tenera*.
[c] Other fatty acid consists of 0.2–4.6.
[d] C_{10} and below consist of 4.4%.
[e] Extremely wide variation exists among the estimated 260 species available.

these palm fruits contain negligible quantities of lauric and myristic acids but significant amounts of oleic (19%–36%), linoleic (15%–39%), and palmitic (23%–39%) acids. This is unusual and is contrary to the general belief that lauric and myristic acids are the principal fatty acids in all palms. Commercialization of these palm fruits is unlikely because of the low lipid content (<1.0%) of the kernel (Idiem-Opute, 1979).

An alternative source of medium-chain fatty acids is the oil from the *Cuphea* seed. This plant belongs to the Lythraceae family, which includes over 260 species. They are grown in Costa Rica, Mexico, Brazil, Peru, Panama, United States, and Nicaragua. Similar to the Palmae family, *Cuphea* seed oil contains high levels of lauric and capric acids, which may in the future augment or replace imports of coconut and palm kernel oils (Wolf et al., 1983; Graham and Kleiman, 1985; Thompson and Kleiman, 1988).

VI. BUTTERS FROM PLANT SOURCES

This group of fats is characterized by their unique melting and crystallization behavior. Their melting ranges are much narrower than those of common vegetable oils, and in the case of cocoa butter, can be as narrow as 35°C–36°C. This unique characteristic is due to the presence of a single dominant triglyceride, palmitooleostearin. There are six or possibly seven polymorphic states for cocoa butter that also contribute to its unique melting behavior. Because of cocoa butter's hardness and nongreasiness at room temperature and sharp melting point at body temperature, it finds many uses in the manufacture of chocolate confections and in coatings for chocolates and candies. Table 11.7 lists examples of butter substitutes.

Because of the high prices of cocoa butter and occasional shortages in supplies, a great deal of effort has been expended in finding replacers or extenders. Borneo tallow nuts are oil-bearing seeds that grow in Sarawak and Borneo (Kershaw, 1987). The fat from these kernels can be used as a cocoa butter substitute or extender. Borneo tallow resembles cocoa butter more than any other natural fat but differs in having a slightly higher and wider melting range, 34°C–39°C, and more stearic and less palmitic acid (Swern, 1964).

Theobroma bicolor is of the same family as cocoa butter, and accordingly its fat should have a similar chemical composition. The fatty acid and triglyceride compositions are very different from those of cocoa butter. The levels of stearic and oleic acids are higher, and that of palmitic acid is lower (Jee, 1984). The monounsaturated triglyceride levels are only 44% compared to 80% for cocoa butter. The high levels of diunsaturated triglycerides are responsible for the observed low melting point and softness of *T. bicolor* fat. Mixtures of more than very small amounts of this fat with cocoa butter will produce an unacceptably soft product.

Shea butter is even more unsaturated than *T. bicolor* and results in a soft and nonbrittle fat at room temperature. The fractionation of this fat has led to a stearin fraction that has been used as a cocoa butter substitute.

A few of the lesser known fats such as sal, kokum, and mowrah have been used as cocoa butter substitutes (Baliga and Shitole, 1981). Nutmeg butter and ucuhuba butter are characterized by high myristic acid levels of 77% and 68%, respectively. Nutmeg butter has been used in pharmaceutical preparations.

Chinese vegetable tallow is obtained from the outer covering of the seed from the Chinese tallow tree. The lipid from the kernel (stillingia oil) is used as a drying oil because of its high-linolenic acid content (20%–28%). The lipid from the shell contains significant amounts of palmitic acid (70%) and greatly reduced levels of linolenic acid (1.5%) (Echols, 1982). The current interest in this oil may arise from its possible use as a renewable energy source.

Cupuassu (*Theobroma grandiflorums*) is a tropical fruit found in the Brazilian forest. The production of cupuassu has increased from 10 MMT in 1998 to 21.4 MMT in 2000. The fruit has the form of a drupe with a strong and pleasant smell. The endocarp contains 25–50 superposed seeds in five rows. The seeds are fermented, roasted, milled, and pressed to obtain the fat in yields

TABLE 11.7
Fatty Acid (%) in Butters of Plant Origin

Common Name	Scientific Name	Lipid (%)	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	Saturation	Unsaturation	References
Aceituno	*Theobroma*[a] *bicolor*	27–38			6.6	42.9	45.1	3.0		2.0	51.5	48.1	Jee (1984); Carpenter et al. (1994)
	Simarouba glauca	60			12.5	27	56				39.5	56	Jeyarani and Reddy (2001)
Borneo tallow[b] nut	*Shorea stenoptera*	46–60			13.6	48.4	33.9	1.1	0.5	2.1	63.7	36.0	Kershaw (1987)
Cacao seed	*Theobroma cacao*	34–54			27.8	34.6	34.0	3.6			62.4	37.6	Lehrian and Kenney (1980); Lehrian et al. (1980); Carpenter et al. (1994)
Chinese Tallow fruit nut shell	*Sepium sebiferum*	53 seed		4.1	37	8	11.3	13.7	24.3		42.1	55	Echols (1982)
		27–33			70	4	10	12	1.5		74	23.5	
Cupuassu	*Theobroma grandiflorum*	60			7.8	33	42.2	3.5	0.2	9.8	53.8	45.9	Lannes (2003)
Dhupa fat	*Vateria indica*				12.2	42.4	40.5	2.2		2.7	57.3	42.7	Baliga and Shitole (1981)
Kokum fat seed	*Garginia indica*	23–30			3.1	56.1	39.1	1.7			59.2	40.8	Baliga and Shitole (1981)
Mowrah fat kernel	*Madhuca latifolia*	50–60			23.7	19.3	43.3	13.7			43.0	57	Swern (1964)
Nutmeg butter kernel	*Myristica officinalis*	25–30	1.5	76.6	10.1	10.5	1.3	4.4			98.7	1.3	Swern (1964)
Pequi	*Caryocar brasiliensis*				44.3	2.6	48.7				46.9	53.1	Segall et al. (2006)
Shea butter kernel	*Butyrospermum parkii*	45–55			5.7	41.1	49.0	4.3			46.7	53.3	Swern (1964)
Shea nut kernel	*Butyrospermum paradoxum*	45–60			2.8	47.6	46.4	2.7	0.5		50.4	49.6	Kershaw and Hardwick (1981)
Ucuhuba butter kernel	*Virola surinamensis*	65	13.7	67.8	6.7	0.8	8.1	1.4			89.0	9.5	Swern (1964)

[a] 0.2% 16:1; 0.1% 20:1.
[b] 0.3% 16:1.

of about 60%. Comparing the fatty acid composition with cocoa butter, the palmitic acid levels in capuassu fat is much lower (7.8%), stearic acid is nearly equal (32.9%), and oleic acid is higher (42.2%). The lower palmitic acid levels in cupuassu fat results in lower POP and POS type triacylglycerols and hence a lower solid fat content at all temperatures when compared to cocoa butter. The cupuassu fat could be modified by fractionation to make it resemble cocoa butter.

The prolific and diverse plant life in Brazil makes it an ideal location to search for new sources of food oils. The pequi tree grows naturally in the dry plains of Brazil and its fruit has been extensively used by the local population as an oil source. The oil is extracted by rendering the pulp, mesocarp, and kernel of the fruit and is used in cooking. The composition of the oil is predominately palmitic (44.2%) and oleic (48.7%) acids with lesser amounts of stearic (2.5%) and linoleic (4.4%) acids. Owing to its OOO, POO, and POP triacylglycerol composition, it can be used as a cocoa butter substitute.

Aceituno is an evergreen tree that is native to El Salvador in Central America. The plant produces brown-colored oval shaped seeds that contain kernels with 60% fat. The fat has a melting point of about 29°C with three major fatty acids: stearic (27%), oleic (56%), and palmitic (12.5%). This fat has the physical and chemical properties to make it a good candidate for use as a cocoa butter extender (Jeyarani and Reddy, 2001).

VII. LESSER KNOWN FRUITS AND FRUIT PRODUCTS

The fruits and fruit products listed in Table 11.8–11.11 are not currently of commercial importance. However, some of them are very important in certain countries and can make a significant impact on the economies of those countries. Where these plants are indigenous, they can sometimes serve as an alternative or supplementary source of edible oils. A number of them are excellent sources of fat and contain over 50% lipid material; examples are *Panda oleosa* (50.5%), *Carapa procera* (53.5%), *Allanblackia floribunda* (67.6%), and *Valenzuela trinervis* (67%). This collection of 50 plants is divided among Tables 11.8–11.11 according to the dominant fatty acid. In all cases, the genus and species are given, and where the common name is unknown the family name is substituted. Because of the wide diversity within this group, we are not certain whether the oils listed are derived from whole fruit or from fruit parts.

The sources of palmitic acid-rich oils are listed in Table 11.8. The levels of this saturated fatty acid range from 30% to 39%, which gives this group a saturated/unsaturated ratio close to 1:1. Oleic and linoleic acids are the other major fatty acids.

The sources of oleic acid-rich oils are listed in Table 11.9. The percentage of oleic acid ranges from 33% to 78%. The lipid content of these seeds is quite high (12%–67%), averaging around 39%. Guindilla seeds are grown in Chile and Argentina. The seed consists of 56% hull and seed coat and 44% cotyledon. The oil contains 12.9% gadoleic acid (20:1) and, as in most Sapindaceae, the oil also contains toxic cyanolipids (Aguilera et al., 1986). Roselle seed oil is very rich in oleic acid (73%). This oil also contains 2.9% sterculic acid, 1.3% malvalic acid, and cyclopropenoid at half the level found in okra seed and one-fifth the level found in cottonseed oil. The ratio of saturated to unsaturated fatty aids in this group is about 1:2.

The linoleic acid group is by far the largest (Table 11.10). The levels of this essential fatty acid range from 35% to 87%. These high-linoleic acid levels coupled with the oleic acid levels gives a saturated/unsaturated ratio of about 1:3. Buffalo gourd oil is unique in having 2.3% and 0.03% conjugated dienoic and trienoic acids, respectively. It is highly unsaturated, with linoleic acid levels ranging from 39% to 77%. In feeding trials, it was found to be free of toxic substances when fed to chicks (Khoury et al., 1982). Karaka is a nut-bearing tree that grows in New Zealand and is eaten by natives. Before being eaten, it must be extensively baked and washed to remove β-nitropropionic acid, a toxic substance formed by hydrolysis of karakin. Acacia seeds are produced on small trees that grow in Australia, Africa, India, and Americas. The oil from this seed contains an epoxy acid

TABLE 11.8
Fatty Acid Composition (%) of Lesser Known Fruit and Fruit Products Rich in 16:0 Fatty Acid

Common Name (Family)	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	Saturation	Unsaturation	References
(Pandaceae)	*Panda oleosa*	50.5	1.5	32.0		7.1	30.2	29.2				40.6	59.4	Foma and Abdala (1985)
(Meliaseae)	*Azadirachta indica*	17.4		35.8		12.1	36.1	15.1		2.0		49.8	51.2	Balogun and Fetuga (1985)
Okra seed	*Hibiscus esculentus*	18.3[a]		39.0	0.2	2.6	17.4	39.8	0.4		0.2	42.0	57.8	Pryde et al. (1981); Lakshminarayana et al. (1984)
(Tilliaceae)	*Desplatzia dewevrei*	20.4	0.2	37.8		7.4	18.1	35.0				45.4	53.1	Foma and Abdala (1985)
(Combretaceae)	*T.*[b] *superba*	14.5	3.0	33.6	0.7	5.6	25.8	28.4	0.5	2.5		46.5	55.4	Balogun and Fetuga (1985)
(Combretaceae)	*T. glucausens*	17.5	0.1	35.0	0.5	4.8	32.7	26.7				40.2	59.8	Balogun and Fetuga (1985)
(Combretaceae)	*T. catappa*	40.1	1.2	30.2		5.8	41.5	19.2	0.9	1.3		38.5	61.5	Balogun and Fetuga (1985)

[a] 0.5% 17:0, trace-1.1% 19:0, and 0.12% 24:4.
[b] T. = *Terminalia.*

TABLE 11.9
Fatty Acid Composition (%) of Lesser Known Fruit and Fruit Products Rich in 18:1 Fatty Acid

Common Name (Family)	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	Satura-tion	Unsatu-ration	References
Seed	*Moringa peregrina*	21–54	T	9.3	2.4	3.5	78.0	0.6	1.6	1.8	2.6	14.7	84.7	Somali et al. (1984)
Asclepiadaceae	*Calotropis asclepiadaceae*	30.8		16.7		9.0	47.2	27.1				25.7	74.3	Foma and Abdala (1985)
Guttiferaceae	*Allanblackia floribunda*	67.6		0.8		55.9	43.3					56.7	43.3	Foma and Abdala (1985)
Moraceae	*Treculla africana*	11.8		25.7	1.6	14.2	32.7	25.8				39.9	58.5	Foma and Abdala (1985)
Roselle seed	*Hibiscus sabdariffa*	21.1		22.2		3.8	73.1	0.3		0.3		26.3	73.4	Al-Wandawi et al. (1984); Sarojini et al. (1985)
Guindilla seed	*Valenzuela trinervis*	67.0[a]		9.6			62.3	10.1				9.6	72.4	Aguilera et al. (1986)
Seed	*Ventilago calyculate*	40.0[b]	1.0	15.9		1.1	63.1	4.5	13.6			18.0	81.2	Grover and Rad (1981)
Annonaceae	*Xylopia aromatica*	30.4[c]		17.5		5.8	47.1	28.3	0.3	0.1		23.4	75.4	Mayworm and Salatino (1996)
Baobab	*Adansonia digitata*		38	20.0		3.2	22.4	16.2				61.2	38.6	Eteshola and Oraedu (1996)
Sandalwood	*Santalum specatum*	40[f]		5		5	50	2	6			10	89	Liu et al. (1997)
Seed oil	*Annona squamosa*	23.0[e]		25.1	3.1	9.3	37.0	10.9		3.3		37.7	51.0	Ansari et al. (1985)
Papilionaceae	*Milletia laurentii*	22.9	0.1	10.5		2.9	44.9	17.6		1.8	10.1	27.6	72.4	Balogun and Fetuga (1985); Foma and Abdala (1985)
Melinceae	*Khaya senegalensis*	45.5	4.6	11.3	0.14	13.8	59.4	9.7	0.6	0.5		30.3	69.8	Balogun and Fetuga (1985)
Melinceae	*Carapa procera*	53.5	0.2	26.8		4.1	62.5	6.3		0.1		31.3	68.7	Balogun and Fetuga (1985)
Melinceae	*Enthendrophragma angolense*	52.0		7.0	17.7	18.8	40.3	24.3		1.7		27.7	82.3	Balogun and Fetuga (1985)
	Brachyehiton acerifolius	35.0		19.5	0.2	0.3	37.8	34.6	0.6	0.8		20.6	73.4	Rao et al. (1989b)
Leguminosae	*Acacia holosericea*	56[d]		29.3	3.1	2.9	54.3	8.1		1.25		33.4	65.5	Prasad and Azeemoddin (1996)

[a] 12.9%, c20:1 if 20:1Δ9.
[b] 0.5%, 1.2%, and 1% of c8, c10, and c14, respectively.
[c] 9.8% Hydroxy acid (isoricinioleic).
[d] Aril fat.
[e] The seed from 28 species of plants grown in Cerrado region of Brazil were analyzed.
[f] Contains ximenynic acid (31%) trans-11-octadecene-9-ionic acid.

TABLE 11.10
Fatty Acid Composition (%) of Lesser Known Fruits and Fruit Products Rich in 18:2 Fatty Acid

Common Name (Family)	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	Saturation	Unsaturation	References
Tobacco seed	*Nicotiana tabacum*	42.6		9.1	tr	2.4	11.6	76.0	0.67	tr	0.21	11.7	88.3	Maestri and Guzman (1993)
Poppy seed	*Papaver somniferum*	44.0		8.6	0.15	1.8	12.8	75.7	0.43	0.37		10.8	89.1	Nergiz and Otles (1994)
Karaka seed	*Corynocapus laevigatus*	9.6[a]	0.1	13.1		7.2	27.2	45.3	1.1	4.2	1.4	26.4	82.6	Body (1983)
Acacia seed	*Leguminosae*	2.1–8.6	0.4–1.5	7–44	1–9.4	13–29	18–72		1–4					Chowdhury et al. (1983); Jamal et al. (1987)
Acacia (leguminosae)	*Acacia arabica*	5.2		14.6		6.2	32.2	39.2	3.1			20.8	74.5	Maity and Mandal (1990)
	Acacia colei	10.9		11.4	0.3	3.7	18.0	55.9		1.4	4.1	21.8	75.3	Adewusi et al. (2003)
	Acacia tumida	8.1		9.4		6.9	22.1	50.1		4.1	4.9	25.3	72.2	Adewusi et al. (2003)
Hemp	*Cannabis sativa* L.	31-33		6.3		2.7	11.7	60.0	19.3			8.9	90.9	Parker et al. (2003) Kriese (2004)
Japonica seed	*Betula playtphylla*	19–28[b]	0.1	1.4	0.2	0.4	8.8	87.5	1.4	tr	0.2	2.1	97.9	Ihara and Tanaka (1980)
Seed oil	*Crotolaria retusa*	4.3[c]		16.9		5.3	13.0	50.2				22.2	77.8	Daulatabad et al. (1989)
Mesta (kenaf)	*Hibiscus sabdariffa*	20–24[d]	1.4	20.6	0.7	3.7	32.6	35.3				25.7	68.6	Rukmini and Vijayaraghavan (1984)
	Hibiscus cannabinus	23.9		22.9		2.8	29.6	44.4				25.7	74.0	Lakshminarayana et al. (1984)
	Hibiscus panduriformia	15.4		12.3		3.2	10.2	74.3				15.5	84.5	Kittur et al. (1982)
	Abutilon pannosum	13.4[e]		21.3		2.8	11.7	60.7				24.1	72.4	Kittur et al. (1982)
Buffalo gourd	*Curcurbit foetidissima*	31–39[f]		10.3		3.0	23.0	62.9				13.3	85.9	Khoury et al. (1982); Vasconcellos et al. (1980)
Columnar	*Cactaceae (family)*	28-31		6.4		4.6	36.2	51.2				11.0	88.6	Nieblas et al. (2001)
Cacti	*Opuntia ficus-indica* L.	6–14	0.2	14.1	1.6	4.4	24.9	53.8	0.3	0.5	0.2	19.4	80.6	Coskuner and Tekin (2003)
Prickly pear	*Opuntia ficus-indica* L.	10.9		9.3	1.4	3.1	16.8	70.3				12.4	88.5	Ennouri et al. (2005)
Prickly pear[g] (peel)	*Opuntia ficus-indica* L.	3.7	1.9	23.1	2.5	2.7	24.1	32.3	0.7		0.5	28.8	69.4	Ramadan and Morsel (2003b)
Sponge gourd	*L. acutangula*	46.9	0.5	20.9		10.8	24.1	43.7				32.2	67.8	Kamel and Blackman (1982); Kamel et al. (1982)
Maple seed	*Acer saccharum*	19.0		8.5		6.3	37.9	39.4	5.8	2.0		16.8	83.4	Kamel et al. (1982)
(Mimosaceae)	*Pentaclethra macrophylla*	45.9[h]	0.2	3.7		2.3	31.3	40.4		2.5	8.5	26.0	74.0	Foma and Abdala (1985); Ikediobi (1981)
(Moraceae)	*Treculia africana*	12–18		19–25	0.5–1.5	10–14	13–33	25–44				29–39	38.5–78.5	Ikediobi (1981)

Continued

TABLE 11.10
(Continued)

Common Name (Family)	Scientific Name	Lipid (%)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	Saturation	Unsaturation	References
Maple seed (*Continued*)														
(Malvaceae)	*Sida veronicifolia*	15.5	0.2	21.8		4.2	5.4	54.9		0.8		27.0	60.3	Rao and Lakshminarayana (1984)
(Malvaceae)	*Sida cordifolio*	11.5	0.2	18.2		4.6	10.7	62.9		0.6		23.6	73.6	Rao and Lakshminarayana (1984)
(Malvaceae)	*Sida ovata*	12.1	0.3	16.5		2.4	6.8	69.4		1.9		21.5	76.2	Rao and Lakshminarayana (1984)
(Malvaceae)	*Sida rhombifolia*	20.2	0.3	16.7		2.6	8.3	65.5		1.7		22.8	73.8	Rao and Lakshminarayana (1984)
(Malvaceae)	*Abutilon crispum*	12.5	0.2	15.7		2.7	12.2	61.2		1.0		19.6	73.4	Rao and Lakshminarayana (1984)
(Malvaceae)	*Cedrella odorata*	18.0		12.5	0.5	4.9	11.6	51.4	19.1			17.4	82.5	Balogun and Fetuga (1985)
(Malvaceae)	*Lovoa trichilloides*	25.9	0.3	12.7	0.2		20.7	63.3	0.7			13.0	84.9	Balogun and Fetuga (1985)
(Sterculiaceae)	*Brachyehiton dicolor*	29.3		13.8	0.5	0.9	31.1	46.7	1.1	0.4		15.1	79.6	Rao et al. (1989b)
(Sterculiaceae)	*B. diversifolius*	32.1		18.8	0.5	1.1	28.6	43.1	1.1	0.4		20.3	73.4	Rao et al. (1989b)
(Malvaceae)	*Urena repanda*	8.0	0.2	28.7	0.3	8.1	16.5	37.9	0.4			39.3	55.1	Ahmad et al. (1983)
(Malvaceae)	*Thespesia lampas*	8.6	0.2	18.4		0.6	14.5	63.6				19.2	78.1	Ahmad et al. (1983)
Seed	*Xylia xylocarpa*	14.8	0.62	22.1		7.2	10.2	51.3	6.3	1.21		31.1	67.8	Siddhuraju et al. (1995)
Onagraceae[i]		14.7	tr	9.7		2	10.3	76.0	tr			12.6	86.3	Zygadlo et al. (1994)

[a] 0.4%, 20:0.
[b] 0.1%, 24:0.
[c] Contains ricinoleic acid 9.8%, malvalic 1.3%, and sterculic 3.5%.
[d] Cyclopropenoid and epoxy acid 2.6% and 2.9%, respectively.
[e] Malvalic and dihydrosterculic at 2.2% and 1.3%, respectively.
[f] Dienoics conjugated fatty acid at 1.9%–2.8%.
[g] 8.6%, 18:3n-6; 1.2%, 24:1n-9.
[h] Oil contains 2.3% and 8.8% of 20:1 and 24:0, respectively.
[i] *Oenothera picensis*, *O. indecora*, *Ludwigia longifolia*, and *L. peruviana*.

TABLE 11.11
Fatty Acid Composition (%) of Lesser Known Fruits and Fruit Products Rich in 18:3 Fatty Acid

Common Name (Family)	Scientific Name	Lipid (%)	16:0	16:1	18:0	18:1	18:2	18:3	Saturation	Unsaturation	References
Spurge fruit	*Euphorbia niciciana*	14.2[a]	8.0			4.9	12.6	74.3	8.0	91.8	Aksoy et al. (1988)
Spurge fruit	*Euphorbia glaerosa*	15.2[a]	6.5			8.4	8.6	76.4	6.5	93.4	Aksoy et al. (1988)
Indian wood apple seed	*Feronia elephantum*	34.0	19.3		7.3	27.2	19.8	26.4	26.6	73.4	Ramakrishna et al. (1979)
China seed	*Salvia hispanica*	25–35[b]	8.0	0.4	7.1	11.9	23.9	46.4	15.6	83.0	Taga et al. (1984)
Gymnosperm	*Athrotaxis cupressoides*	10.5	6.7		2.5	14.3	31.9	33.5	9.5	80.7	Vickery et al. (1984)
Tung	*Aleurites fordii*	17.5	2.0		3.0	13.0		17.0[c]	5.0	86.0	Swern (1964); Sivetz (1963)
Chinese melon	*Momordica charantia*	41–45	1.75	0.14	24.1	3.3	3.85	0.58[d]	25.8	73.6	Chang et al. (1996)

[a] The seed of spurge (wolf's milk) contains 30.1 and 31.6 oil, respectively.
[b] Chia oil contains 1.4%, 0.4%, and 0.5% of c16:0, c22:1, and c22:0, respectively.
[c] Eleostearic acid, isomer of linoleic acid.
[d] 65.7% eleostearic acid.

(9,10-epoxy-*cis*-12-octadecanoic acid) in amounts that range from 3.1% to 6.2% (Chowdhury et al., 1983; Jamal et al., 1987). *Crotolaria retusa* belongs to the Leguminosae family and grows in India, Sri Lanka, and Malaysia. The oil from this seed contains 9.8% ricinoleic, 1.3% malvalic, and 3.5% sterculic acids and cyclopropenoid fatty acids (Daulatabad and Mirajkar, 1989). Many of the seeds from the Meliaceae and Sterculiaceae families contain cyclorproprene acids along with malvalic, sterculic, and dihydrosterulic acids.

The prickly pear plants are potential sources of food in many desert areas of the world. The fruit consists of 48% pericarp and a seed pulp that is consumed fresh in Turkey. An edible oil can be extracted from the seeds in yields of 5.8%–13.6%. The seed oil is highly unsaturated with high amounts of linoleic acid (53.8%–70.3%) (Coskuner and Tekin, 2003; Ennouri et al., 2005). The prickly pear peel on the other hand has 3.7% total lipids with 32.3% linoleic acid and equal amounts of palmitic (23.1%) and oleic (24.1%) acids. Although the α-linolenic was low, this oil has 8.6% γ-linolenic acid. It should be pointed out that the peel represents 48% of the prickly pear and would normally be a waste disposal problem. The extraction of oil from this by-product represents a potential new source of fats (Ramadan and Morsel, 2003b). Another plant that grows in the arid lands of Mexico is the columnar cacti. The fruits of this cactus are berries that contain hundreds of seeds embedded in a sweet pulp. The oil content of the seeds ranges from 28.4% to 30.7% with 51.2% linoleic acid and 36.2% oleic acid. The fruit is an important source of food for the people inhabiting the arid zones of Mexico (Ortega-Nieblas, 2001).

Two Acacia species (*Acacia colei* and *Acacia tumida*) have been investigated for their chemical and nutritional content as a first step prior to their incorporation in the diet of the population in the semi-arid sub-Saharan region of West Africa. The results showed 8.1%–10.9% fat from the seeds with a fatty acid composition of 50.1%–55.9% linoleic acid, 18%–22% oleic acid, and 9.4%–11.4% palmitic acid. The long-chain fatty acids (arachidonic, behenic, and lignoceric) were also detected. It was suggested that the polyunsaturated rich acacia seed oils be blended with oils rich in monounsaturated fatty acid to produce an oil with a ratio of saturated, monounsaturated, and PUFA of 1:1:1, which is considered to be ideal by FAO/WHO 1980 (Adewusi et al., 2003).

Table 11.11 lists the seeds that contain significant amounts (26%–76%) of linolenic acid in their oils. Generally, the presence of this fatty acid renders many of these oils inedible. The exceptions to this are spurge fruit, Indian wood apple, and chia seeds, which are eaten in a number of countries. Two common vegetable oils (soybean and canola) with approximately 10% linolenic acid are used extensively as edible oils. The majority of these high-linolenic acid oils, however, are used for the manufacture of paints, varnishes, linoleum, oilcloth, and printing inks. Tung oil is unique in containing 73% eleostearic acid, a fatty acid with *trans* and conjugated double bonds (18:3Δ9c,11t,13t) (Swern, 1964). Greater demand for linolenic-type oils may develop if the therapeutic benefits of consuming ω-3 fatty acids become established.

VIII. SPICES AND CONDIMENTS

Although spices and condiments are not true fruits, they are widely consumed and do represent a minor source of fatty acids in the diet. The lipid content and fatty acid composition of the seeds and bulbs of these plants therefore deserve investigation (Table 11.12). Coriander seeds contain petroselenic acid (octadeca-*cis*-6-enoic acid) in high amounts (75.1%). The seed matures in 50 days after flowering, during which time the lipids accumulate to 21%. The major change in the fatty acid composition is the steady increase in petroselenic acid and the concomitant decrease in the levels of the other fatty acids (Lakshminarayana et al., 1981).

The seeds of local Ethiopian mustard (*Brassica carinata* Braun) are high in erucic acid (40.6%) and glucosinolates. The *B. carinata* seed contains 29% oil and is in common use in Ethiopia but has not been developed as an oilseed crop elsewhere. Glucosinolate analyses indicate that sinigrin is the major glucosinolate (Mnzava and Olsson, 1990).

Another oilseed that contains high amounts of erucic acid and glucosinolates is taramira (*Eruca sativa*), a member of the Cruciferae family of plants. The erucic acid level is variable and ranges

TABLE 11.12
Fatty Acid Composition (%) of Selected Spices and Condiments

Common Name (Family)	Scientific Name	Lipid (%)	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	Saturation	Unsaturation	References
Coriander seed	*Coriandrum sativum*	21.5		0.4	4.4	0.7	tr	80.6[a]	13.4	tr					4.8	94.7	Lakshminarayana et al. (1981)
Mustards seed	*Brassica carinata*	29.5			3.2	0.2	0.9	13.0	19.9	10.8	0.6	8.5	0.2	40.6	4.9	93.3	Mnzava and Olsson (1990)
Wild mustards	*Cleome viscosa*	26.0			17.2		3.6	11.9	67.2						20.8	79.1	Rukmini et al. (1982)
Black cumin	*Nigella sativa*	32–38[b]		0.8	11.9	0.3	2.3	23.6	59.3	0.3	0.1				16.2	83.5	Nergiz and Otles (1993); Al-Jassir (1992)
Onion	*Allium cepa*	1.0			27.3			2.3	37.2	32.8					29.6	70.0	Al-Khatib et al. (1987)
Onion seed	*Allium cepa*	23.6		0.7	9.1		4.4	34.3	44.6	0.3					14.2	79.2	Rao (1994)
Garlic	*Allium sativum*	0.6	0.5		13.8			6.6	64.3	9.5					15.3	80.4	Kamanna and Chandrasekhara (1980)
		1.0			18.5	5.4	2.3	18.4	37.6		5.0	6.6			26.0	70.6	Al-Khatib et al. (1987)
Basil	*Ocimum sp.*[c]	18–26			8.3	0.3	2.5	10.0	22.5	56.2	0.2				11.0	89.0	Angers et al. (1996)
Nutmeg seed	*Myristica fragrans*	30.0	1.6	79.7	7.3	0.2	0.6	7.7	2.7						89.2	10.6	Al-Khatib et al. (1987)
Fenugreek seed	*Trigonella foenum-graecum*	7.5		1.3	0.4		1.5	52.2	39.8	0.6	0.3	0.1	0.2	3.6	3.7	96.3	Hemavathy and Prabhakar (1988)
Taramira	*Eruca sativa*	27.8–33.7	tr	0.1	6.7	0.1	1.9	13.3	13.0			12.2	1.7	22.2	10.4	89.6	Flanders and Abdulkarim (1985); Kanya and Uvs (1989)

Continued

TABLE 11.12
(Continued)

Common Name (Family)	Scientific Name	Lipid (%)	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	Saturation	Unsaturation	References
Paprika	*Capsicum annuum*	25.6			13.8	0.1	3.7	14.6	67.8	27.6					17.6	82.5	El-Adawy and Taha (2001a,b)
Love in a Mist[d]	*Nigella damascene*	33.6			10.8		19.4	17.4	47.6		0.5				30.3	69.7	Dauksas (2002)
		17.0			6.2	0.1	2.2	8.9	21.7	56.7					9.8	88.0	Azcan (2004)
Oregano	*Orignum onites*																
(Urticaceae)	*Boehmeria spicata*	10.6		0.2	5.2		1.5	7.8	83.5	1.5			0.3		7.2	92.8	Kato and Tanaka (1981)
(Urticaceae)	*Boehmeria nivea*	17.5		tr	6.2		2.3	9.4	80.4	1.2					8.6	91.0	Kato and Tanaka (1981)

[a] 5.5% *C18:1 9 and 75.1% C18:1 6.
[b] 1.1%, 24:0.
[c] Average of seven species.
[d] 4.8%, 20.2.

from a low of 10% in Turkish seeds (Flanders and Abdulkarim, 1985) to over 42% in seeds grown in India (Kanya and Uvs, 1989). These variations in fatty acid composition depend on the maturity of the seed and frequency of irrigation. The average fatty acid composition of seeds from these two sources is given in Table 11.12. The penetrating pungency of taramira seed oil is due to the hydrolysis of glucoerucin (4-thiomethyl 3-butenyl glucosinolate), the major glucosinolate in taramira seeds (Kanya and Uvs, 1989).

Onions, garlic, and nutmeg are used as seasonings and condiments, in folk medicine, and in cosmetics in various Middle Eastern countries. In onions, the oleic and linoleic acids account for 70% and palmitic and stearic acids for 29% of the total identified fatty acids. In garlic oil, the unsaturated fatty acids (palmitoleic, oleic, linoleic, eicosenoic, and arachidonic) account for 70% of the total fatty acids, and palmitic, margaric, stearic, and arachidic acids account for the rest. Nutmeg oil, on the other hand, is much more saturated owing to the presence of 78% myristic acid (Al-Khatib et al., 1987).

Fenugreek is cultivated in India, North Africa, and Mediterranean countries. The leaves are eaten as a vegetable, and the seeds are used as a condiment. The seed contains 7.5% lipid and is highly unsaturated, with 52% oleic acid and 39.8% linoleic acid. The purified oil is golden yellow and has a bitter taste and disagreeable odor. The total lipids consist of 84.1% neutral lipids, 5.4% glycolipids, and 10.5% phospholipids (Hemavathy and Prabhakar, 1989).

Two herbs grown in Japan (*B. spicata* and *Boehmeria nivea*) belong to the family Urticaceae. The achenes from these plants were shown to contain 80%–83% linoleic acid (Kato and Tanaka, 1981).

Paprika is widely used as a flavoring in foods. When processing the flesh, the paprika seeds are separated from the pods and discarded. This waste product has good potential for conversion into nutrients such as oil and protein suitable for human consumption. Paprika seed oil is high in total unsaturates (82.4%) and low in saturates (17.5%). The major fatty acids are linoleic (67.7%), oleic (14.5%), and palmitic (13.8%) acids (El-Adawy and Taha, 2001a,b).

The seeds from *Nigella damascene* are used as a diuretic and for flavoring of foods. The total lipid content was 33.5% with linoleic acid (47.6%–50.8%) as the major fatty acid. Significant levels of eicosodienoic acid (4.76%) were detected along with 35%–51% free fatty acids and 21%–29% elemenes in the volatile oil fraction (Dauksas et al., 2002).

Turkey exported 7400 tons of oregano in the year 2000. Oregano is used as an industrial raw material for producing essential oils, as a tea and as a culinary herb. The seed oil from *Origanum onites* L. contains 14%–20% oil with 57% linolenic and 22% linoleic as the major fatty acids. The distinctive aroma of oregano is due to carvacrol, which is present in the essential oil fraction (Azcan et al., 2004).

IX. FUTURE TRENDS

Vegetable oils are used predominately as a source of food and nutrition but many nonfood applications such as in cosmetics, pharmaceuticals, biobased lubricants, biobased plastics, and biobased fuels as an alternative to petrochemical fuels have emerged. Fatty acids are important for human health and nutrition. These fat-soluble compounds form complexes with protein and are transported to various tissues in the body where they are incorporated into cell membranes, are metabolized into other compounds, or serve as a source of energy. Many of these fruit oils are also excellent sources of nutraceuticals and bioactive compounds. Both argan and sea buckthorn oils have high nutritional and medicinal values. Hemp seed (*Cannabis sativa*) can yield an industrial oil high in erucic acid, which is an intermediate in the production of some polyesters, plastics, and nylons.

Recent studies have shown that hydrogenated and saturated fatty acids can lead to health problems. These findings lead to a reduction in the consumption of animal fats and an increase in the utilization of highly unsaturated vegetable oils. Butter oil consumption underwent a steady decline over the years but the awareness of *trans* fatty acids in hydrogenated oils has put butter in a more favorable light. Over the past 10 years, a number of oils such as grapeseed oil, tomato seed oil, apricot seed oil,

and apple seed oil have gone from emerging oil status to full commercialization. It is expected that similar outcomes are in store for the novel oil from the argan nut, hemp, crambe, and sea buckthorn seeds.

It is clear that India, China, and other developing countries are consuming increasing amounts of oil and this growing demand will put new pressures on finding new sources of edible oils. Although the health conscious consumers in developed countries are turning to specific oils such as olive oil and at the same time reducing their total consumption of fats and oils, this trend may not compensate for the growing demands from the populations in the developing countries. An additional factor that is putting pressure on world seed oil production is the increasing cost of crude oil. With the continual rise in fuel prices, there is an urgent need to develop alternative and renewable fuels. The development of biodiesel from plant and animal fats is one such alternative. Although biodiesel will reduce the reliance on petroleum feedstock, it will siphon off large amounts of vegetable oil that could be used in the edible oil market. This scenario is important because the consumption of vegetable oils is projected to grow 2.9% annually over the next 10 years (Inform 1:286, 2006).

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12 Fatty Acids in Food Cereal Grains and Grain Products

Robert Becker

CONTENTS

I. INTRODUCTION

The fatty acid composition of cereal grains is generally well established and is available to the reader in standard reference sources (Morrison, 1978; USDA, 2005). Typical total lipid contents of cereal grains are listed in Table 12.1; fatty acid compositions of lipids from each grain are given in appropriate sections. Future work can be expected to refine further these values and their ranges and document environmental and processing variations. This discussion focuses on current information to provide a context for a review of knowledge regarding the state of fatty acids in cereal foods as they are consumed, and developments in their effects on food processing, functionality, and nutritional properties.

II. OCCURRENCE

Cereal grain lipids are broadly classified as either polar or nonpolar compounds based on their solubility in different solvent systems. Fatty acids are major components of all lipid classes, but most occur as acyl esters of glycerol, which are nonpolar lipids. Fatty acids may also be bound to a wide variety of other cellular components, for example, long-chain alcohol esters, to form waxes and *N*-acyl compounds that are polar lipids. Unbound or free fatty acids are also usually present in

TABLE 12.1
Total Lipid Content of Cereal Grains

Cereal	Total Lipid Content (%), Moisture-Free Basis[a]	References
Barley, pearled	2.5–4.7 (3.6)	Bhatty and Rossnagel (1980)
Buckwheat	2.6–3.2 (2.9)	Mazza (1988)
Corn	5.2–6.0 (5.6)	Morrison (1978)
Millet, proso	3.9–4.9 (4.5)	Lorenz and Hwang (1986)
Oats	4.9–7.9 (6.4)	Morrison (1978)
Rice	0.9–3.1 (2.9)	Morrison (1978)
Sorghum	3.7–6.0 (4.6)	Morrison (1978)
Wheat, whole kernel	2.1–3.8 (2.9)	Morrison (1978)

[a]Averages in parentheses.

smaller and variable amounts. These result from metabolic accumulations and enzymatic or chemical hydrolysis of the parent compound.

The principal fatty acids in cereal lipids are almost invariably 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3. Trace amounts of other fatty acids may also be present; highly unsaturated fatty acids are unusual, as are hydroxy acids.

Fatty acid lipids occur as components of intercellular membranes, spherosomes (small oil droplets), starch, and protein bodies in all parts of the cereal grain. The lipid contents of the embryonic, bran, and endosperm mill fractions are often reported, frequently without indication of the amount of the material in the parent grain. Thus, embryonic material may be rich in lipid but constitute a small amount of the grain and contain only small amounts of the total lipids. Conversely, endosperm material may contain low levels of lipid but, owing to its preponderance in the kernel, constitute a large percentage of the total lipids. Specific examples are given for individual grains.

III. DEGRADATIVE EFFECTS

In cereal grains, the first step in lipid degradation is usually the liberation of free fatty acids, which then may undergo a variety of oxidative degradation reactions. The fatty acids may be enzymatically liberated by lipases or by chemical saponification (Pomeranz, 1985).

Oxidation of the fatty acids occurs primarily at methylene groups activated by adjacent double bonds. Unless mediated by other oxidants or enzyme systems, oxidation proceeds through a free radical chain mechanism involving initiation, propagation, and termination steps. Consequently, the rate of oxidation increases somewhat geometrically with the increase in the amount of unsaturation in the fatty acids and the number of adjacent double bonds, as are found in polyunsaturated fatty acids. Oxygen reacts with unsaturated fatty acids at relative rates of oleic 1, linoleic 12, and linolenic 25. Trace amounts of heavy metals such as copper, iron, and manganese also promote oxidation, as do conformational effects.

Oils rich in linoleic acid, such as sunflower, safflower, cottonseed, and soy are less stable than peanut, palm, and coconut oils, which contain lesser amounts of unsaturated fatty acids. Marine oils with fatty acids containing more than three double bonds are most susceptible to oxidation. Measurement of lipid oxidation has been reviewed by Gray (1978).

Oxidation may be either chemical, as described above, or enzymatic. Enzyme-mediated oxidation in cereal grains is largely due to lipoxidase and other oxidative enzymes, which occur mainly in the seed germ (Reed and Thorn, 1971). Linoleic/linolenic acids have been shown to be oxidized through a cascade of enzyme reactions (see review by Gardner, 1988). Lipoxidase activity is

preponderant in the germ and has been shown to occur in doughs; oxidized fat affects dough mixing properties and bread structure, flavor, and color.

IV. FLAVOR

The flavor of oils is due to low concentrations of volatile degradation components. Slight oxidation is called *reversion* and may produce desirable flavors; as degradation progresses, the oil becomes less acceptable and is termed *rancid*. The volatile rancidity flavor components are lower molecular weight saturated and unsaturated aldehydes, ketones, and organic acids.

Hydrolytic release of free fatty acids may be extensive without the development of rancid odors and flavors, because C_{16} or longer fatty acids are nonvolatile and are not typically rancid tasting. The flavor of free fatty acids has been described as acidic or soapy.

In addition to contributing to flavor as described above, free fatty acids may initiate other reactions in foods. For example, free fatty acids may hydrolyze sucrose to form glucose and fructose, which in turn may form Maillard-type browning compounds with undesirable colors, off-flavors, and diminished nutritional and functional properties. Alternatively, fatty acid hydroperoxides may from polymers, react with protein or amino acids, or react with carbohydrates, all with undesirable effects.

V. FUNCTIONALITY

The functional effects of lipids in cereal products have been extensively studied, often from an economic perspective. Typical efforts are toward the utilization of liquid oil rather than solid shortening in baked products and the development of theories explaining the role of lipids in the appearance and texture of baked products. Detailed explanations of many such effects are still being pursued. Similar studies of fatty acids, although not as frequent, are also incomplete. Most studies have been focused on wheat lipids and wheat products (Pomeranz, 1985).

In general, addition of fat to doughs will cause a larger volume, the cell walls become more uniform and thinner with a resulting softness and better bite, and crumb characteristics and loaf-keeping quality improve. Reduction of the fat content produces a corresponding coarseness and crumb darkness. The ratio of solid to liquid fat (shortening and oil) is also important to many product characteristics. For example, larger amounts of solid fat will reduce cookie spread. In many baked products, excessive amounts of fat may decrease product quality. In puff pastries, the main function of fat is to separate the thin layers of dough to produce a uniform flaky texture and a high volume. This occurs when the fat does not penetrate into the dough, which keeps the dough layers separate during mixing. Fats have also been implicated in the hydration effects of cereal grain starch granules. Emulsifiers may be added to replace fats.

Mono- and diglycerides of fatty acids and/or their corresponding diacetyltartaric acid esters make useful bread soft, impart better loaf symmetry and favor uniform cell structure and tenderer crusts. These softeners are often incorporated into the shortening. In pound cakes, they increase volume while reducing amounts of eggs and butter and/or shortening needed. Other fatty acid-derived additives include sodium stearyl fumarate, calcium or sodium stearyl-2-lactylate, succinylated mono- and diglycerides, lactylic stearate, tallowyl-β-lactic acid, and propyleneglycol esters of fatty acids. Some of these compounds are proprietary, but all function in much the same manner.

VI. THERMAL DETERIORATION

Thermal and oxidative deterioration of the oil in cereal grains during cooking by boiling, baking, or frying is of significantly less importance than comparable effects in meats, with respect to health as well as the quality of the food prepared (Doolittle et al., 1989). Heating of fats and oils causes

degradation of triglycerides, which may involve hydrolysis into free fatty acids. The liberated free fatty acids may contribute to the taste of the food, or they may become chemical reactants affecting functionality and nutrition. Oleic and linoleic acids are known to inhibit the mutagenicity of heterocyclic amines (Hayatsu et al., 1981). Polyunsaturated fatty acids may also undergo free radical-mediated oxidation reactions with the formation of mutagenic products. In cereals and vegetables, which have low lipid levels, cooking conditions sufficiently severe to cause excessive lipid degradation usually result in unpalatable products, which serve to limit mutagenicity in these products. In meats, many of which have higher lipid levels, cooking to common levels of doneness is usually performed at higher temperatures, especially during boiling and frying. This can generate significant lipid thermal degradation with the accompanying mutagenic pyrolysis products as well as mutagenic heterocyclic amines from degraded proteins.

Since large amounts of vegetable oils are used for cooking, the excessive reuse of oils for deep-fat frying is of special interest. Cooking oils that have been thermally oxidized are thought to be mutagenic (MacGregor et al., 1985) and cause growth retardation, increased liver and kidney weights, and damage to the liver, thymus, and testes (Alexander, 1986). Related studies by Scheutwinkel-Reich et al. (1981) and van Gastel et al. (1984) were negative, but another work has indicated mutagenic activity associated with reused peanut oil (Fong et al., 1980). The mutagenicity of repeatedly reused cooking oils was studied by Hageman et al. (1988) using *Salmonella typhimurium* TA97, a strain not used by previous investigators. Frying oil was heated to 180°C for 10 h/day on four consecutive days. Foods were fried daily, cooking oil samples were collected and fractionated into polar and nonpolar portions, and the lipid peroxidation was measured as thiobarbituric acid reactants. Mutagenic activity was highest after 20 h frying. Rat liver activation enhanced mutagenesis by some fractions but inhibited others. In this initial study, a significant positive correlation was found between mutagenic activity and thiobarbituric acid reactants. In a subsequent study (Hageman et al., 1989), linoleic acid hydroperoxide levels, which are thiobarbituric acid reactants, were correlated with mutagenic activity in the presence of liver mix. Although these studies focus on the mutagenic involvement of lipid oxidation products, other pyrolysates should also be suspected. In any event, extremely thermally oxidized (excessively reused) cooking fats and oils should probably be avoided, and chronic or high consumption of fried foods should be of concern.

An association between lung cancer and heated cooking oils has been suggested by an epidemiological study of 672 Chinese women with lung cancer (Gao et al., 1987). The risk increased with the number of meals cooked by stir frying and deep-fat frying and with the degree of smokiness and frequency of eye irritation during cooking. These studies should be substantiated. Factors such as age, nutritional history, and smoking habits, as well as other pyrolysates and naturally occurring volatiles—for example, those from mushrooms and spices—should also be suspected.

VII. CEREAL GRAINS

A. Wheat

Because of its economic importance and the inherent complexity of many of its manufactured products, no commodity has been studied in as much detail as wheat. The location of wheat kernel lipids, their biochemical purpose, their extraction, functional effects in foods, and nutritional consequences have all been subjects of extensive investigations (Pomeranz, 1971, 1985).

The typical crude lipid and fatty acid composition of wheats and some of their mill fractions are shown in Table 12.2 and 12.3. Even though the endosperm contains only about 1% crude lipid, it contains roughly half of the total lipid content of the whole grain. The bran contains about one-third of the total oil and the germ the remainder. The germ also contains high concentrations of lipase-degrading enzymes.

Of the various changes that occur during grain deterioration, the hydrolytic break-down of fats and the formation of free fatty acids is the most rapid and signals the earliest stage of quality loss.

TABLE 12.2
Crude Lipid Composition of Wheats and Yields of Wheat Mill Fractions

Sample	Fat (%)	Mill Yield (%)	% Total Fat[a]
Wheat, whole grain			
Hard red spring	2.7	100	100
Hard red winter	2.4	100	100
Soft red winter	2.2	100	100
White	2.6	100	100
Durum	3.3	100	100
Wheat mill fractions			
Endosperm flour			
Hard wheat	1.7	83	68
Soft wheat	1.5	83	65
Bran	4.6	14	32
Germ	10.9	2	11

[a] Based on nonstarch lipids.

Source: Compiled from Pomeranz, Y., Chung, O.K., and Robinson, R.J. (1966). *J. Am. Oil Chem. Soc*. *43*: 511–514. With permission.

TABLE 12.3
Fatty Acid Composition of Total Lipids from Wheat and Wheat Mill Fractions

Fatty Acid	Whole Grain	Endosperm	Bran	Germ
Myristic 14:0	0.1	Trace	Trace	Trace
Palmitic 16:0	24.5	18.0	18.3	18.5
Palmitoleic 16:1	0.8	1.0	0.9	0.7
Stearic 18:0	1.0	1.2	1.1	0.4
Oleic 18:1	11.5	19.4	20.9	17.3
Linoleic 18:2	56.3	56.2	57.7	57.0
Linolenic 18.3	3.7	3.1	1.3	5.2
Arachidic 20:0	0.8	Trace	Trace	Trace
Other	1.1	1.1	Trace	0.8

Source: Adapted from Morrison, W.R. (1978). *Adv. Cereal Sci. Technol. 2*: 221–348; Pomeranz, Y. (1971). *Wheat Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, MN. With permission.

This "fat acidity" originates from enzymatic hydrolysis of the fats to release free fatty acids and to a lesser extent from oxidation of the unsaturated fatty acids to form acidic degradation products. This acidity (acid value) can be quantified by titration and has been defined as milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 g of grain. Typically, fresh sound wheat has a fat acidity value of about 11, with a maximum of 20. Deterioration by improper storage, mold growth, high respiratory activity, or spontaneous heating may produce fat acidity values well over 100. Wheat stored under most practical storage conditions for several years will have fat acidity considerably higher than that of fresh sound wheat. Flour milled from deteriorated wheats will have reduced keeping and baking properties. Storage at low temperature and moisture content significantly reduces the changes in fat acidity values.

Flour containing severely deteriorated lipids can be easily detected by its typical rancid or "painty" off-odor. Less severe deterioration may not be as evident but will still be characterized by an increase in free fatty acids.

The practical consequences of deteriorated lipids (increased free fatty acids) are reduced loaf volume and diminished organoleptic parameters. Saturated fatty acids have little effect on doughs or gluten. However, unsaturated fatty acids produce "short," tough glutens and, when exposed to oxygen, significantly damaged baking performance.

B. Durum Wheat

Kobrehel and Sauvaire (1990) have shown that sulfur-rich glutenins from durum wheat have an intrinsic affinity for specific lipids. These protein lipids are tightly bound and have a fatty acid composition substantially different from that of total lipid extracts. As shown in Table 12.4, linoleic acid (18:2) is over 50% of the fatty acid content of the total lipids, whereas in the purified sulfur-rich glutenins, its relative concentration is only about 4%. Lipids from the isolated sulfur-rich glutenin proteins are also significantly richer in C_{14}, C_{16}, and 18:0 fatty aids than the total lipid extracts, with 18:0 being approximately 150% richer in the glutenins. It was suggested that only 16:0, 18:0, and 18:1 were specifically bound to the protein, with the other fatty acids, which occur in lesser amounts, being contaminants.

In addition to the fatty acids, significant quantities of fatty alcohols were observed in the sulfur-rich glutenins. Dodecanol ($C_{12}H_{26}O$) occurs in only trace amounts in the total lipid extract but is a major component in a glutenin extract from Mondur durum wheat. Lesser amounts of tetradecanol ($C_{14}H_{30}O$) and pentadecanol ($C_{15}H_{32}O$) were also observed; another wheat variety had less dodecanol.

The physiological role of these glutenins and their correlation with other low molecular weight proteins is yet to be determined. However, it was observed that the bound lipids appear to promote solubilization of the sulfur-rich glutenins in the presence of sodium salts of fatty acids. Since these sulfur-rich glutenin proteins probably are trypsin and α-amylase inhibitors, the lipid components may facilitate seed wound resistance.

Because of their high sulfide and disulfide content and the presence of bound fatty acids and fatty alcohols, these components also undoubtedly have an important role in technological applications. Other glutenins were shown to aggregate strongly upon heating and to contribute significantly to pasta firmness and elasticity and the surface condition of cooked pasta, primarily through hydrophobic and disulfide bonding (Feillet et al., 1989; Kobrehel and Alary, 1989).

TABLE 12.4
Relative Amounts of Fatty Acids and Dodecanol ($C_{12}H_{26}O$) in Lipids from Isolated Glutenin Proteins and Lipids from Semolina

		Mondur			Kidur		
Fatty Acid	Fatty Alcohol	Frac. I	Frac. II	Semolina Lipid	Frac. I	Frac. II	Semolina Lipid
14:0		9.6	4.5	tr	7.9	10.3	tr
16:0		25.1	34.7	17.9	31.6	28.1	22.2
18:0		28.1	23.0	1.6	27.3	27.2	1.4
21.4			32.5	33.6	20.1	28.3	30.5
18:2		4.5	4.1	57.1	4.8	3.7	51.5
18:3		0.0	0.0	2.8	0.0	0.0	2.9
	C_{12}	19.4	2.0	tr	1.1	1.7	tr

Source: From Kobrehel, K., and Sauvaire, Y. (1990). *J. Agric. Food Chem. 38*: 1164–1171. With permission.

C. Rice

Most of the oil in the rice kernel is located in the bran and embryo, which usually contain about 15%–22% oil and are obtained during the milling process used to produce white rice (Table 12.5). In the intact kernel, the oil is compartmentalized and not available to indigenous enzymes. Milling ruptures the cells, mixes the cellular contents, and initiates autolytic, hydrolytic, and oxidative degradation of the oil, often accompanied by chemical degradative effects.

This oil degradation can be followed by the liberation of free fatty acids. In the intact kernel and immediately after milling, rice oil contains only small amounts of free fatty acids. After milling, the oil in untreated rice bran degrades to produce free fatty acids at a rate of 5%–7% of the oil per day. However, if the enzymes are deactivated, the bran becomes stable and the oil is not degraded. Until recently, processing logistics precluded commercial extraction of the oil from the bran before degradation occurred, so the bran was a waste product or at best was used for animal feed. Bran stabilization has now been economically achieved by extrusion cooking, and rice bran oil has become an important commercial commodity. For discussions of rice bran extrusion and technological assessments, see review by Sayre et al. (1982, 1985) and Saunders (1986).

Rice oil obtained from stabilized bran from good-quality rice has a free fatty acid content of under 2%; proper purification, deodorization, and decolorization virtually eliminate all free fatty acids. Hydrolysis of the total lipids indicates that the oil has a fatty acid content (Table 12.6) similar to those of corn, cottonseed, sesame, and peanut oils, being rich in oleic (40%–60%), linoleic (29%–42%), and palmitic (12%–16%) acids. Rice oil has a very mild flavor, so it is a useful ingredient in lightly seasoned foods and as a flavor carrier.

When used as frying oil, rice bran oil has greater stability than other popular oils, probably because of its high level of unsaponifiables (4.2% vs. 1.3% for corn oil). The unsaponifiables in rice oil are high in the antioxidant ferulic acid esters, contain significant levels of sterols, and have amounts of tocopherols typical of other grains.

TABLE 12.5
Typical Distribution of Rice Lipids in Mill Fractions

Sample	Mill Yield	Fat Content (%)	% Total Fat
Brown rice	100	2.2	100
White rice	89	0.3	13
Rice bran	8	22.0	87

Source: Adapted from Morrison, W.R. (1978). *Adv. Cereal Sci. Technol. 2*: 221–348. With permission.

TABLE 12.6
Fatty Acid Composition of Rice Oil

Sample	14:0	16:0	18:0	18:1	18:2	18:3	20:0
Brown rice	<1	15–28	<3	31–47	29–42	<6	4
Rice bran	<1	12–16	1–4	40–60	29–42	4	2

Source: Adapted from Morrison, W.R. (1978). *Adv. Cereal Sci. Technol. 2*: 221–348. With permission.

A number of experiments have suggested that rice bran and rice bran oil have the effect of lowering serum cholesterol and low-density lipoproteins (LDLs) (Sharma and Rukmini, 1986; Raghuram et al., 1989; Kahlon et al., 1990). The specific dietary components in the oil responsible for these effects appear to be unsaturated fatty acids and unsaponifiables such as tocotrienols and β-sitosterol. In the bran, the effective components are thought to be hemicellulose, β-glucans, and protein.

Other work by Most et al. 2005 found that the unsaponifiables in rice bran oil, not fiber, lowers cholesterol in humans.

D. Maize

The occurrence, composition, chemistry, isolation, purification, properties, and uses of maize (corn) oil have been ably reviewed by Watson and Ramsted (1987). Because of the economics of production and processing, corn is the major cereal grain used for the commercial production of vegetable oil.

Corn oil is localized in the seed germ. The amount of germ in the kernel and oil in the germ are genetically controlled and vary widely. Both dry- and wet-milling processes are used to separate the germ from the remainder of the seed. Dry-milling yields germs containing about 18% (moisture-free basis, MFB) oil, which is rolled (flaked) and hexane extracted. Germ produced by wet milling contains about 56% (MFB) oil, which is heated and screw pressed to remove about 60% of the oil, with the remaining oil being solvent extracted.

Refining crude corn oil removes phosphatides, free fatty acids, pigments, waxes, and trace amounts of undesirable flavors and odors. Refined corn oil is pale yellow and bland and maintains clarity at refrigeration temperatures. Margarines are produced from corn oil by partial hydrogenation, giving products that usually contain more polyunsaturated fatty acids than margarines made from other fats or oils.

The composition of refined maize oil is shown in Table 12.7. Polyunsaturated fatty acids are higher in maize oil than oils from many competing sources, which lead to claims of increased health benefits.

The greater oxidative stability of corn oil is due to its higher levels of tocopherols and lesser amounts of linolenic acid.

TABLE 12.7
Fatty Acid Composition of Maize Oil

Sample	14:0	16:0	18:0	18:1	18:2	18:3	20:0
Total lipid	7–19	1	<4	23–46	35–66	<3	2
Endosperm	11–22	—	2–3	17–39	34–63	1–3	—
Germ	9–18	tr	1–3	20–56	29–68	<1	—
Commercial	9–16	1	4	19–50	34–62	1	1

Source: Adapted from Morrison, W.R. (1978). *Adv. Cereal Sci. Technol. 2*: 221–348. With permission.

E. Oats

Oat groats (caryopsis) have the highest lipid concentration of the common cereal grains (Morrison, 1978) (4.9%–7.9%) (see Table 12.1). These lipids are compositionally similar to other cereal grains, being highly unsaturated and containing considerable amounts of linoleic acid, an essential fatty acid for humans. Owing in part to this lipid content, oats are a good energy source (Webster, 1986).

There is a continuing interest (Youngs, 1986; Branson and Frey, 1989) in increasing the groat oil content to make the crop a source of edible oil (Frey and Hammond, 1975) and antioxidant compounds (Hammond, 1983), as well as a higher energy feed grain. The economics of producing oat oil indicate that a minimum 17% groat oil content is required (Frey and Hammond, 1975); based on production costs and multiple uses of the grain (protein and by-product utilization) and subject to variations in costs of competing sources. Breeding studies indicate that this is attainable (Branson and Frey, 1989) with favorable grain yields and retention of desirable agronomic characteristics. A potent lipase present in the seed may cause rapid hydrolysis and oil quality deterioration unless deactivated or diminished by breeding. Additionally, the higher oil content may make the grain more delicate, presenting shipping and processing problems.

F. Barley

Barley contains 2.5%–4.7% fat, with a fatty acid composition similar to that of other cereals. Crude fat was determined in 92 spring barley samples (81 of two-row and 11 of six-row type) and found to be 3.0% ± 0.2% in the two-row and 3.3% ± 0.3% in the six-row types (Aman et al., 1985). The typical composition of barley lipid is shown in Table 12.8.

The lipid content and composition of barley varies with the date of harvest or days after anthesis (DAA) (deMan and Cauberghe, 1988) as well as genotype and environmental and weather conditions before and after flowering (deMan, 1985). A relationship has also been found between barley fatty acid composition and kernel size as well as a similarity between the lipid composition of small mature grain and immature grain (deMan and Dondeyne, 1985; deMan and Bruyneel, 1987; deMan and Vervenne, 1988).

It was shown that the total fatty acid content (in mg/kernel) of spring barley increases to a maximum at 37 DAA, mainly due to an increase in the free lipid fraction, and diminishes rapidly in the next 5 days. Free and starch lipid contents decrease significantly during the final days of maturation.

Barley dried distillers grain (DDG) is a by-product of industrial "gasohol" production or brewing/distilling processes. Rich in protein, it has traditionally been used for animal feed or discarded. Attempts to incorporate it into food products have been hampered by its sharp, bitter soapy flavor with a distinct bitter or metallic aftertaste. Defatted and/or bleached DDG as an ingredient in oatmeal cookies was evaluated by a taste panel, and the changes in lipid composition correlated with panel acceptance (Dawson et al., 1984). Lipid changes occurring in the gasohol production process were followed, and DDG was tested as an ingredient in granola by Dawson et al. (1987). Unfortunately, the techniques employed in these studies did not satisfactorily address the myriad other compounds that may be present and are known to be related to bitterness (Belitz and Wieser, 1985).

Morrison et al. (1984) examined the lipid and amylose contents of 39 barley starch samples and reported a strong positive correlation between lysophospholipids and amylose overall. The amylose in all starches had similar lipid-binding capabilities, suggesting more uniform α-(1-4)glucan chain length and branching than are found in maize starches. Check feeding experiments indicate barley oil may have hypocholesterolaemic components (Linji et al., 1993).

TABLE 12.8
Fatty Acid Composition of Total Lipid from Barley

Fatty Acid	% Total
Stearic 16:0	2.6
Palmitic 18:0	7.4
Oleic 18:1	26.5
Linoleic 18:2	43.7
Linolenic 18:3	0.4
Unsaponifiable	5.4

Source: From Harris, G. (1962). In *Barley and Malt: Biology, Biochemistry, Technology* (A.H. Cooke, ed.), Academic Press, New York, p. 563. With permission.

G. Buckwheat

Buckwheat is cultivated in most temperature countries for food and for livestock and poultry feed. In North America, it is primarily used as an ingredient in pancake mixes, in Eastern Europe in porridges and soups, and in Japan as an ingredient with wheat in manufactured noodles.

Buckwheat seed and flour deteriorate rapidly with aging, changing from a light green seed and fresh, appetizing flour to a reddish brown seed and bland, somewhat rancid flour. The Japanese

market requires buckwheat flour to be milled within 1 week of use because of the flavor deterioration problem. These economic incentives have favored continued research of lipid and fatty acid composition of modern buckwheat varieties (Taira et al., 1986; Mazza, 1988).

The lipids in whole buckwheat and buckwheat tissues, buckwheat flour, and steamed and stored buckwheat have been reviewed by Pomeranz (1983). The lipid content and fatty acid composition of the three most important North American varieties were reported by Mazza (1988). Variations due to effects of seeding time were studied by Taira et al. (1986). The lipids and the fatty acid compositions of North American varieties are summarized in Table 12.9.

Buckwheat contains about the same amount of total lipids as wheat rye (see Table 12.1). In buckwheat, the neutral lipids constitute about 81%–85% of the total lipids compared to about 35% in wheat and rye. The major classes of fatty acids of all cultivars and all lipid classes were palmitic (16:0), oleic (18:1), and linoleic (18:2). Of the three cultivars examined, there were statistically significant differences only in the oleic and linoleic contents of total lipids, free lipids, and phospholipids. No evidence was found for changes in fatty acid composition during 25 months of room-temperature storage. Taira et al. (1986) found that oleic acid was the dominant fatty acid in early seeded cultures, which also had higher lipid contents, and linoleic acid was dominant in late-seeded cultures.

TABLE 12.9
Lipids of Dehulled Buckwheat Seed and Fatty Acid Composition of the Total Lipid Extract

				Polar Lipids		
Total Lipids	**Free Lipids**	**Neutral Lipids**		**Glycolipids**		**Phospholipid**
2.6–3.2	**2.1–2.6**	**2.2–2.6**		**0.9–0.15**		**0.27–0.33**
Lipid class	16:0	18:0	18:1	18:2	18:3	20:0
Total lipids	13.4–14.6	1.4–1.9	33.9–38.2	34.6–38.7	2.3–3.0	1.3–1.4

Source: From Mazza, G. (1988). *Cereal Chem. 65*: 122–126. With permission.

H. Millet

Because millet is one of the most drought-tolerant food crops, it is an important food in many parts of the world; estimated annual production is about 18 million metric tons (Hoseney et al., 1981). Although several small-seeded grains are called millets, the commonly grown varieties are pearl (*Pennisetum americanum*), proso (*Panicum miliaceum*), and foxtail (*Setaria italica*).

The chemical composition of millet has been well documented and compiled in reviews by Hulse et al. (1980) and Hoseney et al. (1981).

The odor instability of millet flour is a major factor in utilization of the grain (Varriano-Marston and Hoseney, 1983). The off-odor, which is detectable within days after milling, had been attributable to lipid deterioration (Lai and Varriano-Marston, 1980a). However, it was later shown by Kaced et al. (1984) that the odor was not the result of classical lipid oxidative deterioration. Reddy et al. (1986) concluded that the off-odor production is due to enzymatic action on the flavone portion of a *c*-glyco-flavone in the grain.

The free lipid (petroleum ether-extractable) composition of proso millet ranges from 3.03% to 7.40% and is rich in oleic (53.8%), linoleic (34.9%), and palmitic (10.8%) acids. Bound lipids ranged from 0.58% to 0.90% (Lai and Varriano-Marston, 1980b).

Hand-dissection of pearl millet and analysis of the anatomical parts indicated that 88% of the total lipid is in the germ, with the endosperm and bran each containing 6% (Abdelrahman et al., 1984). Osagie and Kates (1984) examined eight solvent systems for extracting lipids from *P. americanum* seeds and concluded that hot-saturated butanol was most efficient in extracting polar, neutral, and total lipids.

Lorenz and Hwang (1986) determined the lipid composition of flours and brans of nine proso millet varieties (Table 12.10). The lipids in glutinous and nonglutinous varieties of foxtail millet were analyzed by Taira (1984), who found that the glutinous varieties have more total lipid and a higher palmitic acid and lower stearic acid content but that there are overlapping ranges between the two varieties (see Table 12.11).

TABLE 12.10
Fatty Acid Composition of Free Lipids from Red Proso Millet Varieties

Sample	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Flour	7.2–10.0	0.1–0.3	1.5–3.4	18.1–24.2	62.2–69.5	1.3–2.2	0.1–0.4
Bran	5.7–8.6	0.2–0.3	1.1–3.1	19.2–25.3	63.4–70.7	1.7–2.2	0.1–0.4

Source: Compiled from Lorenz, K., and Hwang, Y.S. (1986). *Cereal Chem. 63*: 387–390. With permission.

TABLE 12.11
Lipid Content and Fatty Acid Composition of Nonglutinous and Glutinous Foxtail Millet

	Total Lipid	16:0	18:0	18:1	18:2	18:3	20:0
Nonglutinous							
Type A	4.4	7.8	5.6	13.3	68.0	2.8	1.7
Type B	4.5	8.2	1.6	15.6	70.6	2.4	0.9
Glutinous							
Type A	4.8	9.0	4.8	12.5	68.3	3.0	1.7
Type B	4.9	9.4	1.0	16.2	69.4	2.6	0.7
Differences between glutinous and nonglutinous varieties							
Type A	[a]	[a]	[a]	[b]	NS	NS	NS
Type B	[a]	[a]	[a]	NS	NS	NS	[b]

NS = not significant.

[a] Significant at 1% level.
[b] Significant at 5% level.

Source: Compiled from Taira, H. (1984). *J. Agric. Food Chem. 32*: 369–371. With permission.

I. Sorghum

Sorghum is another drought-tolerant crop used for food in Asia and Africa. In Nigeria, it accounts for about 50% of the total cereal production; in South Africa, 90% of the crop is used for home-brewed beer production. Because of its coarseness and the bitterness caused by tannin in bird-resistant varieties, it is used as animal feed in most other countries. The production, evaluation, and nutritional value of traditional sorghum-based foods were reviewed by Rooney et al. (1986). Breeding programs have created varieties with lower tannin contents and improved protein quality.

Sorghum contains rather low levels of total lipids (3.9%), with most occurring in the germ fraction (Table 12.12). Oleic and linoleic acids are 84% of the total fatty acids (Table 12.13), making the lipids highly unsaturated. The improved varieties appear to have less oleic acid than traditional varieties, although the amount of total unsaturated fatty acids is the same.

TABLE 12.12
Typical Distribution of Sorghum Lipids in Mill Fractions

Sample	Mill Yield	Fat Content (%)	% Total Fat
Whole kernel	100	3.2–3.9	100
Endosperm	80–86	0.2–0.8	4–16
Bran	5–12	5–22	5–22
Germ	7–12	20–30	43–92

Source: Adapted from Morrison, W.R. (1978). *Adv. Cereal Sci. Technol. 2*: 221–348. With permission.

TABLE 12.13
Typical Distribution of Sorghum Lipids in Mill Fractions

Sample Variety	16:0	18:0	18:1	18:2	18:3
Traditional	12	1	34	50	3
Improved	14	1	24	59	3

Source: Adapted from Hoseney, R.C., Varriano-Marston, E., and Dendy, D.A.V. (1981). *Adv. Cereal Sci. Technol. 4*: 71–144; Osagie, A.U. (1987). *J. Agric. Food Chem. 35*: 601–604. With permission.

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13 Fatty Acids in Fermented Food Products

Sue Joan Chang and Ching Kuang Chow

CONTENTS

I. INTRODUCTION

Fermentation is the chemical process that converts carbohydrates or sugars into alcohols or acids by microorganisms. The science of fermentation is known as zymology. As fruits and many foods ferment naturally, fermentation actually precedes human history. For centuries, humans have employed the fermentation process to produce and preserve many types of food products. Almost all cultures and ethnic groups have developed some typical forms of fermented foods.

Fermentation as part of food processing has been used for preservation, liberation of predigested nutrients, and production of alcoholic beverages (Solomons, 2002). By using special ingredients and carefully controlling temperature, pH, and other conditions of fermentation, the process allows the growth of "good" microorganisms in raw materials, while it prevents the growth of spoilage-causing microorganisms. Some common fermented food products and their respective ingredients, as well as microorganisms involved are shown in Table 13.1.

The common microorganisms involved in the fermentation process of food products are lactic acid bacteria, yeasts, and molds. In the making of yogurt and pickle vegetable, lactic acid bacteria (*Lactobacillus delbruckii* ssp. *Bulgaricus* and *Streptococcus salivarius* ssp. *Thermophius*) contribute to flavor, shelf life, structure, and consistency. During the production of wine and beer, yeasts (*Saccharomyces cerevisiae*) convert sugars into fruit juice and grains and produce ethanol, CO_2

TABLE 13.1
Some Fermented Products and Raw Materials

Product	Major Ingredients	Functional Microorganisms
Yogurt	Cow's milk	Lactic acid bacteria
Cultured milk	Cow's milk	Lactic acid bacteria
Sauerkrauts	Cabbage	Lactic acid bacteria
Picked olives	Olives	Lactic acid bacteria
Cheese	Cow's milk	Molds and/or lactic acid bacteria
Bread or sourdough bread	Wheat or rye flour	Yeasts and lactic acid bacteria
Yeast-leavened bread	Wheat flour	Yeasts
Lager beer	Barley, hops	Yeasts
White wine	Grapes	Yeasts
Sherry	Grapes	Yeasts
Soy sauce	Soybeans and wheat	Yeasts, molds, and lactic acid bacteria
Tempeh	Soybeans	Yeasts, molds, and lactic acid bacteria

and flavor, and molds (*Aspergillus sojae*) form proteolytic and saccharolytic enzymes, enabling solubilization and flavor production in soy sauce making. Also, bread is made by mixing dough with yeasts, which digest sugars (derived from starches in dough) and produce CO_2, causing the dough to rise.

The benefits of food fermentation include (1) enriching the diet through development of a diversity of flavors, aromas, and textures in food substrates; (2) preserving substantial amounts of food through lactic acid, alcoholic, acetic acid, and alkaline fermentations; (3) enriching food substrates with protein, essential amino acids, essential fatty acids, and vitamins biologically; (4) producing important nutrients or eliminate antinutrients, and making conditions unsuitable for undesirable microorganisms during food-fermentation processing; and (5) decreasing cooking times and fuel requirements (Steinkraus, 1995). Also, by converting lactose to lactic acid during the production of cheeses, yogurts, and acidified milk, the suffering of lactose intolerance by those who have insufficient expression of lactase on the intestinal mucosa can be alleviated (Solomons, 2002). The fatty acids in the fermented drinks, and food products of plant and milk origin, are the focus of this chapter.

II. FERMENTED FOOD PRODUCTS

Fermented foods of plant origin are derived from a variety of raw materials of different chemical composition and biophysical properties. Cereal grains and potatoes have high starch content, legumes and oil seeds have a high protein content, green vegetables have high moisture content, and fruits contain high concentration of reducing sugars. Milk is the main source of fermented foods of animal origin, although meat and fish have also been preserved to prolong the shelf life and to generate certain flavors, and a number of animal-based foods from different parts of the world are labeled as "fermented." However, the term "fermented" is scientifically incorrect because meats (beef, pork, goat, chicken, fish, seafood, etc.) are composed of mainly water, proteins, and fats, and contain very low lactic acid fermentable carbohydrates. Although acidification or fermentation by lactic acid bacteria in meats may be facilitated by adding sugar or starchy foods, the operative processes in the transformation undergone by these animal foods are essentially putrefaction and rancidification. Putrefaction is a general decomposition process of animal proteins, especially by anaerobic microorganisms (Ogielski et al., 1953), and the process usually results in the formation of amines such as putrescine and cadaverine, which have a putrid odor. Rancidification is the decomposition of fats and other lipids by oxidation. Rancid foods and oils develop highly reactive chemicals that produce

unpleasant and obnoxious odors and flavors, and destroy nutrients in food. The end products of animal-based foods resulting from these processes are quite different from those of fermentation, and are often dangerous for human consumption. Accordingly, the fatty acids in the fermented drinks and fermented food products of plant and milk origin are the focus of this chapter.

A. Fermented Drinks

A large number of fermented beverages, such as meads, wines, beers, ciders, vinegar, and tea, are available. As the majority of fatty acids present in the raw materials is consumed or converted to other compounds during fermentation, fermented drinks contain very little fats on a weighed or volume basis. Except for wines and beers, the information concerning fatty acids in fermented drink is rather limited.

a. Wines and Beers

A typical wine is composed of roughly 85% water, 12% ethyl alcohol, and 1% of tartaric, malic, and several other acids. Wine also contains various sugars and carbohydrates, less common alcohols, aromatic aldehydes, ketones, phenolics, enzymes, pigments, vitamins, and some minerals. There are over 300 separate ingredients identified in wine so far, and many other substances are yet to be identified. Most of these elements lend complexity to wine flavors with insignificant nutritional impact on the diet. There are, however, compounds in wine, such as catechins, flavinoids, resveratrol, and quercetin, which may have prophylactic effects against human diseases. A typical beer contains 3%–7% alcohol, over 90% of water, approximately 2% mono- and disaccharides, 1% polysaccharides, and small amounts of proteins, amino acids, minerals, and traces of vitamins. Wines and beers can be a significant source of calories from alcohol and carbohydrates. The caloric content of wines and beers varies between types, depending on color, alcoholic strength, and residual sugar. A 6-oz glass of dry wine (12.5% alcohol) contains about 150 kcal, which is close to a 6-oz glass of beer (110–120 kcal).

Wines and beers contain very little fats. The bulk, if not all, of fatty acids, especially unsaturated ones, originally present in fruit juice or grains are utilized for the growth and survival of yeasts during the fermentation process (Ancin et al., 1996, 1998; Guilloux-Benatier et al., 1998). During the first half of fermentation (50% of the sugar is used up) in Garnacha must, for example, practically all the fatty acids are consumed, with the exception of some medium-chain fatty acids. During the second half of fermentation, 80.1% of the fatty acids are consumed, with 28.8% of the remaining fatty acids being used up during aging. Similarly, during the first half of fermentation in Viura must, 46.9% of the total fatty acid concentration is used, with high consumption of unsaturated long-chain fatty acids (72.3%). During the second half of fermentation, 77.2% of the fatty acids are used, with high consumption of the long-chain saturated and unsaturated acids.

Fatty acids in the medium are taken up by cropped brewer's yeast and incorporated into cellular lipid fractions (Moonjai et al., 2003). The polar lipids of white and red table wines, dessert, and fortified wines account for 30%–40% of total lipids, and about 90% of the polar lipids consist of glycolipids and the minor portion is represented by phospholipids (Zherebin et al., 1981). During the aging of wine, medium-chain fatty acids are excreted by yeasts, and a small amount of unsaturated acids is consumed (Ancin et al., 1998). Biosynthesis of fatty acids by yeasts during alcohol fermentation is influenced by oxygen availabilities on grape must (Maurico et al., 1998). While long-chain fatty acids (C16:0 to C18:3) induce a positive effect on yeast growth and cell viability, medium-chain fatty acids (C10:0 and C12:0), especially in their esterified forms, may be toxic to *Oenococcus oeni* (Guilloux-Benatier et al., 1998).

The levels and types of fatty acids present in wines vary greatly depending on the raw materials and ingredients used, fermentation time and temperature, and type of yeasts used (Torija et al., 2003; Gomez et al., 2004). For example, palmitic acid, myristic acid, and lauric acid are the dominant fatty acids identified in commercial red wines, and the total fatty acids differ considerably (Yunoki et al., 2004). Depending on yeast population and fermentation kinetics and conditions, fatty acids

not found in grape must, such as tridecanoic acid and myristoleic acid, are detected in white wines (Varela et al., 1999). During the production of malt whisky, unsaturated fatty acids are converted to precursors of γ-lactones via hydroxy fatty acids produced by lactic acid bacteria during production (Wanikawa et al., 2000). 10-Hydroxystearic acid and 10-hydroxypalmitic acid are produced from oleic acid and palmitoleic acid, respectively, and then converted to γ-decalactone and γ-dodecalactone by yeast. Both γ-decalactone and γ-dodecalactone contribute to the sweet and fatty flavor, and quality of malt whisky.

Lipase, which is present in the grain of both rice and barley, catalyzes the hydrolysis of triacylglycerides at a lipid–water interface to yield free fatty acids. In brewing, free fatty acids are generally associated with negative effects, including the formation of stale or off-flavor aldehydes. Malt and rice lipases in the insoluble portion of the mash are quite thermostable, suggesting hydrolysis of triacylglycerides, and liberation of free fatty acids may continue through much of mashing (Schwarz et al., 2002). Also, the amount of lipid in barley decreases during malting and changes in the composition of lipid classes are minor, but the amount of free fatty acids increases, which is indicative that lipoxygenase is active during malting (Kaukovirta-Norja et al., 1993).

White must and wine fatty acids are present mainly in the free form or esterified as ethyl esters, and both contribute to flavor and foam properties of wine (Gallart et al., 1997). The free fatty acids, C8:0, C10:0, and C12:0, are negatively correlated with foamability, whereas the ethyl esters of hexanoic, octanoic, and decanoic acids are positively related, and the value of foamability is directly proportional to the ratio of esterified to unesterified fatty acids (Gallart et al., 2002). Fatty acids C6:0 to C10:0 have no impact on the foam stability, but C12:0, C14:0, C18:1, and C18:2 reduce the foam stability and the surface elasticity of beer. Also, C16:0 and C18:0 damage the foam very effectively, but do not influence the surface rheology (Wilde et al., 2003). The foam-damaging effect value of a free fatty acid is related to its extent of adsorption on a foam cell surface, and the addition of free fatty acid increases the foam-damaging effect of beers by decreasing the rate of foam thickness. Di- and trihydroxyoctadecenoic acids derived from linoleic acid are the decisive compounds determining the total foam-damaging effect value of the fatty acids in beers (Kobayashi et al., 2002). As di- and trihydroxyoctadecenoic acids decrease foam adhesion of beers, measurement of these hydroxyl fatty acids can be used to control various mashing conditions or the foam behavior of beer.

The release of lipids during the aging of sparkling wines in contact with yeast influences wine sensory attributes and, especially, foam characteristics. Pueyo et al. (2000) monitored the release of the different classes of lipids during the autolysis of three commercial yeast strains and found that a release of triacylglycerols, 1,3-diacylglycerols, 2-monoacylglycerols, free fatty acids, sterol esters, and sterols, but not phospholipids, during the first 2 days, a period that corresponded to the maximum loss of yeast viability. They also found that the mean lipid content in the autolysates was 8.6% for sterol esters, 3.8% for sterols, 2% for triacylglycerols, and <2% for 1,3-diacylglycerols and free fatty acids. The presence of C18:2 in brewing yeast can be used to distinguish these from closely related yeast species in the brewing industry (Morakile et al., 2002).

Similar to wines, very little of the lipid present in the raw materials survives the brewing process, and less than 0.04% of lipid remains in the finished beer (Anness and Reed, 1985). Spent grains, hot break, and cropped yeast are all effective means of removing lipids. Fatty acids of C14:0–C18:0 in beer are reduced during incubation at 60ºC, and the degradation of C18:1, C18:2 is accelerated by the addition of ferrous ion and is inhibited by ethylenediaminetetraacetic acid (EDTA), suggesting a role of free radical reaction in the degradation of these unsaturated acids (Kaneda et al., 1990).

Methylketones present in the headspace of Cognac are partly responsible for the desirable and complex characteristic called "rancio charentais" or "Cognac rancio," which is found in grape brandies aged in oak barrels for several decades. The ketones, 2-heptanone, 2-nonanone, 2-undecanone, and 2-tridecanone, are formed through β-oxidation and decarboxylation of long-chain fatty acids originating from yeast metabolism (Watts and Butzke, 2003). The concentrations of these ketones increase with Cognac age classification in the 42 brandies analyzed, and 2-heptanone is present at the highest concentration in most samples. The average concentrations and rates of formation

decrease with increasing chain length. The esters propyl octanoate and ethyl octanoate follow the same trend as the methylketones and appear to play an additional role in the formation of the rancio character.

b. Cider

Apple cider is another important fermented drink. Portions of sugars in apple juice are initially fermented to alcoholic apple cider, and the alcohol is then converted into acetic acid, with the help of yeasts and bacteria. Except for water, apple cider mainly consists of alcohol and sugars (Finnish Food Composition Database, 2006). However, a very small amount of fatty acids are present in apple cider in the free or esterified form, and contribute to both the flavor and foam properties of cider. The major fatty acids in cider are caproic, caprylic, capric, and palmitic acids (Blanco-Gomis et al., 2001). Linear discriminant analysis of fatty acids in cider from the region of Asturias (Spain) suggests that palmitoleic, pentadecanoic, linoleic, myristic, and linolenic acids are the most predictive variables to differentiate ciders made from apples grown in the Asturias region and ciders made from apples grown outside this region. Also, oleic, linoleic, caprylic, capric, stearic, and palmitic acids are related to the sweet cider apple category, while pentadecanoic acid is related to the sharp class (Blanco-Gomis et al., 2002). In aged cider, the concentrations of volatile components in distillates such as diethyl butanedioate, ethyl 3-methylbutanoate, ester of long-chain fatty acids, and 1-hexanol are increased, while the acetate esters and long-chain fatty acids are decreased (Mangas et al., 1996).

B. Fermented Plant Products

Fermented soy products are the most important fermented food products of plant origin. Soybeans contain high protein and fat and relatively low carbohydrates. The most abundant fatty acids in soybeans are linoleic acid (54%), oleic acid (23%), and palmitic acid (16%), although the lipid content and fatty acid composition in soybeans vary considerably depending on the season, maturity, and genetic factors (see Chapter 16 by Hammond, this book). The major fermented soy products include miso, soy sauce, soy yogurt, tempeh, and natto. Other ingredients, such as rice and barley, have been employed for miso making. Also, a large number of pickle vegetables are available commercially.

a. Miso

Miso is a rich, salty condiment that characterizes the essence of Japanese cooking, and is popularly used to prepare miso soup and to flavor a variety of foods in other meals. Miso is a rich source of proteins, fiber, minerals, vitamins, and isoflavones. There are many variations of miso, which are all basically made from koji. Koji are grains (mainly rice, but also barley) or soybeans that are mainly fermented with *Aspergillus oryzae* molds, which break down the proteins and carbohydrates, and aged in wooden kegs. The color of miso ranges from creamy white, red to cocoa-brown, and the texture and taste of these variations are just as diverse. Differences in ingredients and variations in length of aging produce different types of miso that vary greatly in flavor, texture, color, and aroma. The most common ones are red miso, white miso, barley miso, and soybean miso.

Red miso is made from white rice, barley, or soybeans, and the natural fermentation takes 1–3 years. Red miso contains the highest levels of protein of all types of miso, and is used for stir-fries, soups, and stews or to marinade meat, poultry, and vegetables. White miso is made by using more rice koji (about 60%) than soybeans in only a few weeks, and can be used as ingredient to make red miso. White miso has a relatively short shelf life, and contains the most carbohydrates and, therefore, tastes the sweetest and has smooth texture. Barley miso is made from barley grains, soybeans, and barley koji, and is naturally fermented for 1–3 years. Barley miso is dark in color and salty, but is rich in taste, and is used for seasoning rich soups, stews, beans, sauces, and spreads. Soybean miso is made from soybean, and the koji is produced from soybeans. Soybean miso has low carbohydrate

TABLE 13.2
Nutritive Values of Soy Miso[a]

Content	g/100 g
Proteins	11.8
Carbohydrates	33.4
Total fat	6.1
Saturated fatty acids	0.9
C16:0	0.6
C18:0	0.3
Monounsaturated fatty acids	1.3
C18:1	1.3
Polyunsaturated fatty acids	3.4
C18:2	3.0
C18:3	0.4

[a]Data are derived from the United States Department of Agriculture Nutrient Database for Standard Reference (1998).

content resulting in a very long fermentation period (>1 year). A special type of soybean miso called "Hatcho miso" is reddish-brown, and is often used to flavor hearty soups. Hatcho miso is normally aged for at least 16 months, and is considered the best miso. The koji for Hatcho miso contains a special mold, *Aspergillus hatcho*, instead of the usual *A. oryzae*.

During maturation of fermented rice-koji miso, triacylglycerol is gradually decomposed into free fatty acid, with distinct formation of fatty acid ethyl esters. Six months after the start of fermentation, the esters, constituted about 50% linoleic acid and 20% oleic acid, account for 35.0% of total lipid, and have no appreciable change in this proportion being observed during maturation (Yamabe et al., 2004). It is thus possible to monitor the maturation of the rice-koji miso by following up the increase with time in fatty acid ethyl ester.

As can be expected, the composition of miso varies greatly depending on the ingredients used and fermentation conditions employed. The nutritive values of a typical soy miso are shown in Table 13.2. Among the total fat (6.1% by weight) in miso, approximately 50% is attributed to linoleic acid (C18:2) and 20% to oleic acid (C18:1). The other significant fatty acids are palmitic acid (C16:0), stearic acid (C18:0), and linolenic acid (C18:3).

b. Soy Sauces

Soy sauce is a dark brown liquid made from fermented soybeans, and is the foundation of oriental cuisine. It is the essential ingredient and the most important condiment used to flavor food and also used to cook with. The soybean fermentation involves a special koji (*A. oryzae*), which converts soy proteins, starches, and fats into amino acids, simple sugars, and fatty acids. Most commercial soy sauces, however, are now made by a chemical process in which cereals and soybeans are mixed with acids. Soy sauce can be stored at room temperature for up to 1 year. However, soy sauce contains a lot of salt, and should be used sparingly to flavor dishes. The nutritive values of a typical soy sauce are shown in Table 13.3. As shown in the table, source sauce contains less than 0.1% of total fats, and 50% of the fatty acids are polyunsaturated.

c. Soy Yogurt

Yogurt production was invented probably by an accident by Balkan tribes thousands of years ago. Soy yogurt is made by fermenting soymilk (water extract of grinded soybean) with friendly

TABLE 13.3
Nutritive Values of Soy Sauce[a]

Content	g/100 g
Water	71.9
Protein	5.2
Carbohydrates	8.5
Fiber	0.8
Ashes	15.1
Total fat	0.08
Saturated	0.01
Monounsaturated	0.01
Polyunsaturated	0.04

[a]Data are derived from the United States Department of Agriculture Nutrient Database for Standard Reference (1998).

TABLE 13.4
Nutritive Values of Soy Yogurt[a]

Content	g/100 g
Water	89.0
Protein	4.7
Carbohydrates	3.2
Fiber	0.2
Total fat	2.7
Saturated	0.5
Monounsaturated	0.6
Polyunsaturated	1.6

[a]Data are derived from the United States Department of Agriculture Nutrient Database for Standard Reference (1998).

bacteria, mainly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. During fermentation, the carbohydrates are converted by the bacteria to lactic acid, which causes the characteristic curd to form, and the bacterial enzymes partially digest the proteins making them easier for absorption. The lactic acid also restricts the growth of food poisoning bacteria. The process is similar to the production of yogurt from cow milk. In some countries, soy yogurt is labeled as "cultured soy milk" because the term "yogurt" is reserved for milk products. Some countries only allow using the name "yogurt" for products that have not been pasteurized to kill the bacteria after fermentation. The nutritive values of a typical soy yogurt are shown in Table 13.4. As expected the total fat content of soy yogurt is low (approximately 2.7%), and over 60% of fatty acids are polyunsaturated, and less than 20% are saturated.

d. Tempeh

Tempeh is a cultured soy food made by removing the hull of cooked soybeans, mixing with a culture, and aging for a day or two. The culture by yeasts, molds, and lactic acid bacteria helps hold the soybean in a cake form. Tempeh can also be made with added ingredients such as vegetables

TABLE 13.5
Nutritive Values of Tempeh[a]

Content	g/100 g
Water	54.9
Protein	19.0
Carbohydrates	17.0
Fiber	4.8
Ash	1.4
Total fat	7.7
Saturated fatty acids	1.1
Monounsaturated fatty acids	1.7
Polyunsaturated fatty acids	4.3

[a]Data are derived from the United States Department of Agriculture Nutrient Database for Standard Reference (1998).

or grains. Since tempeh is made from whole soybeans, it is a rich source of proteins and fiber, and a good source of many nutrients including B-vitamins and isoflavones. The natural tempeh fermentation process also makes the product more digestible. The nutritive values of a typical tempeh are shown in Table 13.5. The fatty acid composition is similar to that of soybean, with over 50% of fatty acids are polyunsaturated.

e. Natto

Natto is a traditional and popular fermented soy food in Japan, and has contributed to the nutrition, health, and well-being of the Japanese for many centuries. Traditional Japanese natto is essentially made by soaking and cooking whole soybeans until they are tender. The beans are then fermented for 14–18 h with *Bacillus* strain bacteria (Wei et al., 2001). The nutritive value of a typical natto is shown in Table 13.6. Natto contains about 11% of fat and over 50% of water. The lipid composition of natto is similar to that of raw soybean, except that much of the fatty acids are liberated from triacylglycerols. For example, free fatty acids account for 5%–18% of total lipids in Yukiwari-natto and 78% in Hama-natto (Kiuchi et al., 1976).

f. Pickle Vegetables

During the fermentations of vegetables, the conversion of carbohydrates to acids, alcohol, and carbon dioxide by the lactic acid bacteria constitutes the most important changes (Vorbeck et al., 1963). Changes in lipid composition also occur during fermentation. These changes are responsible for the unique texture, flavor, and color of fermented products. In addition, the lactic acid produced by the dominant bacteria inhibits the growth of all other microorganisms. Additionally, by consuming spoilage-sensitive parts of the food and releasing chemicals as by-products, the "good" microorganisms help preserving the vegetables, and generating the characteristic flavor and texture of the end products.

Same as other types of fermentation, marked changes occur in all lipid fractions during fermentation of dill pickle. The most striking difference is the decrease in the phospholipid fraction, and marked increases in free fatty acids and fatty acid esters. Pederson et al. (1964), for example, have identified 41 esters in dill pickle. Interestingly, marked increases of linoleic and linolenic acids occur in good pickle, while oleic acid is increased in bloated pickles. Also, tridecenoic acid that is present in cucumber is absent in pickles, and caproic, caprylic, and capric acids normally absent in

TABLE 13.6
Nutritive Values of Natto[a]

Content	g/100 g
Water	55.0
Protein	17.7
Carbohydrates	14.4
Fiber	5.4
Ash	1.9
Total fat	11.0
Saturated fatty acids	1.59
C14:0	0.03
C16:0	1.17
C18:0	0.39
Monounsaturated fatty acids	2.43
16:1	0.03
18:1	2.40
Polyunsaturated fatty acids	6.21
18:2	5.48
18:3	0.73

[a]Data are derived from the United States Department of Agriculture Nutrient Database for Standard Reference (1998).

TABLE 13.7
Nutritive Values of Sauerkraut[a]

Content	g/100 g
Water	91.6
Protein	1.1
Total carbohydrates	7.1
Total fat	0.2
Total fatty acids	0.2
Total free fatty acids	0.2
Saturated	<0.1
Monounsaturated	<0.1
Polyunsaturated	<0.1
C18:2	0.03
C18:3	0.04

[a]Data are derived from the Finnish Food Composition Database (2006).

cucumbers are found in pickles. The hydrolytic alterations of lipids in pickled cucumber are analogous to that of sauerkraut fermentation by lactic acid bacteria (Vorbeck et al., 1963). The marked decrease in phospholipids during fermentation suggests their utilization by the bacteria (*Bacterium acetylcholine*) in cell protoplasma (Stephenson and Rowatt, 1947).

Similar to fresh vegetables, the fat content of fermented vegetable products is very small. For example, less than 0.2% of fat (about 50% saturated and 50% unsaturated) is found in pickle cucumber or dill. The nutritive value of a Finnish sour cabbage (sauerkraut) is shown in Table 13.7. Total fat in sauerkraut accounts for only 0.2% or less, while water accounts for the vast majority of the

weight or volume. The fatty acids in sauerkraut are free forms, and the major polyunsaturated fatty acids detected are linoleic and linolenic acids.

C. Fermented Milk Products

Among the fermented food products from animal origin, those made from milk are most important. Milk is the normal product of mammalian gland secretion. The average milk from cows consists of 88% water, 3.2% protein (mainly casein), 4.7% lactose (milk sugar), 0.74% minerals, and 3.4% fat. Milk fat consists of triacylglycerides (98%), diacylglycerides (0.25%–0.48%), monoacylglycerides (0.02%–0.04%), phospholipids (0.6%–1.0%), cholesterol (0.2%–0.4%), glycolipids (0.006%), and free fatty acids in milk (0.1%–0.4%).

The fatty acid composition of milk varies substantially among mammalian species, and diet plays a major role in modulating the fatty acid composition of milk in cattle (Jensen, 2002), goats (Chilliard et al., 2003), and sheep (Bocquier and Caja, 2001). The milk from ruminant animals, cow, sheep, and goat, contain much higher concentration of short-chain fatty acids (C4:0 and C6:0) than that from nonruminant animals. Milk fat from cow contains roughly 66% saturated fat, 4% polyunsaturated fat (mainly C18:2), and 30% monounsaturated fat (C18:1), and is greatly affected by seasonal variations. Goat's milk contains higher short- (17%) and medium-chain (32%) fatty acids, and lower monounsaturated (17%) fatty acids as compared to that of cow's milk. Also, the seasonal variation in fatty acid levels—goat's milk fat is lower than that of the cow's milk. The grazing conditions for dairy cattle significantly alter the proportion in milk and dairy products of conjugated linoleic acid isomers (Dhiman et al., 1999; Lock and Garnsworthy, 2003). Milk originating from cows, buffaloes, sheep, and goats has high moisture content and a neutral pH.

In general, the nutrient content of cultured or fermented dairy foods is similar to that of the milk from which these products are made. However, factors such as the type and strain of bacteria, milk (whole, low fat, nonfat) used, fermentation conditions, storage, and other treatments such as the addition of milk solids-not-fat, sweeteners, and fruits can influence the nutrient composition of cultured and culture-containing dairy foods. The probiotic bacteria used in commercial production of dairy products are mainly members of the genera *Lactobacillus* and *Bifidobacterium*. *Lactobacillus* species from which probiotic strains have been isolated include *L. acidophilus*, *Lactobacilllus johnsonii*, *Lactobacilllus casei*, *Lactobacilllus rhamnosus*, *Lactobacilllus gasseri*, and *Lactobacilllus reuteri*. *Bifidobacterium* strains include *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis* (Heller, 2001). A major aspect of the production of probiotic fermented dairy products is the interaction between probiotics and starter organisms. Acidification of milk by lactic acid bacterial fermentation, which reduces pH values to <4.5, inhibits the survival and growth of spoilage-causing or disease-associated bacteria. The principal fermented milk products are cheese and yogurt.

a. Cheese

Cheese, a concentrated dairy food made from milk, is the product obtained by draining the whey after coagulation of casein by acid produced by select microorganisms and/or by coagulating enzymes resulting in curd formation. Milk may also be acidified by adding food-grade acidulants in the manufacture of certain varieties of cheese, such as cottage cheese (Kealey and Kosikowski, 1986). Almost all cheese manufactured in the United States is made from cow's milk, although other sources of milk (sheep, goat) have been used in cheese making. Cheese may be unripened (fresh) or ripened (matured). In unripened cheeses such as creamed cottage and cream cheeses, the curd separated from the whey can be used immediately. In ripened cheeses such as Cheddar and Swiss cheese, the curd is further treated by selected strains of bacteria, molds, and yeasts, and ripening agents, resulting in a cheese of a specific flavor, texture, and appearance (Kosikowski and Mistry, 1997a,b; Mistry and Maubois, 2004). Fermentation of milk by lactic acid bacteria, and occasionally

by *propionibacteria*, yeasts, and molds, results in a variety of cheese. Cheeses may also be categorized according to manufacturing procedures such as the method by which the curd is formed (by acid and/or coagulating enzyme), the ripening agent (bacteria, mold, yeast, unripened), or according to rheology, or softness and hardness.

Cheese is an important source of high-quality proteins, vitamins, and minerals. The main protein in cheese is casein, although some lactalbumin and lactalglobulin may also be present, depending on the amount of whey entrapped in the cheese. Fermentation of milk by lactic acid bacteria, and occasionally by *propionibacteria*, yeasts, and molds, results in a variety of cheese. Lactic acid in turn acts with the added enzyme rennet to curdle the milk. Separation of the curd from the whey in cheese making causes a significant partition of nutrients and a considerable change in the nutrient content of cheese compared to that of the original milk. Milk's water-insoluble components, which are primarily retained in the curd, are concentrated in cheese. As about 5 quarts of milk are needed to make 1 pound of milk cheese and 9 pounds of whey, most ripened cheeses contain about ten times the amount of water-insoluble components as in milk. However, as most of milk's water-soluble constituents remain in the whey, these nutrients are lower in cheese than in milk.

The amount of various nutrients retained in the curd and whey largely depends on the type of cheese being manufactured, the type of milk (whole, reduced fat, nonfat) or whey used, the manner of coagulation (enzyme or acid coagulated), and ripening. The chemical composition of the end products is responsible for the characteristic odor and flavor of the finished cheese. As to be expected, the fat content of cheeses varies widely depending on the type of milk and milk products used to make cheese. Cheeses such as Cheddar, Brie, Blue, Limburger, Muenster, Gouda, and Swiss are generally made from whole milk and have about the same amount of fat and protein. A low-fat cheese has a higher protein-to-fat ratio. A small amount of polyunsaturated fatty acids (C18:2 and C18:3) is present in cheese.

The nutritive values of a 50% fat in dry matter Brie cheese are shown in Table 13.8. Brie is a soft cow's milk cheese named after Brie, the French province in which it originated. The majority of fatty acids in Brie cheese is saturated (C14:0, C16:0, and C18:0), and the major monounsaturated fatty acid is oleic acid (C18:1). The quantity of polyunsaturated fatty acids (C18:2 and C18:3) is relatively small.

Emmental is a yellow, medium-hard cheese, with characteristic large holes. Three types of bacteria, *Streptococcus thermophilis*, *Lactobacillus*, and *Propionibacter shermani*, are used in the production of Emmental. In the later stages of cheese production, *Propionibacter shermani* consumes the lactic acid excreted by the other bacteria and releases carbon dioxide, which slowly forms the bubbles that make holes. The nutritive value of Emmental (Swiss) cheese is shown in Table 13.9. Similar to that of Brie cheese, the majority of fatty acids present is saturated, and monounsaturated fatty acids account for about 25% of total fatty acids. Linoleic acid is the major polyunsaturated fatty acid, and almost 1% of the total fatty acids are of the *trans* form.

Seasonal variations in feeding condition are responsible for the variations in fatty acid composition of "Ossolano," a semihard cheese (Zeppaa et al., 2003). Long-chain mono- and polyunsaturated fatty acids are more abundant in the summer cheeses while short- and medium-chain saturated fatty acids are higher in winter products. The ratio of saturated to unsaturated fatty acids is thus lower in summer cheeses as compared with that of winter cheeses. Also, conjugated linoleic acids, n-3 and n-6 acids are higher in summer cheeses. The isomer of conjugated linoleic acid, C18:2 *cis*-9, *trans*-11 (rumenic acid), has potential anticarcinogenic and antiatherogenic property (see Chapter 34 by Huang et al., this book). Conjugated linoleic acid occurs in food as a result of microbial reactions, free radical-type oxidation, and heat treatment. Conjugated linoleic acid in the cheeses varied from 5.3 to 15.80 mg/g of cheese fat, and is dependent primarily on the origin of the milk (season, geography) and the production process (Lavillonniere et al., 1998). Addis et al. (2005) evaluated the effect on milk and cheese fatty acid compositions of feeding different fresh forages to dairy sheep and found that by using appropriate fresh forage-based regimens, the fatty acid profile of sheep dairy may be manipulated to maximize the content of conjugated linoleic acid and vaccenic acid.

TABLE 13.8
Nutritive Values of Brie Cheese (50% Fat in Dry Matter)[a]

Content	g/100 g
Water	49.3
Protein, total	19.8
Carbohydrate, total	0.2
Ash	2.7
Fat, total	27.8
Saturated fatty acids	18.0
C4:0	0.95
C6:0	0.60
C8:0	0.39
C10:0	0.87
C12:0	1.08
C14:0	3.07
C16:0	8.22
C18:0	2.81
Monounsaturated fatty acids	7.5
C14:1	0.39
C16:1	0.60
C18:1	6.17
C20:1	0.32
Polyunsaturated fatty acids	0.8
C18:2	0.58
C18:3	0.21

[a]Data are derived from the Danish Food Composition Databank (2006).

TABLE 13.9
Nutritive Values of Emmental Cheese[a]

Content	g/100 g
Water	39.1
Protein, total	27.9
Carbohydrates, total	3.0
Fat, total	30.0
Fatty acids, total	29.3
Fatty acids, free	28.1
Fatty acids, saturated	18.9
Fatty acids, monounsaturated	7.4
Fatty acid, polyunsaturated	0.8
18:2n-6	0.456
18:3n-3	0.131
Fatty acids, *trans*	0.9

[a]Data are derived from the Finnish Food Composition Database (2006).

The relative abundance of normal-chain free fatty acids in Cheddar-like hard goat cheeses, from greatest to least, is C10:0, C12:0, C8:0, C6:0, C4:0, C9:0, and C11:0 (Attaie and Richter, 1996). Similarly, the relative abundance of branched-chain fatty acids in Cheddar-like hard goat cheeses, from greatest to least, is 4-methyloctanoic, methyl-decanoic, 3-methylbutanoic, and 4-ethyloctanoic acids. These compounds have intense aromas, and even minute quantities can affect the flavor of dairy products. Also, ripening time significantly affects the concentrations of free fatty acids present. The concentrations of free fatty acids are increased during the initial 12 weeks of aging and remain relatively constant for the rest of the 24-week ripening period.

Cheese flavor is obtained through a series of chemical changes that occur in the curd during the early stages of ripening. Lipid hydrolysis leads to the formation of free fatty acids that serve as substrates for further reactions. Peptides and amino acids, which result from proteolysis, also lead to aroma compounds through enzymic and chemical reactions (Molimard and Spinnler, 1996). During cheese ripening, a small amount of the fat is hydrolyzed to volatile fatty acids, butyric, caproic, caprylic, and capric acids and higher carbon chain fatty acids, which contribute to the flavor of cheese. A marked increase in concentration of short-chain free fatty acids occurs during cheese ripening, ranging from 0.35 to 9.33 mg/100 g for butanoic acid, from 0.363 to 4.34 mg/100 g for hexanoic acid, from 0.343 to 2.0 mg/100 g for octanoic acid, and from 1.291 to 3.85 mg/100 g for decanoic acid (Pinho et al., 2002). In Manchego cheese, the free fatty acids that increase most throughout ripening are C4:0, C6:0, and C8:0, and the free fatty acids that are best correlated with ripening time are the short-chain fatty acids (Poveda et al., 1999).

Ripening time affects the content of free fatty acids of cheese. Total amounts of free fatty acids in Idiazabal cheese after 180 days of ripening, for example, are significantly higher in winter than in spring or summer. The major free fatty acids in winter are C10:0, C4:0, C18:1, and C16:0, and the amounts decrease as the cheese-making period progressed from winter to summer with C10:0 exhibiting the largest decrease and C18:1 the smallest decrease (Chávarri et al., 1999). The amounts of free fatty acids shorter than C12:0 are significantly higher in winter than either in spring or summer, and the percentage of volatile (C4–C8), medium (C10–C14), and long-chain free fatty acids (≥C16) changes during 180 days of ripening. Similarly, Macedo and Malcata (1996) monitored the changes in the concentrations of the free fatty acids in Serra cheese over a ripening period of 35 days throughout the cheese-making season, and found that the concentration of stearic acid increased significantly only during the first week of ripening, while palmitic and oleic acids increased significantly during all stages of ripening. They also found that the concentrations of butyric, caprylic, lauric, myristic, and linoleic acids were approximately constant during the first week and increased significantly thereafter, and the concentrations of butyric, caproic, and palmitic acids in cheeses manufactured in February were statistically lower than those in cheeses manufactured in May. The principal free fatty acids throughout ripening are butyric, capric, palmitic, stearic, and oleic acids.

Milk fat of sheep and goat contained significantly more 4-methyloctanoic and 4-ethyloctanoic acids than cow's milk fat. It is, therefore, not surprising that Pyrenees sheep's milk cheese contains significant amounts of methyl- and ethyl-substituted phenols, which contribute the characteristic sheep-like flavor notes to this cheese variety (Ha and Lindsay, 1991).

b. Cottage Cheese

Cottage cheese is a creamy, lumpy acid curd cheese, relying on the natural tendency of warm milk to curdle (no use of rennet). Once the floppy curd is formed, it is cut into pieces and heated gently in whey until it reaches the desired texture. After the whey is removed, the cheese is allowed to ripen for 1 or 2 days. Cottage cheese is an excellent source of calcium, phosphorus, protein, and vitamins. Cottage cheese contains live cultures of *Acidophilus* and *Bifidus*, which are probiotics. As cottage cheese has only trace amounts of lactose, it may not cause an intolerance problem.

Cottage cheese comes in several forms, including small-, medium-, and large-curds. Creamed cottage cheese is produced by adding 4%–8% cream dressing fermented by *B. infantis* to the dry curd,

TABLE 13.10
Nutritive Values of Hytteost Cottage Cheese[a]

Content	g/100 g
Water	79.8
Protein	12.2
Carbohydrates	1.5
Ash	1.4
Total Fat	5.4
Saturated fatty acids	3.50
C4:0	0.18
C6:0	0.12
C8:0	0.08
C10:0	0.17
C12:0	0.21
C14:0	0.60
C16:0	1.60
C18:0	0.55
Monounsaturated fatty acids	1.45
C14:1	0.08
C16:1	0.12
C18:1	1.20
C20:1	0.06
Polyunsaturated fatty acids	0.15
C18:2	0.11
C18:3	0.04
trans Fatty acids	0.23

[a] Data are derived from the Danish Food Composition Databank (2006).

and in low-fat cottage cheese, the cream is skimmed off. The minimum milk fat and maximum moisture content of most cheeses is governed by federal and state regulations. Low-fat and nonfat cottage cheese are now subject to the requirements of FDA's "general standard," which permits foods to be named by a defined nutrient content claim (e.g., low fat) and a standardized term (e.g., "cottage cheese"). Low-fat cottage cheese (0.5%, 1.0%, or 2% milk fat) must contain no more than 3 g total fat per serving (about 4 oz), and nonfat cottage cheese must contain less than 0.5 g total fat per serving. The nutritive value of Hytteost cottage cheese with 20% fat in dry matter is shown in Table 13.10. Over 60% of the total fatty acids are saturated, with C16:0 accounts for about half. Oleic acid is the major monounsaturated fatty acid, and the polyunsaturated fatty acids detected are C18:2 and C18:3. Also, significant amounts of *trans* fatty acids are present in cottage cheese. Essentially all fatty acids in cottage cheese are in free form (Finnish Food Composition Database, 2006).

c. Yogurt

Yogurt, an *acidophilus* cultured food (cow's milk), has been eaten for centuries all over the world. Because of the acidic nature, it helps absorption of iron and supports the growth of valuable intestinal bacteria. Recently, yogurt was allowed to claim to meet all or part of the meat/meat alternate requirements for child and adult nutrition programs because of the high-nutrient value of this dairy food. Yogurt and cultured milk products are important sources of high-quality protein, calcium, phosphorus, magnesium, riboflavin, and vitamins A and B_{12}. The nutritive values of low-fat yogurt

TABLE 13.11
Nutritive Values of Yogurt (g/100 g)

Content	Low Fat Yogurt[a]	Natural Yogurt[b]
Water	85.06	87.8
Protein	5.27	3.8
Carbohydrates	7.06	3.8
Ash	1.78	0.8
Total fat	1.79	3.6
Saturated fatty acids	1.00	2.34
C4:0	0.04	0.12
C6:0	0.03	0.08
C8:0	0.02	0.05
C10:0	0.04	0.11
C12:0	0.05	0.14
C14:0	0.16	0.40
C16:0	0.42	1.07
C18:0	0.15	0.36
C20:0	0.01	—
Monounsaturated fatty acids	0.42	0.97
C14:1	0.01	0.05
C16:1	0.03	0.08
C18:1	0.36	0.80
C20:1	—	0.04
Polyunsaturated fatty acids	0.04	0.10
C18:2	0.03	0.08
C18:3	0.01	0.03
trans Fatty acids	0.03	0.16

[a] Data are derived from the ESHA's Nutrition Databases (2006).
[b] Data are derived from the Danish Food Composition Databank (2006).

and natural yogurt are shown in Table 13.11. Similar to that of cheese and cottage cheese, and the majority of fatty acids in yogurt is saturated, and oleic acid accounts for about 25% of the total fat. Polyunsaturated (linoleic and linolenic acids) and *trans* fatty acids are also present in yogurt.

Milk, yogurt mix, and yogurt from cows fed with fish oil or fish oil and extruded soybeans diets have higher levels of conjugated linoleic acid, omega-3 fatty acids, and *trans*-vaccenic acid (Dave et al., 2002). Unsaturated fatty acids in the milk from cows fed with fish oil, fish oil and extruded soybeans, and extruded soybeans diets are also higher than that of the control diet. The processing of milk (85°C for 30 min) has no effects on fatty acids composition, and changes in fatty acids composition have no effects on the viable numbers of starter bacteria. As fermentation with yogurt and probiotic bacteria and storage does not alter the conjugated linoleic acid, *trans*-vaccenic acid, or omega-3 fatty acids, probiotic yogurt made from milk with increased conjugated linoleic acid and *trans*-vaccenic acid can be produced by changing the diets of cows. Most of the C18 fatty acids, including conjugated linoleic acid, are increased in the milk and cheese of cows fed extruded soybeans and cottonseeds, and processing into cheese does not alter the conjugated linoleic acid content (Dhiman et al., 1999). Similarly, supplementing soy oil or conjugated linoleic acid increases the conjugated linoleic acid contents of the milk and yogurt from Holstein cows (Boylston and Beitz, 2002). However, addition of conjugated linoleic acid to soy-oil-supplemented diets does not affect the conjugated linoleic acid and fatty acid composition of the yogurt.

III. SUMMARY AND CONCLUSION

Fermentation has long been employed to preserve foods, liberate nutrients, and make alcoholic beverages, and the common microorganisms involved in fermentation of food products are lactic acid bacteria, yeasts, and molds. Fermented foods of plant origin are derived from a variety of raw materials of different chemical composition and biophysical properties, and milk is the main source of fermented foods of animal origin. The nutritive value and fatty acids of fermented food products varies greatly depending on the raw materials and ingredients used and fermentation conditions employed.

Most of the fatty acids, especially long-chain and unsaturated ones, present in the fruit juices are utilized by yeasts and lactic acid bacteria during fermentation and aging of wine. The bulk of the fatty acids present in wines, beers, and cider are either in the free form or esterified as ethyl esters, and both contribute to flavor and foam properties. Major fermented food products of plant origin include miso, soy source, soy yogurt, tempeh and natto, and pickle vegetables, while cheese and yogurt are the major fermented products of animal origin. The fatty acid composition of fermented soy products is generally similar to their respective raw materials, except much of the fatty acids present are hydrolyzed to free fatty acids or form ethyl esters, which contribute to the characteristic flavor of the products. Fermentation of milk by lactic acid bacteria, and occasionally by *propionibacteria*, yeasts, and molds, results in a variety of cheese. The fat content of cheeses varies widely depending on the type of milk and milk products used to make cheese. During cheese ripening, a portion of the fat is hydrolyzed to volatile fatty acids and higher carbon chain fatty acids, which contribute to the flavor of cheese. Similarly, essentially all fatty acids in cheese are in free form, and the majority of the total fatty acids are saturated.

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14 Fatty Acid Content of Convenience Foods

Maria G. Boosalis

CONTENTS

Even though it is not an exact definition, the term *convenience* foods refers to foods that have been either processed or created de novo to make them more appealing to the consumer (Briggs and Calloway, 1979). These types of foods include mixes, snack foods, desserts, preprepared or frozen items, including entrees, to name a few. The convenience foods selected to appear in this chapter are grouped together by the type of food items (e.g., snacks or pudding and custards) or similarities in the type of food products (e.g., French toast, pancakes, and waffles or doughnuts and muffins) and then listed alphabetically by their specific groupings as follows: baby foods; cakes and frostings; candies and syrups; cookies; doughnuts and muffins; French toast, pancakes, and waffles; frozen desserts, gelatin, and dessert topping; pastries and pies; pizza and entrees; puddings and custards; and snacks (chips, crackers, popcorn, pretzels, and trail mix).

Grouping similar types of convenience foods together in their respective tables does not necessarily mean that they have similar lipid content. The lipid composition data in convenience foods presented in this chapter were obtained and adapted from the U.S. Department of Agriculture (USDA), Agricultural Research Service, Nutrient Database for Standard Reference, Release 19 (USDA, 2006). This database is developed and maintained by the Nutrient Data Laboratory (NDL), one of the six units in the Beltsville Human Nutrition Research Center of the Agricultural Research Service. This laboratory and its predecessor organizations in the USDA have been compiling and developing food composition databases for over a century. The Standard Reference and the Primary Data Set food composition data serve as the numerical basis for most of the public and private work in the field of human nutrition. Most of these food composition data were previously published in the form of Agricultural Handbook 8 (AH-8), which is no longer available in printed form. Instead, the information contained in AH-8 is now provided via Internet access in the principle database the USDA Nutrient Database for Standard Reference (SR). This database includes 21 sections and 4 supplements of AH-8 that are updated on a timely basis. The most recent update of the SR is number 19, issued in August 2006, which is the database used for this chapter's fatty acid content.

As shown in the Table 14.1 through 14.11, the lipid content of the particular food items is reported as total cholesterol, total fat, total saturated, monounsaturated, polyunsaturated, and *trans* fat, as well as the individual fatty acid content. Except for cholesterol (which is given in milligrams), all levels are given as grams of fatty acid per 100 grams (g) of food. With respect to the amount of fatty acid levels listed, their totals may not add up to the total lipid value, because the latter may also include some nonfatty acid materials such as glycerol, phosphate, sugar, or sterol. As an example, in the case of a vegetable oil that is 100% triglyceride, 95.6% is fatty acids and the remaining 4.4% is glycerol; therefore, its lipid conversion factor is 0.956. For other fats, the percentage of fatty acids present will be even lower. Zero values in the tables should be understood to mean that there

TABLE 14.1
Assorted Babyfood

	Total per 100 g									
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0	10:0
Apple yogurt dessert, strained	6	1.600	1.034	0.438	0.044		0.049	0.035	0.020	0.044
Apples with ham, strained	8	0.90	0.250	0.360	0.111		0	0	0	0.002
Baked finger snacks cereal	0	9.90	1.646	3.130	3.638		0	0	0	0
Banana juice with low fat yogurt	3	0.80	0.517	0.209	0.022		0.025	0.017	0.010	0.022
Carrots and beef, strained	8	2.50	0.960	1.060	0.130		0	0	0	0.002
Cereal, barley prepared with whole milk		3.30								
Cereal, egg yolks and bacon, junior	94	5.00	1.640	2.207	0.641		0	0	0	0.005
Cereal, high protein, with whole milk		3.80								
Cereal, mixed, prepared with whole milk	11	3.50	1.927							
Cereal, oatmeal, prepared with whole milk	11	4.10	2.258							
Cereal, rice, prepared with whole milk	11	3.60	2.325							
Cereal, with egg yolks, junior	63	1.80	0.610	0.790	0.300		0	0	0	0
Cherry cobbler, junior		0.10	0.020	0.020	0.030		0	0	0	0
Dessert, banana pudding, strained	29	0.80	0.264	0.277	0.111		0.002	0	0.001	0.001
Dessert, banana yogurt, strained	1	0.50	0.370	0.120	0.030		0.120	0.008	0.005	0.010
Dessert, blueberry yogurt, strained	4	0.70	0.452	0.192	0.019		0.022	0.015	0.009	0.019
Dessert, cherry vanilla pudding, junior	10	0.2	0.058	0.070	0.036		0	0	0	0
Dessert, custard pudding, vanilla junior	38	0.98	0.325	0.364	0.128		0.003	0	0.001	0.002
Dessert, fruit pudding, orange, strained	3	0.90	0.545	0.257	0.038		0.029	0.015	0.010	0.021
Dessert, peach cobbler, junior	0	0	0	0	0		0	0	0	0
Dessert, peach yogurt	4	0.18	0.117	0.048	0.005		0.006	0.004	0.002	0.005
Dinner, apples and chicken, strained	5	1.38	0.347	0.411	0.350		0	0	0	0
Dinner, beef and rice, toddler		2.90								
Dinner, beef lasagna, toddler		2.10								

Grams Fatty Acids per 100 g Food												
12:0	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.054	0.167	0.438	0.158	0.025	0.364	0	0.030	0.015	0	0	0	0
0.001	0.012	0.162	0.079	0.028	0.346	0.004	0.100	0.006	0	0.005	0	0
0.041	0.02	1.484	0.102	0.020	3.110	0	3.476	0.163	0	0	0	0
0.027	0.084	0.219	0.079	0.017	0.182	0	0.015	0.007	0	0	0	0
0.002	0.068	0.546	0.285	0.090	0.923	0.002	0.067	0.010	0.009	0	0	0
0.004	0.042	1.104	0.483	0.168	2.038	0	0.546	0.051	0.025	0	0	0
0	0.010	0.440	0.150	0.050	0.740	0	0.260	0.010	0.030	0	0	0
0	0.001	0.014	0.005	0	0.024	0	0.014	0.013	0	0	0	0
0.001	0.006	0.183	0.058	0.024	0.250	0.002	0.090	0.008	0.010	0	0	0.003
0.013	0.041	0.134	0.039	0.010	0.093	0	0.021	0.010	0	0	0	0
0.024	0.073	0.192	0.069	0.015	0.159	0	0.013	0.007	0	0	0	0
0	0.001	0.041	0.014	0.004	0.065	0	0.025	0.008	0.002	0	0	0.001
0.001	0.009	0.222	0.078	0.030	0.331	0.003	0.108	0.004	0.013	0	0	0.003
0.023	0.088	0.233	0.104	0.022	0.225	0	0.027	0.014	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0.007	0.020	0.053	0.019	0.004	0.044	0	0.004	0.002	0	0	0	0
0.001	0.012	0.226	0.078	0.047	0.350	0.003	0.304	0.027	0.010	0	0.002	0.002

(*Continued*)

TABLE 14.1
Continued

	Total per 100 g									
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**	**10:0**
Dinner, beef stew, toddler	13	1.20	0.580	0.440	0.100		0	0	0	0
Dinner, beef with vegetables	12	6.93	2.840	3.120	0.900		0	0	0	0
Dinner, broccoli and chicken, strained	6	1.47	0.414	0.568	0.367		0	0	0	0
Dinner, chicken and noodle with vegetables, toddler	28	1.70	0.461	0.632	0.392		0	0	0	0
Dinner, chicken and rice	10	0.90	0.211	0.392	0.188		0	0	0	0
Dinner, chicken noodle, junior		1.18	0.338	0.467	0.272		0	0	0	0
Dinner, chicken stew, toddler	29	3.70	1.100	1.700	0.770		0	0	0	0.010
Dinner, macaroni and cheese, junior	6	2.00	1.175	0.534	0.132		0.057	0.029	0.015	0.033
Dinner, macaroni, tomato, beef, junior	4	1.10	0.411	0.447	0.079		0.001	0	0	0.001
Dinner, potatoes, cheese, ham, toddler	6	2.00	1.100	0.733	0.163		0.031	0.015	0.008	0.020
Dinner, spaghetti, tomato, meat, toddler		1.00								
Dinner, sweet potatoes, chicken	11	2.17	.572	0.980	0.480		0	0	0	0
Dinner, turkey and rice, junior	4	0.92	0.239	0.304	0.233		0	0	0	0
Dinner, vegetables and bacon, strained	4	2.95	1.063	1.347	0.377					
Dinner, vegetables and beef, junior	8	1.85	0.683	0.761	0.143		0	0	0	0.002
Dinner, vegetables and chicken, junior	7	1.12	0.302	0.402	0.269		0	0	0	0
Dinner, vegetables, dumplings, beef, junior		0.80								
Dinner, vegetables and ham, junior	3	1.89	0.483	0.811	0.448		0	0	0	0.001
Dinner, vegetables and lamb, junior	5	1.70	0.696	0.690	0.154		0	0	0	0.004
Meat, beef, junior	28	4.90	2.590	1.850	0.160		0	0	0	0
Meat, chicken sticks, junior	78	14.40	4.094	6.267	2.986		0	0	0	0
Meat, chicken, junior	59	9.60	2.470	4.330	2.330		0	0	0	0
Meat, ham, junior		6.70	2.240	3.180	0.910		0	0	0	0
Meat, meat sticks, junior	70	14.60	5.820	6.480	1.590		0	0	0	0.010
Meat, pork, strained	48	7.10	2.40	3.580	0.780		0	0	0	0

Grams Fatty Acids per 100 g Food												
12:0	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0.030	0.280	0.220	0.030	0.400	0	0.090	0.010	0.010	0	0	0
0.051	0.214	1.463	1.112	0.321	2.778	0.022	0.747	0.149	0.004	0	0	0
0.002	0.011	0.302	0.085	0.070	0.477	0.013	0.267	0.083	0.006	0.001	0.001	0.002
0.002	0.012	0.343	0.091	0.081	0.529	0.014	0.340	0.020	0.013	0.001	0.002	0.004
0.001	0.006	0.152	0.043	0.038	0.342	0.008	0.165	0.008	0.006	0.001	0.001	0.002
0.002	0.010	0.247	0.066	0.056	0.394	0.011	0.241	0.017	0.005	0.001	0.001	0.002
0.010	0.050	0.740	0.220	0.140	1.540	0.010	0.730	0.030	0.020	0	0	0
0.029	0.181	0.557	0.221	0.055	0.453	0	0.105	0.027	0	0	0	0
0.001	0.029	0.241	0.119	0.038	0.392	0.001	0.066	0.007	0.004	0	0	0
0.020	0.112	0.493	0.219	0.070	0.650	0	0.131	0.032	0	0	0	0
0.001	0.016	0.437	0.118	0.095	0.803	0.014	0.487	0.024	0.005	0	0	0
0.001	0.008	0.157	0.052	0.032	0.261	0.002	0.203	0.016	0.007	0	0.001	0.002
0.002	0.041	0.408	0.205	0.058	0.676	0.005	0.120	0.016	0.005	0	0	0
0	0.01	0.191	0.071	0.047	0.340	0.004	0.238	0.013	0.009	0	0	0.002
0.003	0.013	0.314	0.151	0.033	0.777	0	0.403	0.039	0.004	0	0	0
0.007	0.064	0.358	0.221	0.047	0.626	0	0.122	0.026	0.005	0	0	0
0.010	0.140	1.240	1.01	0.130	1.660	0	0.110	0.030	0.020	0	0	0
0.037	0.133	3.045	0.813	0.887	5.277	2.764	0.111	0	0	0	0	
0	0.070	1.800	0.500	0.350	3.950	0.020	2.250	0.050	0	0	0	
0.010	0.080	1.400	0.680	0.180	2.910	0.070	0.820	0.030	0.05	0	0	
0.020	0.250	3.570	1.780	0.460	5.890	0.070	1.460	0.070	0.060	0	0	0
0	0.080	1.530	0.720	0.230	3.270	0.070	0.690	0.030	0	0	0	0

(*Continued*)

TABLE 14.1
Continued

	Total per 100 g									
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0	10:0
Meat, turkey sticks, junior	65	14.20	4.143	4.671	3.615		0	0	0	0
Meat, turkey, junior	53	7.10	2.310	2.640	1.760		0	0	0	0
Meat, veal, strained	26	4.80	2.290	2.050	0.160		0	0	0	0
Ravioli, cheese filled with tomato sce	7	2.20	0.960	0.570	0.480		0.051	0.019	0.012	0.026
Pretzels	0	2.00	0.316	0.188	0.833		0	0	0	0
Teething biscuits	0	5.00	1.534	1.425	0.863		0.049	0.013	0.015	0.032

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.2
Cakes and Frostings

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Bars/Cakes									
Corn cakes	0	2.400	0.420	0.760	0.930		0	0	0
Corn cakes, very low sodium	0	2.400	0.420	0.760	0.930		0	0	0
Crisped rice bar, chocolate chip	0	13.500	5.240	3.990	3.650		0	0.010	0.080
Granola bar, hard, almond	0	25.500	12.510	7.740	3.760		0	0.080	0.940
Granola bar, hard, chocolate chip	0	16.300	11.410	2.630	1.270		0	0.060	0.760
Granola bar, hard, peanut	0	21.400	2.520	5.760	11.880		0	0	0
Granola bar, hard, peanut butter	0	23.800	3.200	7.000	12.080		0	0	0
Granola bar, hard, plain	0	19.800	2.370	4.380	12.050		0	0	0
Granola bar, soft, coated milk chocolate, chocolate chip	5	24.900	14.220	7.770	1.820		0.100	0.040	0.130
Granola bar, soft, coated milk chocolate, peanut butter	12	31.100	17.010	6.540	1.900		0.070	0.040	0.420
Granola bar, soft, uncoated, chocolate chip	1	16.600	10.180	3.530	1.970		0	0.060	0.550
Granola bar, soft, uncoated, chocolate chip graham and marshmallow	1	15.500	9.180	2.920	2.550		9.180	0.060	0.620
Granola bar, soft, uncoated, plain	1	17.200	7.240	3.810	5.320		0	0.050	0.460
Granola bar, soft, uncoated, peanut butter	1	15.800	3.650	6.590	4.270		0	0.010	0.150
Granola bar, soft, uncoated, raisin	1	17.800	9.570	2.840	3.210		0	0.070	0.730
Granola bar, soft, uncoated, nut and raisin	1	20.400	9.540	4.220	5.520		0	0.060	0.670
Granola bar, soft, uncoated, peanut butter and chocolate chip	1	20.000	5.590	8.360	4.610		0	0.010	0.100

Grams Fatty Acids per 100 g Food												
12:0	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.020	0.102	2.539	1.015	0.772	3.798	0.020	3.006	0.163	0.284	0	0.041	0.061
0.040	0.100	1.410	0.660	0.220	2.370	0.020	1.610	0.080	0.070	0	0	0
0.020	0.240	1.180	0.670	0.190	1.790	0	0.100	0.030	0.020	0	0	0
0.028	0.134	0.494	0.158	0.039	0.509	0	0.424	0.035	0.002	0	0	0.001
0	0	0.300	0.023	0.003	0.185	0	0.789	0.044	0	0	0	0
0.033	0.161	0.801	0.379	0.065	1.331	0	0.805	0.058	0	0	0	0

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.010	0.360	0.040	0.010	0.760	0	0.890	0.030	0	0	0	0
0	0	0.010	0.360	0.040	0.010	0.760	0	0.890	0.030	0	0	0	0
0.060	0.460	0.200	2.460	1.980	0.030	3.970	0	3.450	0.200	0	0	0	0
0.750	5.600	2.120	2.430	0.530	0.050	7.690	0	3.660	0.100	0	0	0	0
0.610	4.550	1.720	2.160	1.520	0.010	2.610	0	1.220	0.050	0	0	0	0
0	0.010	0.010	1.630	0.780	0.010	5.680	0.060	11.850	0.030	0	0	0	0
0	0.020	0.020	2.210	0.900	0.010	6.910	0.080	11.950	0.110	0.020	0	0	0
0	0.010	0.010	1.540	0.760	0.010	4.370	0	11.990	0.060	0	0	0	0
0.150	0.630	0.570	6.040	6.570	0.190	7.580	0	1.710	0.110	0	0	0	0
0.490	5.500	2.150	4.420	3.920	0.120	6.390	0.030	1.830	0.070	0.010	0	0	0
0.440	3.250	1.240	2.580	2.060	0.050	3.480	0	1.870	0.100	0	0	0	0
0.490	3.630	1.380	2.000	1.000	0.050	2.870	0	2.410	0.130	0	0	0	0
0.360	2.680	1.040	2.120	0.540	0.050	3.770	0	5.020	0.300	0	0	0	0
0.120	0.890	0.340	1.670	0.460	0.010	6.410	0.160	4.220	0.030	0.020	0	0	0
0.570	4.270	1.620	1.850	0.460	0.050	2.790	0	3.040	0.170	0	0	0	0
0.530	3.990	1.540	2.090	0.660	0.040	4.140	0.030	5.360	0.170	0	0	0	0
0.080	0.590	0.240	2.730	1.850	0.020	8.170	0.180	4.540	0.030	0.030	0	0	0

(*Continued*)

TABLE 14.2
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Granola bar, oats, fruits, and nuts	0	6.300	0.730	0.170	4.830		0	0	0
Breakfast bars, oats, sugar, raisins, coconut (include granola bar)	0	17.600	12.690	1.933	1.100		0	0.081	1.005
Rice cake, cracker (include hain minirice cakes)	0	4.300	0.875	1.582	1.520		0	0	0
Rice cakes, brown rice, plain	0	2.800	0.570	1.030	0.990		0	0	0
Rice cakes, brown rice, sesame seed	0	3.800	0.540	1.100	1.150		0	0	0
Rice cakes, brown rice, buck wheat	0	3.500	0.640	1.110	1.110		0	0	0.020
Rice cakes, brown rice, corn	0	3.200	0.630	1.140	1.130		0	0	0
Rice cakes, brown rice, multigrain	0	3.500	0.560	1.160	1.440		0	0	0
Rice cakes, brown rice, rye	0	3.800	0.580	1.310	1.550		0	0	0
Cakes									
Commercially prepared									
Chocolate, commercially prepared with chocolate frosting	46	16.400	4.642	8.993	1.911		0.001	0.001	0.001
Angelfood, commercially prepared	0	0.80	0.121	0.071	0.367				
Boston cream pie	37	8.5	2.445	4.544	1.009		0.020	0.012	0.007
Pound, butter	221	19.9	11.559	5.900	1.067		0	0.334	0.195
Pound, other than butter, enriched	58	17.9	4.647	9.935	2.251		0.001	0	0.001
Sponge	102	2.70	0.802	0.949	0.448		0.001	0	0
Coffeecake, crème-filled with chocolate	69	10.8	2.833	5.664	1.465		0.001	0	0.001
Fruitcake, commercially prepared	5	9.10	1.048	4.200	3.323		0	0	0
Sweet rolls, cinnamon, refrigerator frosting	0	13.20	3.348	7.421	1.728		0.001	0.001	0
Yellow, vanilla frosting	55	14.50	2.371	6.107	5.150		0	0	0
Yellow with chocolate frosting	55	17.400	4.721	9.671	2.088		0.001	0	0.001
Dry mix									
Cherry Fudge with chocolate frosting	42	12.500	5.066	4.400	2.340		0.212	0.124	0.073
Snack cakes									
Crème-filled, chocolate with frosting	17	14.500	3.233	5.343	4.178		0.001	0.001	0
Crème-filled, sponge	16	11.400	2.561	4.073	3.303		0.001	0	0
Cupcakes, chocolate low-fat frosting	0	3.700	1.084	1.849	0.487		0.001	0	0
Fudge cake-type (includes trolley cakes)	0	3.700	1.112	1.813	0.466		0	0	0
Frostings									
Shortening, special purpose for cakes and frosting, soybean (hydrogenated)	0	100	20.001	36.555	37.991		0	0	0
Coconut-nut, ready-to-eat	0	24	8.631	10.786	3.210		0	0.034	0.417

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0.006	0.011	0.540	0.170	0.001	0.171	0	4.666	0.166	0	0	0	0
0.802	6.135	2.357	1.701	0.562	0.007	1.926	0.001	1.574	0.011	0	0	0	0
0	0	0.015	0.737	0.077	0.015	1.57	0	1.490	0.015	0	0	0	0
0	0	0.010	0.480	0.050	0.010	1.020	0	0.970	0.010	0	0	0	0
0	0	0.010	0.430	0.080	0.010	1.090	0	1.110	0.040	0	0	0	0
0.010	0.010	0.200	0.510	0.050	0.020	1.090	0	1.050	0.070	0	0	0	0
0	0	0.010	0.540	0.060	0.010	1.130	0	1.090	0.050	0	0	0	0
0	0	0.010	0.460	0.080	0.010	1.150	0	1	0.440	0	0	0	0
0	0	0.010	0.450	0.110	0.010	1.300	0	1.490	0.060	0	0	0	0
0.001	0.001	0.079	2.374	2.184	0.032	8.958	0.003	1.801	0.092	0.013	0	0	0
		0.001	0.110	0.005	0.002	0.068		0.346	0.021				
0.005	0.013	0.093	1.265	1.006	0.031	4.504	0.002	0.941	0.055	0.010	0	0.003	0.003
0.437	0.489	1.757	5.11		0.457	5.176	0.01	0.76	0.267	0.032	0.001	0.001	0.008
0.001	0.001	0.087	2.478	2.069	0.049	9.880	0.004	2.114	0.108	0.023	0.001	0	0.006
0.001	0.001	0.011	0.58	0.193	0.07	0.87	0.007	0.389	0.015	0.034	0.001	0	0.009
0.001	0.001	0.050	1.571	1.195	0.054	5.603	0.005	1.365	0.069	0.024	0.024	0.001	0.006
0	0	0.020	0.743	0.267	0.044	4.100	0.032	2.959	0.336	0.003	0.000	0.000	0.001
0.001	0.001	0.067	1.734	1.544	0.001	7.420		1.641	0.088				
0	0	0.018	1.590	0.754	0.092	5.998	0.004	4.766	0.347	0.018	0.001		0.005
0.001	0.001	0.084	2.45	2.19	0.034	9.634	0.003	1.97	0.101	0.015	0	0	0.004
0.163	0.181	0.660	2.38	1.12	0.183	4.11	0.001	2.08	0.240	0	0	0	0.002
0.001	0.030	0.153	2.11	0.916	0.191	5.07	0.066	3.75	0.424	0.004	0	0	0.001
0.001	0.023	0.119	1.648	0.718	0.156	3.843	0.033	2.912	0.369	0.006	0	0	0.001
0.001	0.001	0.017	0.553	0.509	0.001	1.85	0	0.462	0.025	0	0	0	0
0	0	0.002	0.548	0.545	0.005	1.81	0	0.453	0.013	0	0	0	0
0	0	0	9.666	10.335	0	36.555	0	35.790	2.201	0	0	0	0
0.332	2.644	1.056	2.183	1.863	0.011	10.752	0.023	3.075	0.135	0	0	0	0

(*Continued*)

TABLE 14.2
Continued

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Chocolate, creamy, dry mix, prepared with margarine	0	12.870	1.74	3.943	2.797		0	0	0
Chocolate, creamy, dry mix, prepared with butter	24	13.100	5.610	2.600	0.330		0.290	0.170	0.100
Chocolate, creamy, ready-to-eat	0	17.600	5.526	9.020	2.130		0	0	0
Cream cheese-flavor, ready-to-eat	0	17.300	4.545	3.755	6.153		0	0	0
White, fluffy, dry mix, prepared with water	0	0	0						
Vanilla, creamy, dry mix, prepared with margarine	0	12.740	1.740	3.942	2.797		0	0	0
Vanilla, creamy, ready-to-eat	0	16.230	2.974	4.860	7.951		0	0	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.3
Candies and Syrups

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Candies									
3 Musketeers bar	5	12.75	8.646	2.373	0.316	0.129	0.216	0.078	0.045
Almond Joy bites	11	34.500	20.600	8.850	1.460				
Baking chocolate, Mexican, squares	0	15.59	8.606	5.044	1.162		0	0	0
Baking Chocolate, unsweetened, liquid	0	47.70	25.290	9.230	10.700			0.130	1.590
Baking chocolate, unsweetened squares	0	52.31	32.351	16.106	1.555		0	0	0
Butterscotch	9	3.300	2.065	0.845	0.122		0.130	0.081	0.048
Candies, hard	0	0.200	0	0	0		0	0	0
Candies, jellybeans	0	0.050	0	0	0		0	0	0
Candies, krackel chocolate bar	11	26.580	15.920	6.250	0.570				
Candies, marshmallows	0	0.200	0.056	0.080	0.047		0	0	0
Candies, milk chocolate	23	29.660	14.23	13.21	0.853		0.502	0.182	0.104
Candies, dark chocolate-coated coffee beans	13	30.00	15.00	0	0		0	0	0
Candies, milk chocolate-coated coffee beans	20	26.090	12.490	5.833	0.854		0.434	0.157	0.090
Candies, nougat with almonds	0	1.670	1.667	0	0		0	0	0
Candies, peanut bar	0	33.70	4.678	16.721	10.650		0	0	0
Candies, Kit Kat bites	8	26.410	17.100	4.500	0.430				

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.022	1.07	0.650	0	3.94	0	2.68	0.121	0	0	0	0
0.230	0.250	0.910	2.370	1.090	0.200	2.270	0	0.200	0.130	0	0	0	0
0	0	0.1	2.410	3.060	0	9.020	0	2.130	0.096	0	0	0	0
0	0	0.075	3.54	0.934	0.026	3.73	0	5.79	0.361	0	0	0	0
							0			0	0	0	0
0	0	0.022	1.07	0.650	0	3.94	0	2.68	0.121	0	0	0	0
0	0	0.020	2.38	0.579	0.178	4.68	0	7.050	0.901	0	0	0	0

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.085	0.093	0.295	3.699	3.911	0.017	2.346	0.001	0.306	0.010	0	0	0	0
0	0	0.010	3.628	4.705	0.037	5.007	0	0.942	0.072	0.148	0	0	0
1.270	9.450	3.63	6.06	3.160	0.050	9.180							
0	0	0.033	13.340	18.274	0.100	16.006	0	1.438	0.117	0	0	0	0
0.102	0.104	0.299	0.872	0.402	0.039	0.802	0.004	0.110	0.013	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0.001	0.040	0.015	0.004	0.076	0	0.045	0.001	0.001	0	0	0
0.197	0.216	0.663	5.95	6.1	0.124	6.500	0	0.728	0.041	0	0	0	0
0	0	0.022	6.552	8.407	0	0	0	0	0	0	0	0	0
0.170	0.187	0.572	5.29	5.3	0.107	5.654	0	0.847	0.007	0	0	0	0
0	0	0.002	1.340	0.324	0	0	0	0	0	0	0	0	0
0	0	0.022	3.528	0.752	0.010	16.260	0.495	10.647	0.002	0	0	0	0

(*Continued*)

TABLE 14.3
Continued

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Candies, Kit Kat wafer bar	11	25.990	17.95	5.844	0.895	0.099	0.136	0.117	0.502
Caramels	7	8.100	2.476	1.542	3.478		0.047	0.012	0.013
Caramels, chocolate flavor, roll	2	3.310	0.967	1.929	0.266		0	0	0.067
Carob, unsweetened	3	31.360	29.02	0.483	0.295	0	0.163	0.130	1.55
Chocolate covered, caramel with nuts	0	21.000	4.657	9.444	5.834		0	0.005	0.080
Confectioner's coating, butterscotch	0	29.050	24.100	1.734	1.041		0.020	0.125	1.42
Confectioner's coating, peanut butter	0	29.800	13.120	12.71	2.123		0.010	0.018	0.290
Confectioner's, coating yogurt	1	27.000	24.1	0.526	0.524	0	0.025	0.044	0.678
Choc covered dietetic low calorie	21	39.40	21.910	10.942	4.354		0.005	0.049	0.786
Candies, fudge, vanilla with nuts	13	13.68	3.320	2.246	6.489		0.114	0.098	0.066
Candies, hard dietetic or low calorie	0	0	0	0	0		0	0	0
Candies, gumdrops starch jelly pieces	0	0	0	0	0		0	0	0
Halavah, plain	0	21.520	4.127	8.194	8.481		0	0	0
Heath bites	19	30.380	15.620	8.800	2.550				
Hershey, 5th Avenue bar	6	23.98	6.650	10.600	3.390		0.128	0.045	0.028
Hershey, Almond Joy bar	4	26.93	17.590	5.260	1.180				
Hershey, Caramello bar	27	21.19	12.720	50.290	0.630				
Hershey, Mound's bar	2	26.60	20.580	0.390	0.140		0.000	0.101	1.160
Hershey, Reece's Pieces	0	24.77	16.420	4.460	1.880		0.002	0.039	0.639
Hershey, Rolo Caramels in milk chocolate	18	20.000	12.200	6.300	0.600		0.030	0.018	0.019
Hershey Twizzler, cherry bits	0	1.170	0.250	0.490	0.040	0.540			
Hershey Twizzler, strawberry	0	2.32	0.400						
Hershey, Whatchamacallit bar	10	23.68	17.040	3.780	0.830	0.120	0.403	0.169	0.334
Hershey's Golden Almond Solitaires	13	37.130	15.230						
Hershey's Kit Kat Big Kat bar	9	27.840	17.990	4.910	0.510	0.000			
Hershey's milk chocolate with almond bites	19	35.730	17.390	14.380	2.420	0.150			
Hershey's pot of gold almond bar	13	38.460	16.67						
Hershey's Reesesticks Crispy Wafers, peanut butter, milk chocolate	6	31.340	13.210	10.820	4.680		0.119	0.044	0.040
M&M Mars peanut butter chocolate candies	7	29.320	18.485	4.448	0.542	0.210	0.153	0.101	0.626
M&M Peanut Chocolate	8	26.13	10.169	8.086	3.456	0.328	0.300	0.108	0.062
M&M Plain Chocolate	14	21.130	13.080	3.373	0.387	0.167	0.462	0.167	0.095
Mars Milky Way midnight bar	10	17.500	11.474	4.639	0.514	0.176	0.130	0.078	0.046
Mars almond bar	17	23.000	7.268	10.69	3.980		0.165	0.081	0.054
Mars milky way bar	9	17.230	12.016	2.230	0.260	0.206	0.182	0.093	0.301
Milk chocolate-coated peanuts	9	33.500	14.600	12.920	4.330		0.130	0.030	0.040
Milk chocolate-coated raisins	3	14.800	8.800	4.740	0.510		0.080	0.020	0.030
Milk chocolate with almonds	19	34.400	16.980	13.490	2.280		0.160	0.040	0.050
Milk chocolate with rice cereal	19	26.500	15.890	8.640	0.780		0.150	0.040	0.050
Nestle 100 Grand bar	13	17.300	11.900	3.203	0.775	0.105	0.347	0.177	0.371

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.508	4.91	1.75	4.98	4.8	0.149	5.7	0	0.821	0.075	0	0	0	0
0.026	0.016	0.190	1.880	0.293	0.095	1.45	0	3.45	0.025	0.007	0	0	0
0.025	0	0	0.467	0.396	0	1.907	0	0.250	0	0	0	0	0
1.22	12.958	4.32	3.07	5.54	0	0.48	0	0.259	0.034	0	0	0	0
0.090	1.140	0.407	2.178	0.551	0.003	9.19	0.246	5.83	0.001	0	0	0	0
1.19	13.415	4.88	2.340	0.694	0.007	1.73	0	0.959	0.082	0	0	0	0
0.362	6.200	2.21	2.23	1.69	0.020	12.7	0.009	2.054	0.069	0	0	0	0
0.847	14.505	5.15	2.27	0.571	0.003	0.523	0	0.478	0.046	0	0	0	0
0.883	11.179	3.950	3.589	1.363	0.035	10.753	0.126	4.303	0.042	0	0	0	0
0.118	0.120	0.367	1.660	0.710	0.037	2.187	0.022	5.234	1.255	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	2.11	1.85	0.025	8.13	0.036	8.42	0.060	0	0	0	0
0.053	0.058	0.213	3.532	1.974	0.054	8.355	0.145	3.305	0.084	0	0	0	0
0.925	6.775	2.515	3.065	2.945	0.030	0.388	0.010	0.135	0.005	0	0	0 0	0
0.717	9.105	3.184	1.984	0.747	0.019	4.434	0.001	1.769	0.107	0	0	0	0
0.034	0.160	0.645	5.799	5.406	0.020	6.266	0	0.017	0	0	0	0	0
0.449	3.660	1.801	5.260	4.715	.055	5.803	0.001	2.961	0.027	0	0	0	0
0.060	0.222	0.236	6.01	6.16	0.053	9.32	0.113	4.59	0.049	0	0	0	0
0.537	6.611	2.465	2.954	4.904	0.079	4.314	0.002	0.515	0.026	0	0	0	0
0.118	0.129	0.402	4.811	3.910	0.062	7.853	0.131	0.803	0.021	0	0	0	0
0.181	0.199	0.609	5.465	5.605	0.06	3.269	0.000	0.366	0.021	0	0	0	0
0.101	0.108	0.362	4.788	5.601	0.063	4.574	0.002	0.467	0.048	0	0	0	0
0.164	0.294	0.637	3.52	2.19	0.215	10.3	0.009	3.700	0.261	0	0	0	0
0.335	3.951	1.593	2.558	2.884	0.060	2.148	0	0.231	0.029	0	0	0	0
0.090	0.090	0.440	7.000	6.790	0.210	12.550	0.160	4.280	0.050	0	0	0	0
0.060	0.060	0.290	3.920	4.350	0.140	4.600	0	0.450	0.050	0	0	0	0
0.110	0.110	0.580	7.660	8.270	0.310	13.170	0.010	2.170	0.110	0	0	0	0
0.110	0.110	0.520	7.010	7.910	0.260	8.380	0	0.720	0.060	0	0	0	0
0.368	1.695	1.137	4.192	3.432	0.044	1.815	0.001	0.686	0.072	0	0	0	0

(*Continued*)

TABLE 14.3
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Nestle After Eight mints	0	11.900	8.330			0.000			
Nestle Baby Ruth bar	0	21.600	12.100	5.557	2.638	0.050	0.013	0.061	0.676
Nestle Bit-O'-Honey candy chews	0	7.500	5.500	1.030	0.338	0.000	0.005	0.047	0.496
Nestle Butterfinger bar	0	18.900	9.500	5.119	3.164	0.040	0.007	0.022	0.282
Nestle Crunch bar and dessert topping	13	26.000	16.000			0.137			
Nestle Chunky bar	10	27.500	12.500			0.160			
Nestle Goobers chocolate covered peanuts	12	34.000	12.100			0.07			
Nestle Oh Henry bar	7	23.000	9.600	5.530	2.706	0.050	0.124	0.055	0.160
Nestle Raisinettes chocolate covered raisins	11	17.000	11.000			0.080			
Reese's Bites	7	29.850	18.000	7.060	1.820	0.050			
Reese's Nutrageous candy bar	3	32.090	8.860	12.630	8.090				
Semisweet chocolate	0	30.000	17.750	9.966	0.966		0	0	0
Sweet chocolate-coated fondant	0	9.30	5.460	3.090	0.300		0	0	0
Sweet chocolate	0	34.200	20.08	11.220	0.990		0	0	0
Semisweet chocolate made with butter	18	29.700	17.530	9.930	0.950		0.480	0.285	0.165
Sesame crunch	0	33.300	4.460	12.570	14.580		0	0	0
Skittles original bites size candies	0	4.370	4.115	0	0	0.074	0.003	0.011	0.145
Skor toffee bar	53	32.180	18.780	9.290	1.280				
Snickers bar	13	23.850	9.069	7.875	3.014	0.436	0.318	0.142	0.082
Soft fruit and nut squares	0	9.520	0.898	1.297	6.840		0	0	0
Star Brites peppermint mints	0	0.200							
Starburst fruit chews, original flavor	0	8.210	7.778	0	0	0.141	0.006	0.027	0.321
Sugar-coated almonds	0	17.500	1.948	12.08	3.904		0	0	0
Symphony milk chocolate bar	24	30.570	18.340	7.910	0.690				
Tootsie roll, chocolate-flavor	2	3.310	0.967	1.929	0.266		0	0	0.067
Twix caramel cookie bars	7	24.850	18.960	2.182	0.329	0.342	0.182	0.101	0.531
Twix peanut butter cookie bars	6	32.670	15.761	10.626	3.012	0.505	0.259	0.094	0.058
White chocolate candies	14	32.090	19.41	9.004	1.000	0.000	0.106	0.075	0.328
York Bites candies	1	7.320	4.430	0.480	0.100				
York Peppermint Pattie	1	7.170	4.340	0.410	0.110				
Syrups									
Chocolate, fudge type	2	8.90	3.980	3.857	0.278		0	0	0.018
Corn light	0	0.020	0	0	0		0	0	0
Hershey's genuine chocolate flavor lite	0	0.52	0	0	0		0	0	0
Maple syrups	0	0.200	0.036	0.064	0.1		0	0	0
Table blends, pancake	0	0	0	0	0		0	0	0
Table blends, pancake, with butter	4	1.60	1.010	0.470	0.060		0.050	0.030	0.020
Sorghum syrups	0	0	0	0	0		0	0	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.569	6.372	2.327	1.505	0.530	0.007	5.450	0.096	2.634	0.003	0	0	0	0
0.352	2.798	1.050	0.560	0.189	0.005	1.025	0.000	0.336	0.002	0	0	0	0
0.273	4.183	1.406	1.763	1.445	0.002	4.983	0.134	3.163	0.002	0	0	0	0
0.156	0.853	0.471	2.869	1.815	0.024	5.376	0.116	2.805	0.001	0	0	0	0
0	0	0.020	7.347	10.007	0.062	9.904	0	0.898	0.068	0	0	0	0
0	0	0.020	2.500	2.870	0	3.090	0	0.300	0	0	0	0	0
0	0	0.030	8.690	11.350	0.070	11.150	0	0.960	0.030	0	0	0	0
0.375	0.420	0.050	8.030	9.230	0.360	9.930	0	0.950	0.240	0	0	0	0
0	0	0.080	2.980	1.400	0.100	12.420	0.050	14.330	0.250	0	0	0	0
0.140	2.151	0.720	0.334	0.599	0	0	0	0	0	0	0	0	0
0.167	0.181	0.575	4.181	3.145	0.107	7.625	0.110	2.966	0	0	0	0	0
0	0	0	0.647	0.241	0	1.28	0.019	5.53	1.32	0	0	0	0
0.259	3.524	1.226	0.686	1.708	0	0	0	0	0	0	0	0	0
0	0.002	0.106	1.48	0.364	0.090	12	0	3.9	0	0	0	0	0
0.025	0	0	0.467	0.396	0	1.91	0	0.250	0	0	0	0	0
0.547	7.433	2.688	3.249	4.114	0.037	2.120	0	0.313	0.015	0	0	0	0
0.102	0.154	0.436	7.491	6.158	0.039	10.492	0.075	2.971	0.041	0	0	0	0
0.442	6.404	2.55	4.78	4.53	0.157	8.85	0.000	0.895	0.105	0	0	0	0
0.047	0.056	0.237	1.801	0.756	0.067	3.780	0.010	0.249	0.024	0.005	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0.036	0.004	0	0.064	0	0.100	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.040	0.050	0.160	0.430	0.200	0.040	0.410		0.040	0.020	0			
0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 14.4
Cookies

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Cookies									
Animal crackers (includes arrowroot, tea biscuits	0	13.800	3.463	7.666	1.873		0	0	0
Brownies, commercially prepared	17	16.300	4.235	8.965	2.259		0.001	0	0
Brownies, dry mix, special dietary, prepared	0	11.100	5.096	4.573	0.806		0	0	0
Butter, commercially prepared	117	18.800	11.051	5.522	0.982		0.546	0.323	0.188
Chocolate chip, refrigerated, baked	27	22.600	7.759	11.26	2.319			0	0
Chocolate chip, commercially prepared regular, lower fat	0	15.400	3.810	6.095	4.649		0	0	0
Chocolate chip, commercially prepared, regular, higher fat-enriched	0	24.73	7.838	13.282	1.383		0	0	0
Chocolate chip, commercially prepared, special dietary	0	16.800	4.183	6.730	5.035		0	0	0
Chocolate wafer	2	14.200	4.241	4.865	4.153		0.001	0.011	0.128
Chocolate sandwich, crème filling, regular	0	19.08	3.680	10.634	2.257	5.308	0	0	0
Chocolate sandwich, crème filling, special dietary	0	22.100	3.825	9.228	7.940		0.001	0	0
Chocolate sandwich, crème filling, regular, chocolate coated	0	26.400	7.444	14.42	3.065		0.002	0.001	0.001
Chocolate sandwich, extra crème filling	0	25.200	4.808	14.72	3.304		0	0	0
Fig bars	0	7.300	1.123	3.003	2.772		0.002	0	0
Fortune	2	2.70	0.669	1.345	0.466		0	0	0
Fudge, cake-type (includes trolley cakes)	0	3.700	1.112	1.813	0.466		0	0	0
Gingersnaps	0	9.800	2.451	5.365	1.372		0	0	0.001
Graham crackers, plain, honey or cinnamon	0	10.100	1.519	4.086	3.832		0	0	0.005
Graham crackers, chocolate coated	0	23.20	13.380	7.687	1.038		0	0	0.002
Ladyfingers with lemon juice and rind	221	9.100	3.477	4.253	1.611		0	0	0.003
Marshmallow chocolate coated (includes marshmallow pies)	0	16.900	4.722	9.345	1.943		0.004	0.001	0.001
Molasses	0	12.800	3.212	7.130	1.729		0	0	0
Oatmeal, commercially prepared, reg.	0	18.100	4.519	10.025	2.543		0	0	0
Oatmeal, commercially prepared, fat free	0	1.500	0.314	0.388	0.635		0	0	0
Oatmeal, commercially prepared, soft-type	5	14.700	3.630	7.988	2.204		0	0	0
Oatmeal, commercially prepared, special dietary	0	18.000	2.693	7.550	6.784		0	0	0
Oatmeal, refrigerated, baked	26	21.000	5.339	11.729	2.976				0
Peanut butter, commercially prepared, regular	1	23.600	4.485	12.368	5.518		0	0	0
Peanut butter, commercially prepared, soft-type	0	24.400	6.149	13.844	3.181				
Peanut butter, refrigerated, baked	30	27.500	6.199	14.53	4.889				0.001
Peanut butter sandwich, regular	0	21.100	4.995	11.191	3.795		0.006	0.001	0.001
Peanut butter sandwich, special dietary	0	34.000	4.937	15.391	12.026		0	0	0
Raisin, soft-type	2	13.600	3.460	7.649	1.756		0	0	0

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.067	1.797	1.591	0.003	7.663	0	1.776	0.096	0	0	0	0
0	0	0.078	2.176	1.973	0.011	8.946	0.006	2.09	0.159	0.004	0	0	0.001
0	0.095	0.096	3.100	1.803	0.003	4.570	0	0.759	0.046	0	0	0	0
0.422	0.473	1.700	4.849	2.179	0.420	4.845	0.005	0.681	0.256	0.023	0.011	0	0.007
0	0	0.086	3.77	3.900	0.043	11.213	0.003	2.190	0.111	0.014	0		0.004
0	0.007	0.029	2.074	1.655	0.058	6.027	0	4.320	0.318	0	0	0	0
0	0.323	0.197	3.655	3.519	0	13.257	0.025	1.334	0.049	0	0	0	0
0	0.008	0.032	2.263	1.832	0.063	6.655	0	4.677	0.346	0	0	0	0
0.102	0.760	0.305	1.796	1.125	0.048	4.807	0.001	3.866	0.268	0.006	0.003	0	0.002
0	0	0.015	1.957	1.550	0	10.609	0.025	2.203	0.054	0	0	0	0
0	0.181	0.025	2.392	1.393	0.084	9.123	0	1.4	0.084	0	0	0	0
0.002	0.002	0.126	3.808	3.5	0.051	14.367	0.004	2.89	0.148	0.022	0.001	0	0.006
0	0	0.001	2.579	2.087	0.025	14.691	0	7.375	0.544	0	0	0	0
0.001	0	0.013	0.747	0.355	0.029	2.966	0	2.583	0.182	0	0	0	0
0	0	0.012	0.378	0.276	0.008	1.337	0.001	0.438	0.024	0.003	0	0	0.001
0	0	0.002	0.548	0.545	0.005	1.807		0.453	0.013				
0.002	0.004	0.048	1.279	1.111	0.002	5.363	0	1.298	0.073	0	0	0	0
0	0.006	0.010	1.029	0.471	0.041	4.036	0	3.563	0.259	0.001	0	0	0
0	0.045	0.113	5.859	7.098	0.071	7.623	0	0.973	0.064	0	0	0	0
0.003	0.003	0.038	2.503	0.873	0.332	3.877	0.031	1.366	0.043	0.158	0.004	0	0.041
0.001	0.001	0.086	2.367	2.249	0.003	9.341	0	1.847	0.096	0	0	0	0
0	0	0.062	1.666	1.480	0.002	7.128	0	1.641	0.088	0	0	0	0
0	0.003	0.086	2.383	2.031	0.003	10.022	0	2.413	0.129	0	0	0	0
0	0	0.005	0.286	0.020	0.005	0.384	0	0.635	0.039	0	0	0	0
0	0.005	0.068	1.956	1.581	0.006	7.981	0	2.093	0.109	0.002	0	0	0
0	0.001	0.019	1.798	0.867	0.071	7.462	0	6.303	0.464	0	0	0	0
0	0	0.102	2.871	2.365	0.015	11.713	0.001	2.818	0.152	0.005	0	0	0.001
0	0	0.057	2.772	1.504	0.013	12.174	0.179	5.440	0.067	0.006	0.003	0	0.002
	0.002	0.089	3.01	2.27	0.001	13.169	0.098	4.24	0.113	0.018			
0.001	0.004	0.099	3.54	2.55	0.044	14.372	0.110	4.700	0.131	0.052	0.001	0	0.005
0.002	0.006	0.107	2.649	1.980	0	11.126	0.059	3.688	0.107	0	0	0	0
0	0	0.028	3.426	1.335	0.087	15.110	0.172	11.454	0.551	0	0	0	0
0	0	0.067	1.795	1.594	0.006	7.642	0	1.660	0.092	0.014	0	0	0.004

(*Continued*)

TABLE 14.4
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Sugar, commercially prepared, regular (includes vanilla)	51	21.100	5.435	11.836	2.660		0.001	0	0
Sugar, commercially prepared, special dietary	0	13.000	1.872	5.243	4.755		0	0	0
Sugar, refrigerated, baked	32	23.100	5.906	13.010	2.891		0	0	0
Sugar wafers, crème filling, regular	0	24.300	3.622	10.331	9.158		0	0	0
Sugar wafers, crème filling, special dietary	0	25.700	3.829	10.932	9.684		0	0	0
Vanilla sandwich, crème filling	0	20.000	2.979	8.437	7.551		0	0	0
Vanilla wafers, high fat	0	19.400	4.936	11.076	2.437		0.001	0	0
Vanilla wafers, lower fat	51	15.200	3.838	6.544	3.887		0	0	0
Babyfood cookies, arrowroot	1	14.30	3.330	8.980	0.840				
Babyfood cookies	0	13.200	3.700	7.380	1.110		0	0	0
Archway, Apple Filled Oatmeal	6	11.780	2.700	4.760	1.020	3.300			
Archway, Apricot Filled	6	13.170	5.120	4.110	1.100	2.830			
Archway, Cherry Filled	21	14.120	5.570	4.500	1.090	2.950			
Archway, Chocolate Chip Drop	12	15.330	5.290	5.400	1.000	3.520			
Archway, Chocolate Chip Ice Box	13	24.400	8.080	8.940	1.430	5.830			
Archway, Coconut Macaroon	0	22.550	20.100	1.610	0.810				
Archway, Cookies Jar Hermits	10	9.770	2.310	3.880	0.840	2.740			
Archway, Dark Molasses	0	12.590	2.880	5.020	0.980	3.680			
Archway, Date Filled Oatmeal	6	12.050	2.760	4.820	1.110	3.360			
Archway, Dutch Cocoa	7	14.990	3.620	5.940	1.180	4.240			
Archway, Fat Free Devil's Food cookie	0	0.900	0.320	0.290	0.280				
Archway, Fat Free Oatmeal Raisin	44	1.510	0.320	0.580	0.610				
Archway, Frosty Lemon	0	17.110	5.830	5.750	0.920				
Archway, Fruit and Honey bar	17	12.540	2.880	5.090	0.0940	3.620			
Archway, Gourmet Apple' n Raisin	7	16.640	3.610	6.360	2.420	4.250			
Archway, Gourmet Oatmeal Pecan	8	23.130	7.580	8.610	2.210	4.730			
Archway, Gourmet Rocky Road	10	19.710	5.130	6.820	3.480	4.250			
Archway, Gourmet Ruth's Golden Oatmeal	8	16.440	3.290	5.670	4.110	3.370			
Archway, Iced Molasses	12	14.430	4.160	4.750	2.440	3.080			
Archway, Iced Oatmeal	8	16.530	4.610	5.610	2.720	3.580			
Molasses	25	12.060	2.810	4.860	1.050	3.340			
Archway, Oatmeal	10	14.080	3.200	5.660	1.330	3.880			
Archway, Oatmeal Raisin	8	12.080	2.750	4.850	1.160	3.320			
Archway, Old Fashioned Molasses	25	11.830	2.770	4.800	1.000	3.250			
Archway, Old Fashioned Windmill	1	17.550	3.750	7.820	1.670	4.300			
Archway, Peanut Butter	37	24.280	5.390	10.210	4.230				
Archway, Pecan Ice Box	31	26.460	5.670	11.550	2.450	6.800			
Archway, Raspberry Filled	6	13.260	5.160	4.140	1.100	2.850			
Archway, Reduced Fat Ginger Snaps	1	11.140	2.600	3.580	1.650	3.310			
Archway, Ruth's Oatmeal	16	15.880	3.530	5.630	2.370				
Archway, Soft Sugar Drop	41	13.870	3.330	5.710	1.020	3.800			
Archway, Strawberry Filled	6	13.260	5.160	4.140	1.100	2.850			
Archway, Sugar	16	12.800	2.960	5.040	1.200	3.600			
Archway, Sugar Free Chocolate Chip	0	22.710	6.300	9.130	1.350	5.930			

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.104	2.87	2.469	0.030	11.802	0.003	2.500	0.130	0.016	0.008	0	0.005
0	0	0.013	1.247	0.608	0.050	5.180	0	4.416	0.327	0	0	0	0
0	0	0.112	3.081	2.709	0.028	12.979	0.003	2.731	0.143	0.013	0	0	0.003
0	0	0.025	2.394	1.200	0.097	10.210	0	8.503	0.632	0	0	0	0
0	0	0.026	2.530	1.270	0.103	10.804	0	8.991	0.668	0	0	0	0
0	0	0.020	1.976	0.979	0.080	8.338	0	7.011	0.520	0	0	0	0
0	0	0.097	2.526	2.307	0.002	11.074	0	2.314	0.123	0	0	0	0
0	0	0.060	2.244	1.543	0.001	6.508	0.003	3.625	0.231	0.017	0.008	0	0
		0.020	1.540	1.670		8.980		0.780	0.040				
0	0.010	0.040	2.420	1.140	0.010	7.370	0	0.990	0.040	0	0	0	0

(*Continued*)

TABLE 14.4
Continued

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Archway, Sugar Free Oatmeal	0	20.920	4.900	7.860	1.310				
Archway, Sugar Free Rocky Road	0	20.520	5.050	7.190	4.160	4.120			
Shortbread, commercially prepared plain	20	24.100	6.106	13.420	3.233		0	0	0
Shortbread, commercially prepared pecan	33	32.50	8.204	18.628	4.122		0	0	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.5
Doughnuts and Muffins

	Total per 100 g							
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0
Doughnuts								
Cake-type, plain, unsugared, old-fashioned	9	23.55	7.127	12.732	2.659		0	0
Cake-type, plain, sugared or glazed	32	22.900	5.926	12.697	2.909		0.001	0
Cake-type, chocolate, sugared or glazed	57	19.900	5.132	11.278	2.477		0.001	0
Cake-type, plain, chocolate coated or frosted	19	25.25	13.470	8.618	1.960		0	0.025
French crullers, glazed	11	18.300	4.667	10.45	2.285		0	0
Yeast-leavened, glazed, enriched (includes honey buns)	30	19.10	5.440	9.960	2.864		0	0
Yeast-leavened, jelly filling	26	18.700	4.843	10.226	2.377		0	0
Yeast-leavened, crème filling	24	24.500	5.430	12.080	3.082		0	0
Muffins								
Blueberry, commercially prepared	40	19.240	3.549	5.152	9.703		0	0
Blueberry, dry mix	0	10.00	2.489	5.507	1.393			
Blueberry, prepared from recipe, made with low-fat (2%) milk	37	10.80	2.030	2.596	5.388		0.025	0.015
Blueberry, toaster-type, toasted	5	10.100	1.551	2.371	5.477		0.001	0.001
Corn, dry mix, prepared	62	10.200	2.797	5.249	1.250		0.017	0.010
Corn, commercially prepared	26	8.400	1.354	2.104	3.215		0	0
Corn, toaster-type	13	11.3	1.681	2.625	6.324		0	0
Oat bran	0	7.400	1.087	1.695	4.129		0	0
Plain, prepared from recipe, made with low fat (2%) milk	39	11.40	2.156	2.757	5.721		0.027	0.016
Wheat bran, dry mix	0	12.00	2.939	6.403	1.904		0	0
Wheat bran, toaster-type with raisins, toasted	9	9.400	1.500	2.221	4.918		0.001	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.115	3.208	2.775	0.022	13.395	0.002	3.057	0.155	0.011	0.016	0	0.004
0	0	0.158	4.209	3.830	0.035	18.618	0.047	3.913	0.206	0.001	0	0	0

Grams Fatty Acids per 100 g Food													
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.201	3.748	2.863	0.157	12.486	0	2.456	0.203	0	0	0	0
0.001	0.001	0.001	0.112	3.153	2.643	0.057	12.632	0.005	2.735	0.141	0.026	0.001	0	0.007
0	0.001	0	0.099	2.649	2.376	0.013	11.263	0.001	2.343	0.123	0.007	0.002	0	0.002
0.379	0.294	3.710	1.358	3.675	3.792	0.164	8.352	0	1.794	0.166	0	0	0	0
0	0	0	0.091	2.395	2.176	0.006	10.440	0	2.167	0.115	0.002	0	0	0.001
0	0	0	0.124	2.873	2.271	0.080	9.810	0.018	2.606	0.257	0	0	0	0
0	0	0	0.087	2.637	2.111	0.110	10.107	0.006	2.214	0.110	0.033	0.008	0	0.010
0	0	0.001	0.103	2.867	2.452	0.073	12.008	0.002	2.900	0.162	0.012	0	0	0.003
0	0	0	0.018	2.257	1.155	0.063	5.045	0.045	8.469	1.234	0	0	0	0
			0.048	1.294	1.142	0.002	5.505	0.001	1.307	0.086				
0.009	0.020	0.022	0.090	1.349	0.494	0.059	2.515	0.020	4.752	0.621	0.011	0	0	0.003
0.001	0.001	0.001	0.015	1.14	0.397	0.037	2.31	0.019	4.84	0.627	0.009	0	0	0.002
0.006	0.013	0.015	0.096	1.51	1.12	0.054	5.19	0.004	1.159	0.064	0.020	0.001	0	0.005
0	0	0	0.021	1.05	0.278	0.031	2.070	0.002	3.02	0.182	0.009	0	0	0.002
0	0	0	0.012	1.220	0.426	0.030	2.562	0.022	5.586	0.722	0.004	0	0	0.001
0	0	0.002	0.008	0.803	0.252	0.016	1.66	0.013	3.664	0.458	0	0	0	0
0.009	0.021	0.024	0.095	1.433	0.525	0.063	2.671	0.021	5.046	0.659	0.012	0	0	0.003
0	0	0	0.056	1.562	1.312	0.005	6.398	0	1.795	0.108	0.001	0	0	
0	0.001	0.001	0.014	1.110	0.372	0.044	2.16	0.018	4.350	0.551	0.013	0	0	0.003

TABLE 14.6
French Toast, Pancakes, and Waffles

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
French Toast									
Fast foods, French toast sticks	53	20.600	3.340	8.970	7.050		0	0	0
French toast, frozen, ready-to-heat	82	6.100	1.533	2.040	1.227		0.022	0.013	0.008
Pancakes									
Plain, dry mix complete, prepared	12	2.500	0.507	0.881	0.820		0.005	0.003	0.002
Plain, dry mix incomplete, prepared	71	7.700	2.045	2.070	2.923		0.048	0.029	0.017
Plain, frozen, ready-to-heat (includes buttermilk)	18	5.17	0.810	1.891	1.081		0	0	0
Plain, frozen, ready-to-heat, microwave (includes buttermilk)		4.73	0.949	2.721	1.070				0
Buckwheat, dry mix incomplete	0	2.70	0.439	0.581	1.000		0	0	0.011
Special dietary, dry mix	0	1.40	0.202	0.276	0.611				
Whole wheat, dry mix, incomplete prepared	61	6.500	1.749	1.742	2.406		0.041	0.025	0.014
Waffles									
Plain, frozen, ready-to-heat microwave	16	9.910	1.580	5.195	2.240				0
Buttermilk, frozen, ready-to-heat	15	9.220	1.890	4.530	1.539		0.005	0.003	0
Plain, frozen, ready-to-heat, toasted	15	9.610	1.638	4.914	2.197		0.001	0	0
Plain, frozen, ready-to-heat	14	9.700	1.531	5.129	2.165		0.001	0	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.7
Frozen Novelties, Gelatin, and Dessert Toppings

	Total per 100 g							
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0
Ice cream cones, cake or wafer type	0	6.90	1.222	1.845	3.264		0	0
Ice cream cones, sugar, rolled type	0	3.80	0.573	1.470	1.452		0	0
Frozen novelties, ice cream type, chocolate or caramel covered with nuts	1	20.20	12.658	4.977	1.469		0.271	0.199
Eskimo pie bar, vanilla ice cream, with dark chocolate coating	28	24.10	14.50					
Frozen novelties, ice cream type, sundae, prepackaged	13	6.00	3.090	1.872	0.663		0	0.092
Frozen novelties, ice cream type vanilla ice cream, light, no sugar added chocolate coated	10	10.10	5.528	2.700	1.359		0.075	0.063

Grams fatty acids per 100 g food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.021	2.154	1.010	0.112	8.531	0.001	6.338	0.458	0.004	0	0	0
0.017	0.019	0.077	0.971	0.380	0.101	1.917	0.007	1.103	0.076	0.036	0.001	0	0.009
0.004	0.005	0.018	0.344	0.125	0.023	0.857	0.001	0.764	0.048	0.006	0	0	0.002
0.037	0.043	0.159	1.24	0.464	0.088	1.96	0.013	2.57	0.321	0.022	0.001	0	0.006
0	0	0.015	0.512	0.253	0.012	1.865	0.013	1.016	0.003	0	0	0	0.001
0	0.005	0.019	0.615	0.275	0.017	2.689	0.015	1.018	0.042	0.003			
0.005	0.004	0.010	0.364	0.029	0.014	0.564	0	0.945	0.054	0.001	0	0	0
	0.001	0.001	0.003	0.018	0.003	0.273		0.586	0.025				
0.032	0.037	0.137	1.06	0.391	0.077	1.650	0.010	2.12	0.264	0.019	0	0	0.005
0	0	0.012	0.909	0.576	0.026	5.099	0.070	1.856	0.371	0.005			
0	0	0.021	1.03	0.772	0.022	4.47	0.039	1.41	0	0.009	0.012	0	0.007
0	0	0.012	0.920	0.624	0.024	4.83	0.061	1.87	0.312	0.001	0.005	0	0.003
0	0	0.011	0.880	0.560	0.025	5.063	0.068	1.798	0	0.005	0.004	0	0.003

Grams Fatty Acids per 100 g Food														
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.018	0.976	0.228	0.012	1.833	0	3.076	0.188	0	0	0	0
0	0	0	0.003	0.400	0.170	0.013	1.457	0	1.355	0.097	0	0	0	0
0.557	0.631	3.244	2.052	3.654	1.824	0.186	4.626	0.041	1.345	0.124	0	0	0	0
0.057	0.113	0.338	0.451	1.267	0.654	0.110	1.690	0.011	0.576	0.063	0.024	0	0	0
0.259	0.245	1.453	0.761	1.567	1.042	0.067	2.594	0	1.239	0.118	0	0	0	0

(*Continued*)

TABLE 14.7
Continued

	Total per 100 g							
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**
Agutuk, fish with shortening (alaskan ice cream; alaska native)	26	43.50	8.600	15.600	17.300			
Agutuk, fish/berry with seal oil (alaskan ice cream; alaska native)	10	31.80	7.700	14.400	8.400			
Agutuk, meat-caribou (alaskan ice cream; alaska native)	89	18.60	5.100	8.700	3.600			
Ice cream, chocolate	34	11.000	6.800	3.210	0.410		0.340	0.200
Fruit and juice bars	0	0.100	0	0	0.030		0	0
Ice cream, french vanilla, soft-serve	91	13.000	7.480	3.490	0.450		0.390	0.230
Ice cream, vanilla	44	11.000	6.790	2.969	0.452		0.360	0.210
Ice cream, vanilla rich	92	16.200	10.329	4.460	0.680		0.491	0.290
Ice cream strawberry	29	8.400	5.190					
Ice cream, chocolate, light	28	7.190	4.380	2.055	0.241		0.226	0.077
Ice cream, chocolate, light no sugar added	16	5.740	3.610	0.930	0.527		0.172	0.105
Ice cream, chocolate, rich	60	16.980	10.38	4.794	0.658		0.517	0.312
Ice creams, french vanilla, soft serve	91	13.00	7.480	3.490	0.450		0.390	0.230
Ice creams, vanilla, fat free	0	0	0	0	0		0	0
Ice creams, vanilla, light, no sugar added	27	7.45	4.048	1.855	0.740		0.213	0.116
Ice creams, vanilla, light	27	4.83	2.927	1.281	0.219		0.141	0.099
Ice creams, vanilla, light, soft serve	12	2.60	1.630	0.760	0.100		0.090	0.050
Ice milk, vanilla, soft-serve, with cone	27	5.94	3.429	1.763	0.348		0	0.121
Ice type, pop	0	0.24	0.013	0.051	0.016		0	0
Ice type, pop, with low-calorie sweetener	0	0	0	0	0		0	0
Ice type, fruit, no sugar added	0	0.10	0				0	0
Ice type, sugar free, orange, cherry, and grape Popsicle pop	0	0	0					
Juice type, orange	0	0	0	0	0		0	0
Fat free Fudgesicle bars	3	0.66	0.480					
No sugar Added, Fudgesicle pops	2	0.97	0.570					
Sugar free, Creamsicle pops	0	2.33	2.00					
No sugar Added Creamsicle pops	3	0.86	0.470					
Ice type, lime	0	0	0	0	0		0	0
Ice type, pineapple-coconut	0	2.600	2.304	0.111	0.029			
Sherbet, orange	0	2.000	1.160	0.530	0.080		0.060	0.030
Frozen yogurts, chocolate	13	3.60	2.272	0.961	0.102		0.096	0.067
Frozen yogurts, chocolate, nonfat milk, sweetened without sugar	4	0.80	0.505	0.214	0.030		0.027	0.009
Frozen yogurts, chocolate, soft-serve	5	6.000	3.630	1.750	0.220		0.160	0.090
Frozen yogurts, flavors, not chocolate	13	3.60	2.326	0.986	0.100		0.111	0.078
Frozen yogurts, vanilla, soft-serve	2	5.600	3.420	1.590	0.210		0.180	0.100
Gelatins								
Dry mix prepared with water	0	0	0	0	0		0	0
Shake								
Shake, fast food vanilla	23	6.52	3.962	1.785	0.316		0.198	0.152
Shake, fast food, strawberry	11	2.80	1.734					
Shake, fast food, chocolate	13	3.700	2.313	1.075	0.140		0.121	0.073

Grams Fatty Acids per 100 g Food														
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
									0.500	16.100				
									0.530	7.100				
									0.310	0.510				
0.120	0.260	0.290	1.070	3.060	1.460	0.240	2.970	0	0.250	0.150	0	0	0	0
0	0	0	0	0	0	0	0	0	0.022	0.008	0	0	0	0
0.130	0.300	0.340	1.240	3.360	1.470	0.270	3.220	0	0.280	0.180	0	0	0	0
0.120	0.280	0.310	1.130	3.060	1.316	0.206	2.763	0	0.275	0.175	0	0.003	0	0
0.169	0.380	0.425	1.526	4.168	1.899	0.360	4.098	0.002	0.437	0.222	0.014	0.001	0	0.004
0.079	0.140	0.091	0.705	2	0.846	0.199	1.86	0	0.168	0.074	0	0	0	0
0.065	0.135	0.149	0.543	1.62	0.706	0.066	0.863	0	0.434	0.092	0	0	0	0
0.188	0.403	0.450	1.62	4.41	2.14	0.361	4.43	0	0.409	0.250	0	0	0	0
0.130	0.300	0.340	1.240	3.360	1.470	0.270	3.220	0	0.280	0.180	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.067	0.149	0.153	0.495	1.439	0.956	0.088	1.388	0.012	0.165	0.032	0.011	0.002	0	0
0.074	0.117	0.127	0.471	1.260	0.570	0.070	1.211	0	0.134	0.085	0	0	0	0
0.030	0.070	0.070	0.270	0.730	0.320	0.060	0.700	0	0.060	0.040	0	0	0	0
0.046	0.115	0.149	0.501	1.500	0.868	0.155	1.516	0	0.249	0.074	0.025	0	0	0
0	0	0	0	0.013	0	0	0.051	0	0.015	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.020	0.050	0.050	0.200	0.510	0.230	0.050	0.490	0	0.050	0.030	0	0	0	0
0.038	0.086	0.106	0.327	0.971	0.443	0.067	0.817	0	0.073	0.029	0	0	0	0
0.009	0.018	0.009	0.071	0.241	0.104	0.018	0.187	0	0.021	0.009	0	0	0	0
0.050	0.120	0.130	0.500	1.610	0.960	0.110	1.640		0.140	0.070				
0.044	0.100	0.122	0.377	0.986	0.355	0.078	0.820	0	0.067	0.033	0	0	0	0
0.060	0.140	0.160	0.570	1.520	0.680	0.130	1.460	0	0.130	0.080	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.066	0.154	0.174	0.593	1.724	0.795	0.116	1.607	0.004	0.261	0.029	0.007	0	0.003	0
							0.701		0.063					
0.043	0.094	0.103	0.373	0.978	0.449	0.022	0.935	0	0.085	0.055	0	0	0	0

(*Continued*)

TABLE 14.7
Continued

	Total per 100 g							
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0
Milk shakes, thick chocolate	11	2.70	1.681	0.780	0.100		0.088	0.052
Milk shakes, thick vanilla	12	3.03	1.886	0.875	0.113		0.098	0.058
Dessert Topping								
Powdered, 1.5 oz prepared with 1/2 cup milk	10	12.41	10.684	0.843	0.201		0.077	0.046
Pressurized	0	22.30	18.912	1.927	0.241		0	0
Semisolid-frozen	0	25.310	21.783	1.616	0.523		0	0

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.8
Pastries and Pies

	Total per 100 g							
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0
Puff Pastry								
Frozen, ready-to-bake, baked	0	38.500	5.502	8.828	22.23		0	0
Toaster Pastries								
Brown sugar, cinnamon	0	14.200	3.639	8.032	1.802		0.005	0.001
Fruit, frosted (apple, blueberry, cherry, strawberry)		10.08	2.672	5.733	1.235	2.319	0	0
Fruit (apple, blueberry, cherry, strawberry)	0	10.56	2.618	6.404	1.082	2.6680	0	0
Kellogg's Pop Tart, frosted chocolate fudge	0	9.300	1.900	5.200	2.200			
Kellogg's Pop Tart, frosted chocolate vanilla crème	0	10.200	1.940	6.190	1.980			
Kellogg's Pop Tart, s'mores	0	10.500	2.800	5.900	1.800			
Kellogg's Pop Tart, apple cinnamon	0	10.200	1.690	5.870	2.600			
Kellogg's Pop Tart, blueberry	0	13.300	2.020	6.500	4.810			
Kellogg's Pop Tart, cherry	0	10.400	1.670	5.730	3.000			
Kellogg's Pop Tart, frosted blueberry	0	10.000	1.980	6.830	1.130			
Kellogg's Pop Tart, frosted cherry	0	10.200	1.940	6.690	1.580			
Kellogg's Pop Tart, frosted grape	0	9.800	1.730	5.90	2.150			
Kellogg's Pop Tart, frosted raspberry	0	10.600	1.880	6.170	2.560			
Kellogg's Pop Tart, frosted strawberry	0	9.600	2.600	5.600	1.400			
Kellogg's Pop Tart, frosted wild berry	0	9.200	2.600	5.400	1.300			
Kellogg's Pop Tart, strawberry	0	10.500	2.900	6.200	1.500			

Grams Fatty Acids per 100 g Food														
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.030	0.068	0.076	0.272	0.710	0.327	0.060	0.679	0	0.061	0.039	0	0	0	0
0.034	0.076	0.085	0.305	0.797	0.367	0.068	0.762	0	0.068	0.044	0	0	0	0
0.194	0.302	3.704	1.682	1.993	2.650	0.053	0.754	0	0.166	0.034	0	0	0	0
0.449	0.642	8.014	3.246	2.408	4.166	0	1.929	0	0.241	0	0	0	0	0
0.638	0.905	8.84	3.756	3.092	4.580	0.241	1.38	0	0.305	0.218	0	0	0	0

Grams Fatty Acids per 100 g Food														
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.039	4.02	1.44	0.078	8.67	0.075	19.640	2.59	0	0	0	0
0.001	0.002	0.001	0.078	1.871	1.675	0.003	8.027	0	1.711	0.091	0	0	0	0
0	0	0	0	1.402	1.231	0	5.719	0	1.182	0.053	0	0	0	0
0	0	0	0	1.475	1.108	0	4.079	0	1.042	0.040	0	0	0	0

(*Continued*)

TABLE 14.8
Continued

	Total per 100 g							
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0
Pies								
Apple, commercially prepared, enriched flour	0	11.00	3.797	4.388	2.198		0	0
Banana cream, prepared from mix, no bake type	29	12.90	6.904	4.559	0.764		0.320	0.189
Blueberry, commercially prepared	0	10.00	1.679	4.244	3.524		0	0
Cherry, commercially prepared	0	11.00	2.562	5.837	2.055		0	0
Chocolate crème, commercially prepared	5	19.40	4.968	11.117	2.398		0	0
Chocolate mousse, prepared from mix, no-bake type	35	15.40	8.195	5.084	0.814		0.387	0.229
Coconut crème, prepared from mix no-bake type	23	17.60	8.934	6.554	1.217		0.248	0.162
Coconut crème, commercially prepared	0	16.60	6.976	7.260	1.544		0.001	0.025
Coconut custard, commercially prepared	35	13.20	5.854	5.495	1.170		0.054	0.049
Egg custard, commercially prepared	33	11.60	2.349	4.797	3.723		0.024	0.011
Lemon meringue, commercially prepared	45	8.70	1.766	2.685	3.648		0	0
Peach	0	10.00	1.508	4.241	3.749		0	0
Pecan, commercially prepared	32	18.50	3.545	10.741	3.182		0	0
Pumpkin, commercially prepared	20	9.50	1.785	4.032	3.150		0.007	0.003
Fried pies, cherry	0	16.10	2.457	7.449	5.376		0.001	
Fried pies, fruit	0	16.100	2.457	7.447	5.376		0.001	0
Fried pies, lemon	0	16.100	2.457	7.449	5.376		0.001	
Pie crust, standard, frozen, ready-to-bake, baked	0	32.800	10.583	15.72	4.034		0	0
Pie crust, standard, dry mix, prepared, baked	0	30.400	7.711	17.29	3.847			
Nabisco Nilla Pie Crust, ready to use	10	27.10	5.150	18.700	1.350			

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.9
Pizzas and Entrees

	Total per 100 g									
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0	10:0
Pizza										
Pizza, cheese topping, regular crust, frozen, cooked	14	12.28	4.272	4.246	1.903		0.275	0.053	0.083	0.112
Pizza, cheese topping, rising crust, frozen, cooked	16	8.78	3.825	2.198	1.381		0.197	0.038	0.059	0.122

Grams Fatty Acids per 100 g Food														
8:0	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0.021	0.126	2.062	1.534	0.146	4.109	0	2.066	0.123	0	0	0	0
0.110	0.247	0.277	1.006	2.991	1.550	0.221	4.191	0	0.600	0.164	0	0	0	0
0	0	0	0.016	1.072	0.585	0.023	4.215	0	3.281	0.239	0	0	0	0
0	0	0	0.042	1.380	1.138	0.013	5.825	0	1.917	0.138	0	0	0	0
0.002	0	0	0.096	2.535	2.331	0.002	1.116	0	2.278	0.120	0	0	0	0
0.133	0.299	0.335	1.214	3.697	1.996	0.267	4.732	0	0.633	0.192	0	0	0	0
0.266	0.336	1.362	1.259	3.191	1.944	0.171	6.269	0	1.060	0.157	0	0	0	0
0.303	0.241	1.917	0.820	1.967	1.700	0.001	7.258	0	1.469	0.075	0	0	0	0
0.228	0.208	1.368	0.732	1.805	1.373	0.038	5.431	0	1.092	0.078	0	0	0	0
0.008	0.018	0.019	0.088	1.469	0.685	0.099	4.673	0.004	3.438	0.249	0.021	0.001	0	0.006
0	0.001	0	0.027	1.345	0.384	0.060	2.619	0.004	3.414	0.205	0.022	0.001	0	0.006
0	0	0	0.011	0.995	0.501	0.038	4.193	0	3.485	0.255	0	0	0	0
0	0	0	0.055	2.020	1.434	0.063	10.604	0.051	3.014	0.140	0.015	0	0	0.004
0.002	0.005	0.006	0.039	1.127	0.586	0.041	3.981	0.001	2.951	0.186	0.007	0	0	0.002
	0.001	0.001	0.032	1.516	0.834	0.012	7.299	0.089	4.808	0.568				
0	0.001	0.001	0.032	1.516	0.834	0.012	7.299	0.089	4.808	0.568	0	0	0	0
	0.001	0.001	0.032	1.514	0.834	0.012	7.299	0.089	4.808	0.568				
0	0.023	0.045	0.295	6.35	3.81	0.622	15.093	0	3.79	0.245	0	0	0	0
			0.149	3.963	3.599		17.289		3.65	0.193				
0.014	0.014	0.014	0.066	2.659	2.269	0.038	13.972	0.086	1.980	0.081	0.014			

Grams Fatty Acids per 100 g Food												
12:0	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.152	0.453	2.039	1.105	0.129	4.092	0.025	1.680	0.167	0.014	0	0	0
0.164	0.517	1.820	0.907	0.121	2.059	0.018	1.155	0.187	0.010	0	0	0

(*Continued*)

TABLE 14.9
Continued

	Total per 100 g									
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0	10:0
Pizza, meat and vegetable topping, regular crust, frozen, cooked	16	14.43	4.800	5.832	2.511					0.112
Pizza, meat and vegetable topping, rising crust, frozen, cooked	19	11.75	4.255	4.296	1.836					
Pizza, pepperoni topping, regular crust, frozen, cooked	15	15.2	4.867	6.865	2.356					
Entrees										
Miscellaneous										
Beef Macaroni, frozen entrée	6	0.93	0.280	0.500	0.140		0	0	0	0
Breakfast burrito, ham and cheese flavor, frozen entrée	194	7.00	2.010	2.100	1.820					
Cinnamon Swirl French toast with sausage, frozen breakfast	63	14.900	4.680	6.040	2.200					
Chicken pot pie, frozen entrée	19	13.41	4.455	5.750	2.068		0	0	0	0.008
Lasagna with meat and sauce, frozen entrée	15	4.73	2.245	1.781	0.336				0.008	0.047
Lasagna with meat and sauce, low-fat, frozen entrée	7	2.23	0.967	0.698	0.279				0.002	0.016
Spaghetti with meat sauce, frozen entrée	6	1.01	0.350	0.330	0.320		0	0	0	0.001
Beef pot pie	19	12.300	4.300	4.890	1.350					
Fish fillet, battered or breaded, fried	34	12.290	2.820	2.580	6.270					
Turkey pot pie	16	8.800	2.880	3.460	1.380					

Abbreviations: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.10
Puddings and Custards

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Puddings									
Banana, dry mix, instant	0	0.600	0.090	0.140	0.350		0	0	0
Banana, dry mix, instant, prepared with 2% milk	6	1.700	1.010	0.480	0.120		0.050	0.030	0.020
Banana dry mix, regular	0	0.400	0.090	0.030	0.160		0	0	0

Grams Fatty Acids per 100 g Food												
12:0	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.121	0.442	2.663	1.462	0.273	5.559		2.291	0.220				
0.126	0.426	2.418	1.286	0.281	4.015							
0.135	0.398	2.726	1.608	0.257	6.608							
0	0.015	0.177	0.072	0.032	0.449	0	0.125	0.013	0.001	0	0	0
0.018	0.124	2.738	1.538	0.267	5.459	0.012	1.890	0.114	0.030	0	0.003	0.009
0.070	0.273	1.160	0.583	0.123	1.547	0.033	0.292	0.039				
0.029	0.119	0.516	0.240	0.049	0.602		0.244	0.034				
0.001	0.020	0.224	0.092	0.024	0.297	0	0.287	0.028	0.003	0	0	0
	0.130	2.260	0.430		2.580		5.780	0.490				

Grams Fatty Acids per 100 g Food												
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.060	0.020	0	0.14	0	0.310	0.040	0	0	0	0
0.040	0.050	0.170	0.440	0.200	0.040	0.440	0	0.090	0.030	0	0	0	0
0	0	0	0.080	0	0	0.030	0	0.160	0.010	0	0	0	0

(*Continued*)

TABLE 14.10
Continued

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Banana dry mix, instant, with added oil	0	4.400	0.790	1.300	2.120		0	0	0
Banana, dry mix, instant, prepared with whole milk	11	2.900	1.740	0.820	0.170		0.090	0.050	0.030
Banana, dry mix, regular, prepared with 2% milk	7	1.700	1.060	0.0480	0.090		0.060	0.030	0.020
Banana, dry mix, regular, prepared with whole milk	12	30	1.830	0.850	0.140		0.090	0.060	0.030
Banana dry mix, regular, with added oil	0	50	0.900	1.480	2.410		0	0	0
Banana, ready-to-eat	0	3.600	0.560	1.530	1.330		0	0	0
Chocolate, dry mix, instant	0	1.900	0.640	0.720	0.420		0	0	0
Chocolate dry mix, regular prepared with whole milk	12	3.150	1.809	0.814	0.179		0.064	0.064	0.064
Chocolate, dry mix, instant-prepared with 2% milk	6	1.900	1.100	0.580	0.130		0.050	0.030	0.020
Chocolate, dry mix, instant-prepared with whole milk	11	3.100	1.830	0.920	0.180		0.090	0.050	0.030
Chocolate, dry mix, regular-prepared with 2% milk	7	1.990	1.189	0.565	0.072		0.053	0.031	0.018
Chocolate, ready-to-eat	3	40	0.710	1.700	1.430		0	0	0
Coconut cream, dry mix, instant, prepared with 2% milk	6	2.300	1.370	0.620	0.190		0.050	0.030	0.050
Coconut cream, dry mix, instant, prepared with whole milk	11	3.500	2.100	0.960	0.240		0.090	0.050	0.060
Coconut cream, dry mix, regular prepared with whole milk	12	3.800	2.570	0.880	0.120		0.090	0.060	0.090
Coconut cream, dry mix, regular prepared with 2% milk	7	2.500	1.800	0.520	0.070		0.060	0.040	0.080
Lemon, dry mix, instant prepared with 2% milk	6	1.700	1.010	0.500	0.100		0.050	0.030	0.020
Lemon, dry mix, instant, prepared with whole milk	11	2.900	1.740	0.850	0.150		0.090	0.050	0.030
Lemon, dry mix, regular, prepared with sugar, egg yolk, water	53	1.300	0.390	0.480	0.170		0	0	0
Lemon, dry mix, with added oil, potassium, sodium	0	1.500	0.0270	0.440	0.720				
Lemon, ready-to-eat	0	30	0.450	1.300	1.140		0	0	0
Rice pudding, dry mix, prepared with 2% milk	6	1.600	1.010	0.450	0.060		0.050	0.030	0.020
Rice pudding, dry mix, prepared with whole milk	11	2.820	1.682	0.767	0.104		0.091	0.054	0.031
Rice pudding, ready-to-eat	1	7.500	1.170	3.210	2.790		0	0	0
Tapioca, dry mix, prepared with whole milk	12	2.890	1.724	0.786	0.107		0.093	0.055	0.032
Tapioca, dry mix, prepared with 2% milk	6	1.670	0.991	0.452	0.061		0.53	0.032	0.018
Tapioca, ready-to-eat	1	3.700	0.931	2.251	0.324		0.001	0	0
Vanilla, dry mix, instant, prepared with whole milk	11	2.900	1.740	0.840	0.150		0.090	0.050	0.030
Vanilla, dry mix, regular, prepared with 2% milk	7	1.730	1.017	0.462	0.088		0.054	0.032	0.018

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.620	0.160	0	1.290	0	1.990	0.120				
0.070	0.080	0.290	0.770	0.070	0.760	0	0.120	0.050	0	0	0	0	0
0.040	0.050	0.170	0.470	0.210	0.040	0.440	0.060	0.030	0	0	0	0	0
0.070	0.080	0.310	0.820	0.370	0.070	0.780	0	0.090	0.050	0	0	0	0
0	0	0	0.700	0.180	0	1.470	0	2.270	0.140	0	0	0	0
0	0	0.010	0.360	0.180	0.020	1.520	0	1.240	0.090	0	0	0	0
0	0	0	0.310	0.320	0.010	0.720	0	0.400	0.030	0	0	0	0
0.064	0.066	0.254	0.803	0.431	0	0.814	0	0.115	0.064	0	0	0	0
0.040	0.050	0.170	0.490	0.250	0.040	0.540	0	0.100	0.030	0	0	0	0
0.070	0.080	0.290	0.820	0.410	0.070	0.850	0	0.130	0.050	0	0	0	0
0.041	0.046	0.164	0.521	0.316	0.037	0.528	0	0.049	0.024	0	0	0	0
0	0	0.010	0.440	0.260	0.020	1.390	0	1.330	0.100	0	0	0	0
0.060	0.210	0.230	0.500	0.230	0.040	0.580	0	0.160	0.030	0	0	0	0
0.090	0.250	0.350	0.830	0.380	0.07	0.890	0	0.190	0.050	0	0	0	0
0.120	0.460	0.450	0.880	0.420	0.070	0.810	0	0.080	0.050	0	0	0	0
0.090	0.420	0.320	0.530	0.260	0.040	0.470	0	0.050	0.030	0	0	0	0
0.040	0.050	0.170	0.440	0.210	0.040	0.460	0	0.080	0.030	0	0	0	0
0.070	0.080	0.290	0.770	0.360	0.070	0.780	0	0.100	0.050	0	0	0	0
0	0	0	0.280	0.100	0.040	0.440	0	0.150	0	0.020	0	0	0
		0	0.210	0.050	0.010	0.440		0.680	0.040				
0	0	0	0.300	0.150	0.010	1.280	0	1.060	0.080	0	0	0	0
0.040	0.050	0.170	0.440	0.200	0.040	0.420	0	0.040	0.020	0	0	0	0
0.070	0.079	0.282	0.737	0.339	0.063	0.704	0	0.063	0.041	0	0	0	0
0	0	0.020	0.760	0.380	0.030	3.170	0	2.590	0.190	0	0	0	0
0.072	0.081	0.289	0.755	0.348	0.064	0.722	0	0.064	0.042	0	0	0	0
0.041	0.046	0.166	0.434	0.200	0.037	0.415	0	0.037	0.024	0	0	0	0
0.001	0.001	0.008	0.422	0.465	0.004	2.245	0.001	0.316	0.008	0	0	0	0
0.070	0.080	0.290	0.770	0.360	0.070	0.780	0	0.100	0.050	0	0	0	0
0.042	0.047	0.168	0.453	0.203	0.037	0.425	0	0.062	0.026	0	0	0	0

(*Continued*)

TABLE 14.10
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Vanilla, dry mix, regular, prepared with whole milk	9	2.900	1.643	0.714	0.197		0.065	0.065	0.065
Vanilla, dry mix, regular, with added oil	0	1.100	0.200	0.320	0.530				
Vanilla, ready-to-eat	7	3.600	1.512	1.483	0.410		0.004	0.001	0.001
Custards									
Egg, dry mix, prepared with whole milk	53	4.100	2.122	1.201	0.244		0.098	0.059	0.033
Egg, dry mix, prepared with 2% milk	56	2.740	1.357	0.852	0.197		0.057	0.035	0.019
Egg, dry mix	258	6.400	2.030	2.400	0.850		0.010	0.010	0
Flan, caramel custard, dry mix, prepared with 2% milk	7	1.720	1.035	0.472	0.064		0.056	0.033	0.019
Flan, caramel custard, dry mix, prepared with whole milk	12	30	1.800	0.821	0.111		0.097	0.057	0.033

Abbreviation: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

TABLE 14.11
Snacks

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Chips									
Banana chips	0	33.600	28.970	1.950	0.630		0	0.200	2.510
Corn-based, extruded, plain	0	28.41	3.612	7.913	14.010		0	0	0
Corn-based, extruded, cones, nacho-flavor	5	31.700	26.790	2.140	0.840		0.030	0.200	2.280
Corn-based, extruded, onion-flavor	0	22.600	4.340	13.360	3.140		0.020	0	0
Corn-based, extruded, barbecue flavor	0	32.700	4.460	9.480	16.170				
Corn-based, extruded, puffs or twists, cheese-flavor	4	35.760	5.512	10.44	18.65	0.806	0.030	0	0
Cornnuts, barbecue flavor	0	14.300	2.580	7.360	3.220				
Cornnuts, nacho-flavor	2	14.200	2.560	7.310	3.200				
Potato chips, cheese flavor	4	27.200	8.600	7.720	9.560				
Potato chips from dried potatoes, cheese flavor	4	37.00	9.570	7.140	18.600		0.050	0.030	0.010
Potato chips from dried potatoes, sour cream and onion flavor	3	37.00	9.470	7.120	18.780		0.040	0.020	0.010
Potato chips, plain, made with partially hydrogenated soybean oil, salted	0	34.600	5.430	18.000	9.160				

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.065	0.067	0.259	0.738	0.319	0	0.714	0	0.130	0.067	0	0	0	0
			0.160	0.040		0.320		0.500	0.030				
0.003	0.004	0.019	0.716	0.674	0.005	1.464	0.014	0.331	0.079	0	0	0	0
0.077	0.086	0.309	1.015	0.444	0.097	1.100	0.003	0.180	0.047	0.014	0	0	0.003
0.045	0.050	0.181	0.680	0.290	0.069	0.780	0.003	0.151	0.028	0.014	0	0	0.003
0.010	0.010	0.050	1.430	0.510	0.190	2.190	0.020	0.71	0.020	0.090	0	0	0.020
0.043	0.048	0.173	0.453	0.209	0.039	0.434	0	0.039	0.025	0	0	0	0
0.075	0.084	0.301	0.788	0.363	0.067	0.753	0	0.067	0.044	0	0	0	0

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
2.010	14.910	5.620	2.790	0.940	0	1.950	0	0.620	0.010	0	0	0	0
0	0	0	2.891	0.570	0.027	9.570	0	15.230	1.260	0	0	0	0
1.830	13.520	5.170	2.810	0.960	0.030	2.120	0	0.830	0.020				
0	0.040	0.160	2.800	1.310	0.080	13.270	0	3.010	0.130	0	0	0	0
			3.510	0.960	0.090	9.390		14.940	1.230				
0.038	0.045	0.158	4.13	0.942	0.056	9.69	0.080	18.2	0.300	0	0	0	0
		0.030	1.600	0.950	0.010	7.340		3.170	0.050				
		0.030	1.590	0.940	0.010	7.290		3.150	0.050				
0	0.060	0.240	7.320	0.870	0.140	7.470	0.120	9.410	0.150				
0.040	0.040	0.470	7.780	1.160	0.360	6.780		18.310	0.350				
0.030	0.030	0.450	7.770	1.140	0.360	6.760		18.430	0.350				
			3.970	1.460		17.930	0.070	8.900	0.260				

(*Continued*)

TABLE 14.11
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Potato chips, plain, salted	0	37.47	10.960	9.840	12.170		0	0	0
Potato chips, sour-cream-and-onion-flavor	7	33.900	8.890	6.120	17.420		0.010	0.010	0
Potato chips, barbecue-flavor	0	32.400	8.050	6.540	16.370		0	0	0
Potato chips, made from dried potatoes, light	0	25.700	5.130	5.920	13.490		0	0	0
Potato chips from dried potatoes, plain	0	38.400	9.450	7.270	19.980		0	0	0
Potato chips, reduced fat	0	20.80	4.160	4.800	10.940		0	0	0
Potato chips, fat-free, made with olestra	0	0.70	0.240	0.180	0.304		0	0	0
Potato chips, made from dried potatoes, fat-free, made with olestra	0	0.85	0.355	0.300	0.190		0	0	0.001
Potato chips, white, restructured, baked	0	18.20	2.631	9.910	4.234		0	0	0
Potato sticks	0	34.40	8.880	6.160	17.900		0	0	0
Taro	0	24.900	6.430	4.430	12.880		0	0	0
Torilla chips, taco flavor	5	24.200	4.640	14.290	3.360		0.020		
Tortilla chips, nacho flavor, made with enriched masa flour	3	25.600	4.900	15.090	3.540		0.020	0	0
Tortilla chips, nacho flavor, reduced fat	3	15.200	2.910	8.950	2.100		0.010	0	0
Tortilla chips, plain, white corn	0	23.36	2.827	7.748	4.768	3.057	0	0	0
Tortilla chips, light (baked with less oil)	3	15.20	2.837	6.341	5.024		0.034	0.014	0.007
Tortilla chips, low fat, made with olestra, nacho cheese	2	3.93	1.054	1.379	1.495		0.004	0.002	0.004
Tortilla chips, low fat, unsalted	0	5.70	0.850	1.660	2.870		0	0	0
Tortilla chips, nacho cheese		26.07	3.936	7.067	13.324				0
Tortilla chips, unsalted, white corn	0	23.36	2.458	9.520	9.956		0	0	0
Tortilla chips, ranch-flavor		24.63	3.539	7.047	13.528	0.345			0
Crackers									
Animal crackers (includes arrowroot, tea biscuits)	0	13.80	3.463	7.666	1.873		0	0	0
Graham crackers, chocolate coated	0	23.20	13.380	7.687	1.038		0	0	0.002
Graham crackers, plain or honey (includes cinnamon)	0	10.10	1.519	4.086	3.832		0	0	0.005
Cracker meal	0	1.70	0.271	0.152	0.722		0	0	0
Cheese, regular	13	25.300	9.371	12.105	2.472		0.268	0.135	0.071
Cheese, sandwich-type with cheese filling	6	24.41					0	0	0
Cheese, sandwich type with peanut butter filling	0	25.12	4.406	13.003	5.093		0	0	0
Crispbread, rye	0	1.300	0.145	0.165	0.557		0	0	0
Matzo, egg	83	2.100	0.548	0.612	0.473				0
Matzo, egg, and onion	45	3.9	0.936	0.987	1.001				0.001

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0.080	0.300	9.320	1.110	0.180	9.510	0.150	11.980	0.190	0	0	0	0
0.010	0.010	0.300	7.730	0.820	0.280	5.840	0	17.340	0.080				
0	0.010	0.250	7.000	0.750	0.250	6.260	0.030	16.280	0.090	0	0	0	0
0	0	0.150	4.340	0.630	0.620	5.300	0	13.250	0.240	0	0	0	0
0	0	0.360	7.990	1.090	0.360	6.900	0	19.620	0.360	0	0	0	0
0	0	0.120	3.520	0.510	0.500	4.300	0	10.750	0.190	0	0	0	0
0	0.007	0.009	0.172	0.037	0.006	0.174	0	0.275	0.029	0	0	0	0
0.001	0.009	0.002	0.162	0.060	0	0.300	0	0.160	0.030	0	0	0	0
0	0.014	0.003	1.812	0.802	0	9.910	0	4.037	0.197	0	0	0	0
0	0	0.270	7.810	0.800	0.270	5.880	0	17.800	0.070	0.030	0	0	0
0	0	0.200	5.650	0.570	0.200	4.230	0	12.820	0.060	0	0	0	0
	0.050	0.170	3.000	1.410	0.090	14.200		3.220	0.140				
0	0.050	0.180	3.170	1.480	0.100	15.000	0	3.400	0.140	0	0	0	0
0	0.030	0.110	1.880	0.880	0.060	8.890		2.020	0.090				
0	0	0.012	1.842	0.808	0.015	7.704	0.029	4.639	0.129	0	0	0	0
0.018	0.023	0.094	1.648	0.373	0.028	6.149	0.152	4.977	0.047	0	0	0	0
0.017	0.031	0.105	0.669	0.222	0.020	1.337	0.019	1.445	0.050	0	0	0	0
0	0	0	0.684	0.090	0.005	1.500	0	2.524	0.078	0	0	0	0
0.031	0.036	0.129	2.988	0.597	0.042	6.331	0.059	12.265	0.237	0			
0	0.045	0.002	1.856	0.418	0.034	9.242	0.184	8.860	1.096	0	0	0	0
0.022	0.022	0.022	2.769	0.548	0.022	6.719	0.059	13.195	0.235	0			
0	0	0.067	1.797	1.591	0.003	7.663	0	1.776	0.096	0	0	0	0
0	0.045	0.113	5.859	7.098	0.071	7.623	0	0.973	0.064	0	0	0	0
0	0	0.010	1.029	0.471	0.041	4.036	0	3.563	0.259	0.001	0	0	0
0	0	0	0.259	0.012	0	0.152	0	0.683	0.039	0	0	0	0
0.154	0.154	0.875	4.368	3.007	0.270	11.700	0	2.339	0.132	0	0	0	0
0	0	0.200	2.703	1.790	0	14.177	0.130	2.293	0.033	0	0	0	0
0	0	0	3.307	0.550	0	12.770	0.233	5.093	0	0	0	0	0
0	0	0.002	0.138	0.005	0.012	0.146	0.006	0.479	0.079	0	0	0	0
0	0	0.005	0.423	0.119	0.043	0.564	0.004	0.427	0.020	0.021	0.001		0.005
0.001	0.001	0.008	0.742	0.184	0.066	0.914	0.006	0.916	0.045	0.031	0.001		0.008

(*Continued*)

TABLE 14.11
Continued

	Total per 100 g								
	CHOL (mg)	**Lipids (g)**	**SFAs (g)**	**MUFAs (g)**	**PUFAs (g)**	**TFAs (g)**	**4:0**	**6:0**	**8:0**
Matzo, plain	0	1.400	0.226	0.127	0.603		0	0	0
Matzo, whole-wheat	0	1.50	0.243	0.194	0.653		0	0	0
Melba toast, plain	0	3.20	0.445	0.782	1.282		0	0	0
Melba toast, wheat	0	2.30	0.337	0.544	0.910				
Milk	11	15.800	2.633	6.479	5.749		0.016	0.005	0.005
Rusk toast	78	7.200	1.376	2.755	2.310		0	0	0
Rye, wafers, plain	0	0.900	0.108	0.152	0.397		0	0	0
Rye, wafers, seasoned	0	9.20	1.287	3.270	3.608		0	0	0
Rye, sandwich-type with cheese filling	9	22.300	5.987	12.236	2.864		0.031	0.015	0.008
Saltines (includes oyster, soda, soup)	0	11.35	1.656	7.004	1.246	3.598	0	0	0
Standard, snack-type, regular	0	25.30	3.776	10.640	9.545		0	0	0
Standard, snack-type, sandwich with cheese filling	2	21.100	6.125	11.254	2.567		0.031	0.017	0.009
Standard, snack-type sandwich with peanut butter filling	0	24.54	4.913	13.776	4.655		0	0	0
Wheat, regular	0	20.600	5.178	11.448	2.796		0	0	0.008
Wheat, sandwich with cheese filling	7	25	4.129	10.351	9.166		0.027	0.014	0.011
Wheat, sandwich with peanut butter filling	0	26.700	4.602	11.766	8.862		0	0	0.003
Whole-wheat	0	17.200	3.393	5.880	6.596		0	0	0.016
Popcorn									
Air-popped	0	4.54	0.570	1.100	1.900		0	0	0
Air-popped, white popcorn	0	4.20	0.570	1.100	1.900				
Cakes	0	3.100	0.480	0.920	1.350		0	0	0
Caramel coated without peanuts	5	12.800	3.610	2.880	4.480		0.110	0.070	0.040
Caramel coated with peanuts	0	7.800	1.040	2.730	3.270		0	0	0
Cheese flavored	11	33.200	6.410	9.700	15.370		0.030	0.020	0.010
Microwave, low fat and sodium	0	9.50	1.415	4.085	3.572		0	0	0
Microwave, 94% fat free	0	7.44	1.161	2.605	2.496	0.931	0	0	0
Oil-popped, microwave, regular flavor	0	43.55	6.820	9.809	23.191		0	0	0
Oil-popped, unsalted	0	28.100	4.890	8.170	13.420		0	0	0
Oil-popped, white popcorn	0	28.100	4.890	8.170	13.420				
Pretzels									
Hard, plain, salted	0	2.63	0.344	1.292	0.787		0	0	0
Hard, whole wheat	0	2.600	0.560	1.030	0.830				0
Hard, confectioner's coating, chocolate flavor	0	16.700	7.680	5.390	2.150		0	0.010	0.210
Soft	3	3.10	0.695	1.071	0.948		0	0	0
Trail Mix									
Regular, with chocolate chips, salted nuts and seeds	4	31.900	6.100	13.540	11.290			0.010	0.210
Regular	0	29.4	5.550	12.530	9.650			0.010	0.200
Tropical	0	17.10	8.480	2.490	5.160			0.050	0.640

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0	0	0	0.216	0.010	0	0.127	0	0.570	0.032	0	0	0	0
0	0	0.002	0.222	0.012	0.010	0.184	0	0.618	0.033	0.002	0	0	0
0	0	0.002	0.355	0.088	0.020	0.762	0	1.210	0.071	0	0	0	0
	0	0.002	0.275	0.056	0.018	0.526		0.858	0.050	0.001			
0.011	0.012	0.068	1.700	0.795	0.087	6.367	0.001	5.335	0.395	0.003	0.001	0	0.001
0	0	0.011	0.973	0.386	0.065	2.678	0.005	2.114	0.143	0.026	0.013	0	0.008
0	0	0.001	0.101	0.006	0.003	0.145	0.004	0.349	0.048	0	0	0	0
0	0	0	0.921	0.352	0.037	3.215	0.011	3.283	0.318	0	0	0	0
0.018	0.017	0.202	3.063	2.602	0.031	12.191	0.001	2.702	0.161	0	0	0	0
0	0	0.007	0.937	0.600	0.008	6.975	0.020	1.190	0.057	0	0	0	0
0	0	0.025	2.516	1.235	0.098	10.517	0	8.865	0.655	0	0	0	0
0.020	0.020	0.196	2.911	2.446	0.058	11.165	0	2.415	0.153	0	0	0	0
0	0	0	3.077	1.250	0	13.559	0.217	4.655	0	0	0	0	0
0	0	0.099	2.693	2.371	0.006	11.442	0	2.653	0.140	0.001	0	0	0
0.017	0.017	0.107	2.639	1.271	0.119	10.196	0	8.505	0.637	0	0	0	0
0	0.005	0.064	2.809	1.335	0.061	11.594	0.097	8.436	0.411	0	0	0	0
0	0	0.003	1.744	1.617	0.012	5.868	0	6.218	0.376	0.002	0	0	0
0	0	0	0.592	0.084	0.006	1.028	0.015	2.499	0	0	0	0	0
			0.500	0.070	0	1.100							
0	0	0	0.420	0.050	0.010	0.910	0	13.100	0.040	0	0	0	0
0.090	0.100	0.370	2.120	0.720	0.100	2.780	0	4.070	0.400	0	0	0	0
0	0	0	0.820	0.160	0.010	2.670	0.050	3.210	0.060	0	0	0	0
0.030	0.020	0.220	4.800	1.280	0.100	9.600	0	14.500	0.880	0	0	0	0
0	0	0.010	0.931	0.475	0.038	4.037	0	3.315	0.247	0	0	0	0
0	0	0.06	0.818	0.287	0.008	2.853	0.013	2.427	0.069	0	0	0	0
0	0	0	4.610	1.873	0.037	9.654	0.118	22.339	0.853	0	0	0	0
0	0	0.080	3.930	1.040	0.060	8.230	0	12.730	0.790	0	0	0	0
		0.070	3.860	0.960	0.050	8.120		12.680	0.730				
0	0	0	0.253	0.085	0.001	0.643	0.005	0.685	0.051	0	0	0	0
0	0.030	0.020	0.340	0.170		1.030		0.790	0.040				
0.230	2.920	1.060	1.940	1.310	0.020	5.370	0	1.990	0.160	0	0	0	0
0	0	0.009	0.446	0.243	0	1.070	0	0.892	0.055	0	0	0	0
0.180	1.260	0.540	2.750	1.150	0.070	13.280	0.190	11.210	0.080				
0.160	1.170	0.480	2.530	1.000	0.070	12.290	0.170	9.580	0.070				
0.510	3.860	1.490	1.270	0.640	0.010	2.460	0.010	5.120	0.040				

(*Continued*)

TABLE 14.11
Continued

	Total per 100 g								
	CHOL (mg)	Lipids (g)	SFAs (g)	MUFAs (g)	PUFAs (g)	TFAs (g)	4:0	6:0	8:0
Miscellaneous Snacks									
Beef jerky, chopped and formed	48	25.60	10.850	11.305	1.011		0	0	0
Beef sticks, smoked	133	49.60	20.800	20.470	4.420				
Pork skins, Barbecue-flavor	115	31.800	11.560	15.030	3.450				
Pork skins, plain	95	31.300	11.370	14.780	3.640		0	0	0
Oriental mix, rice-based	0	25.58	3.785	9.86	10.64		0	0	0
Sesame sticks, wheat-based, salted	0	36.7	6.48	10.91	17.42		0	0	0
Sweet potato chips	0	24.7	3.5	9.12	12.24		0	0	0

Abbreviation: CHOL, cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans* fatty acids.

is either no content of fatty acid or that trace amounts may still be present as reported in the USDA Nutrient Database for Standard Reference (USDASR); blanks indicate that no data were provided by the USDASR.

A word concerning the *trans* fatty acid content of the various convenience foods. As of January 1, 2006, food manufacturers have been required to include the content of *trans* fatty acids in their respective products' food labels if one serving of that product contains 0.5 g or more of *trans* fatty acids (or *trans* fat) per serving size (for a description of labeling law see http://www.cfsan.fda.gov/). To date, only a fraction of the 7293 foods currently included in the USDA, Agricultural Research Service, Nutrient Database for Standard Reference, Release 19 (USDA, 2006) have their *trans* fatty acid content included. For this reason, only a limited amount of convenience foods in this chapter have the total amount of *trans* fatty acid (as total *trans* fat) included in their respective tables.

This section for babyfood (Table 14.1) has been expanded considerably since the previous edition of this chapter (Boosalis, 1999). Included in this section are prepared babyfoods that contain lipids. Excluded are foods such as fruits, juices, and vegetables that would not naturally contain or have added lipids. Other than several of the meat products that are included, the total lipid content of most of the other foods is relatively low.

In contents of the "Bars" section of Table 14.2, as a group, have a low cholesterol content (per 100 g of foodstuff) but their percentage of total lipid ranges from 2.4% to 31%, primarily in the form of 18:1, 18:2, 12:0, and 16:0 fatty acids, whereas the "Cakes" themselves have a higher content of cholesterol, yet a lower percentage of total lipid (range 14.8%–18.6%) with 18:1 and 16:0 being the predominant fatty acids in that grouping.

In Table 14.3, the "Candies" section, the cholesterol content varies depending on whether caramel or chocolate is present in the product, and the total percentage of the product that is lipid also varies tremendously (1.6%–52.3%), with 18:1, 18:0, 16:0, and 12:0 being the predominant fatty acids. A small number of candy bars also have their content of *trans* fatty acids included.

In Table 14.4, the cholesterol content of "Cookies" is relatively low per 100 g with the exception of the ladyfingers. The percentage of lipid in the cookies also varies, from 1.5% to 34%, with 18:1, 18:0, 16:0, and 18:2 fatty acids being predominant. In fact, as one looks especially at this table, one can see that small changes (likely due to a change in the ingredients) can make a difference in the fatty acid content of the food. As examples, "chocolate chip, commercially prepared, regular,

Grams Fatty Acids per 100 g Food													
10:0	**12:0**	**14:0**	**16:0**	**18:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:5**	**22:6**
0.015	0.162	0.819	5.720	3.897	1.135	10.099	0.071	0.810	0.185	0.016	0	0	0
0.010	0.330	1.430	11.770	7.260	2.300	17.910	0.250	4.030	0.380	0.010			
0.020	0.070	0.400	6.960	4.100	0.990	13.780	0.250	3.410	0	0.040	0	0	0
0.020	0.070	0.390	6.850	4.040	0.970	13.560	0.250	3.350	0.260	0.030	0	0	0
0	0	0.06	2.52	1.06	0.09	9.71	0.03	10.3	0.32	0	0	0	0
0.01	0.01	0.11	5	1.35	0.07	10.8	0	16.4	0.97	0	0	0	0
0	0	0	1.4	0.81	0.02	8.84	0.19	11.1	1.15	0	0	0	0

lower fat" compared to "chocolate chip, commercially prepared, regular, higher fat" have a total lipid content that is about one-third less, owing primarily to a decrease in both the saturated and monounsaturated fatty acid content of the former vs. the latter product. When "chocolate sandwich, crème-filling, special dietary" is compared to the "regular" version of the same cookie, the total lipid and saturated fatty acid content of the products are about the same. The difference in those products seems to be in the type of fat used. The "special dietary" version seems to have more of its fatty acids in the form of polyunsaturated fatty acids compared to the "regular" version of the product, which is also reported to contain *trans* fatty acids.

In Table 14.5, the total cholesterol per 100 g of food ranges between 0 and 62 mg and the percentage of total lipids varies between 9.4% and 25.25%, with 18:1, 18:2, 16:0, and 18:0 fatty acids being predominant.

In Table 14.6, the cholesterol content per 100 g varies between 0 and 82 mg and the percentage of total lipid varies between 1.4% and 20.6%, with 18:1, 18:2, and 16:0 being the predominant fatty acids.

In Table 14.7, the cholesterol content per 100 g of foodstuff varies between 0 and 92 mg, whereas the percentage of the product that is total lipid varies between 0% and 43.5%, with 16:0, 18:1, 18:0, and 14:0 being the predominant fatty acids. Of note, the products that have the most total lipid per 100 g (31%–43.5%) are two types of Alaskan ice creams made from fish with either shortening or seal oil. As predicted, these products also have a relatively high content of both mono- and polyunsaturated fatty acids. One of the highest content of saturated fatty acids is found in semisolid frozen dessert topping per 100 g, but keep in mind that a 1-tsp serving weighs only 4 g.

In Table 14.8, the lipid content varies, primarily because of the total lipid content of puff pastry and pie crust.

In Table 14.9, "Pizza" is approximately 9%–15% total lipid, depending on whether cheese, cheese and meat, or pepperoni is added. For most products, depending on how you make them, the fatty acid content will also be affected.

As shown in Table 14.10 with respect to pudding mixes, the lipid content seems directly related to the type of milk used in their preparation. The data are reported using either whole milk or 2% milk. Although data are not provided on the USDASR for puddings prepared with skim milk, using the latter would further reduce the total lipid content and alter the fatty acid profile.

In Table 14.11, "Snacks," the total lipid content varies with the product, with the subcategory of "Chips" products containing the greatest percentage of total lipid. The fatty acid profile of these products also varies widely, depending on the particular product.

Since space does not permit the inclusion of a typical serving size in the tables, the author recommends that the readers either check the nutrition label on the product of concern to determine its serving size or consult the USDA website (http://www.mypyramid.gov/pyramid/index.html) to obtain specific information on the particular serving size for each food group of My Pyramid.

With respect to the clinical and public health implications of choosing convenience foods with respect to their fat intake, the author recommends that the readers become familiar with the various types of fat and fatty acids listed in these products and then compare the products with the type of fat or fats used in the ingredients as listed on their labels. In general, when making daily food choices, the author recommends that the readers follow the recommendations as outlined on the USDA website (http://www.mypyramid.gov/) (USDA, 2005) with the number of servings per group selected being appropriate for the individual's activity level, age, and gender. In addition, the acceptable macronutrient distribution range (AMDR) for total daily intake of fat is 20%–35% of total energy with the intake of saturated fatty acids is not more than 10% of the day's total kilocalorie intake (National Academy of Sciences, 2005). Owing to the observation of a positive linear trend between *trans* fatty acid intake and total and low-density lipoprotein (LDL) cholesterol, it is recommended "that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet" (National Academy of Sciences, 2002). The information presented in this chapter should aid the readers in achieving these suggested goals.

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15 *Trans*-Fatty Acids in Foods

Margaret C. Craig-Schmidt and Carmen A. Teodorescu

CONTENTS

I. INTRODUCTION

Hydrogenated fat has been an important component of the Western diet. The use of margarine and other products made from hydrogenated fat increased as consumers became concerned about their intake of saturated fat and cholesterol. Vegetable oils used in the manufacture of margarine contain polyunsaturated fatty acids that are modified in structure during processing. Similarly, dairy products and meats from ruminant animals contain fat that has been modified by biohydrogenation. The term *isomeric* in reference to dietary fat generally refers to those fatty acids that are formed as a result of partial hydrogenation of unsaturated fat. Whether the process occurs in a commercial plant or in the rumen of an animal, double bonds originally present are modified in both conformation and position.

The question of whether or not isomeric fatty acids should be termed *unnatural* has been debated (Applewhite, 1981; Sommerfeld, 1983; Wood, 1983). Isomeric fatty acids are formed by biohydrogenation in the rumen of animals and thus appear "naturally" in meat and dairy products. On the other hand, the commercial process of partial hydrogenation by which margarines and shortenings are manufactured results in the presence of isomeric fatty acids considered to be "unnatural," because they were not present in the original vegetable oils. The issue, however, is not one of semantics but one of whether current amounts of these isomeric fatty acids in the food supply are having an effect on the health of populations consuming hydrogenated fat. Concerns have been raised about the possible effects of these isomeric fatty acids in promoting the incidence of cardiovascular disease (Kummerow, 1975, 1979; Vergroesen and Gottenbos, 1975; Kritchevsky, 1983; Mensink and Katan, 1990; Siguel and Lerman, 1993; Willett et al., 1993; Judd et al., 1994; Aro, 1998; Mensink and Zock, 1998; Stender and Dyerberg, 2004; Mozaffarian et al., 2006) and cancer (Enig et al., 1978). Additionally, there has been concern about possible detrimental effects of *trans*-fatty acids in infant development (Koletzko, 1992, 1994; Berra, 1993; van Houwelingen and Hornstra, 1994; International Life Sciences Institute Expert Panel on *Trans*-Fatty Acids and Early Development, 1997; Craig-Schmidt, 2001). On the other hand, conjugated linoleic acid (CLA; predominantly 9-*cis*, 11-*trans*-octadecadienoic acid) found principally in ruminant products in small, but significant, quantities is believed to be antiathrogenic and anticarcinogenic. This active area of research has been reviewed by Pariza et al. (1991), Parodi (1994), Ip et al. (1994), Chardigny et al. (1996a), Ip (1997), and Banni and Martin (1998). The physiological effects of isomeric fatty acids as they are metabolized and incorporated into membranes have been reviewed by a number of authors (Aaes-Jørgensen, 1966; Kummerow, 1974, 1975, 1979, 1986; Kaunitz, 1976; Alfin-Slater and Aftergood, 1979; Emken, 1979, 1981, 1983, 1984; Applewhite, 1981; Brisson, 1981; Kinsella, 1981; Kinsella et al., 1981; Beare-Rogers, 1983a; Gottenbos, 1983; Holman et al., 1983; Hølmer, 1998; Sebedio and Chardigny, 1998) and are beyond the scope of this chapter.

The studies on the physiological effects of isomeric fatty acids, however, cannot be properly interpreted unless they are put in the context of consumption data. The purpose of this chapter is thus to summarize what is known to date about the amounts and types of isomeric fatty acids in various foods and to review current estimates of isomeric fatty acids in the food supply. Earlier reviews of this topic include the Canadian Report of the Ad Hoc Committee on the Composition of Special Margarines (Spence et al., 1980), an article by Sommerfeld (1983) that emphasizes work done in Germany and other European countries, and a report by the Life Sciences Research Office for the U.S. Food and Drug Administration (Senti, 1985). Hunter and Applewhite (1986), Enig et al. (1990a), Craig-Schmidt (1998), and Craig-Schmidt (2006) review estimates of isomeric fatty acids in the diet but do not summarize the food composition data of individual food items. Additional reviews include (1) a report by the International Life Science Institute Expert Panel on *Trans*-Fatty Acids and Coronary Heart Disease (1995); (2) the American Society for Clinical Nutrition/American Institute of Nutrition Task Force on *Trans*-Fatty Acids (1996); (3) the International Life Sciences Institute Expert Panel on *Trans*-Fatty Acids and Early Development (1997); (4) two reports by the British Nutrition Foundation (1987, 1995); (5) the Danish Nutrition Council Report by Stender et al. (1994, 1995); (6) the FAO/WHO Report of the Joint Expert Consultation on Fats and Human Nutrition (FAO, 1993) and reviews by Gurr (1983, 1986, 1996), Emken (1984), Sanders (1988), Schaafsma (1992), Enig (1993, 1995), Wahle and James (1993), Mensink and Katan (1993), Precht and Molkentin (1995), and Becker (1996).

II. FORMS AND SOURCES OF DIETARY ISOMERIC FATTY ACIDS

A. Forms of Dietary Isomeric Fatty Acids

The major fatty acid isomers formed in the process of hydrogenation are classified into two types: positional and geometric isomers (Dutton, 1979). The term isomeric fat refers to both these types as well as to minor amounts of other fatty acids found in hydrogenated fat.

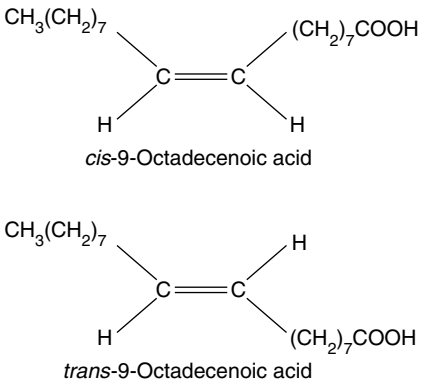

FIGURE 15.1 Geometrical isomerism in unsaturated fatty acids.

Positional isomers are formed in the hydrogenation process when double bonds shift from their original position in the unprocessed oil to other positions in the molecule. For example, fatty acids containing double bonds at the Δ9 and Δ12 positions are changed to isomeric forms containing double bonds ranging from positions Δ4 to Δ16 (Elson et al., 1981; Marchand, 1982), with the greatest concentration clustered in the vicinity of the original double bond (Scholfield et al., 1967; Carpenter and Slover, 1973; Parodi, 1976a; Emken, 1981; Sampugna et al., 1982).

Geometric isomers or *cis*/*trans*-isomers are formed when the "naturally" occurring *cis*-double bonds in vegetable oils are isomerized to the more thermodynamically stable *trans*-configuration; for example, *cis*-9-octadecenoic acid or oleic acid is transformed into *trans*-9-octadecenoic acid or elaidic acid (Figure 15.1). *Trans*-fatty acids have a higher melting point than the corresponding *cis*-fatty acids and thus contribute to the hardness of partially hydrogenated fat. Fatty acids containing a *cis*-double bond have a "bent" structure, whereas those containing a *trans*-double bond are more linear and thus resemble a saturated fatty acid rather than an unsaturated one. Because of their similarities in conformation, some investigators (Beare-Rogers et al., 1979; Enig et al., 1990b) believe that the sum of the *trans*- and saturated fatty acids is the physiologically meaningful value and have expressed their data as such. Enig et al. (1990b) term this sum "saturated-equivalents" and report that some snack and fast food items such as cheese corn chips and pizza crust contain as much as 60%–70% of the fatty acids as saturated-equivalents.

The composition of the hydrogenated fat depends in part on the composition of the original oil. If soybean oil and other vegetable oils are used as the source oil, then the hydrogenated product contains primarily isomers of octadecenoic acid (18:1), although much smaller amounts of isomeric fatty acids with shorter chain lengths are formed in some hydrogenated fats (Heckers and Melcher, 1978). If marine oil is used as the raw material, then the hydrogenated fat can contain isomers of fatty acids with chain lengths of 20–22 carbons (Hølmer and Aaes-Jørgensen, 1969; Beare-Rogers, 1983b).

In addition to the positional and geometrical isomers that are present in hydrogenated fat, small amounts of other isomeric fatty acids are found in these fats. Cyclic monomers, as well as intramolecular linear dimers, are known to be present (FAO, 1980). These minor components, however, have not been well studied.

B. Sources of Dietary Isomeric Fatty Acids

The primary source of isomeric fatty acids in the food supply is commercial hydrogenation of vegetable oils. Emken (1984) estimated that up to 90%–95% of isomeric fatty acids appearing in the U.S. diet at that time was contributed by commercially hydrogenated fat. These hardened fats and oils have been found in margarine, shortening, and frying fats and in various processed foods that contain

these fats. Commercial hydrogenation of vegetable oils results in a product that has a higher melting point appropriate for margarine and shortening products, is less susceptible to oxidative rancidity, and has improved flavor stability (Dutton, 1979). By controlling the conditions of the hydrogenation process, the manufacturers can provide the consumer with margarines and shortenings that have the desired consistency and spreadability. At the same time, however, the partial hydrogenation process results in the conversion of some naturally occurring fatty acids to isomeric forms in which both the conformation and position of the double bonds in the fatty acids have been altered.

A secondary source of isomeric fatty acids is biohydrogenation, which occurs in ruminant animals as a result of bacterial fermentation in the rumen (Reiser, 1951; Hartman et al., 1954, 1955). Isomeric fatty acids similar to those formed in commercial hydrogenation are formed by biohydrogenation (Hay and Morrison, 1970). The complex enzyme systems of rumen microflora transform the monounsaturated and polyunsaturated fatty acids in feedstuffs into more saturated products and into geometric and positional isomers not originally present in the feed. Thus, dairy products and other foods of animal origin contain small amounts of isomeric fatty acids (Hay and Morrison, 1970; Parodi and Dunstan, 1971; Smith et al., 1978; deMan and deMan, 1983; Wolff et al., 1998).

Finally, as summarized by Sommerfeld (1983), a number of plant species naturally contain small amounts of *trans*-unsaturated fatty acids in the seed fats and in the leaves. For example, vegetables such as leeks, peas, spinach, and lettuce contain *trans*-3-hexadecenoic acid (16:1Δ3t). Rapeseed oil is reported to contain brassidic acid (22:1Δ13t) and gondoic acid (20:1Δ11t) (Turchetto and Lorusso, 1977; Sommerfeld, 1983), but Fogerty et al. (1978) found neither of these fatty acids in rapeseed oil. The other vegetable oils commonly used in margarine and shortening do not naturally contain *trans*-isomers (Carpenter and Slover, 1973; Lanza et al., 1980; Sommerfeld, 1983).

III. POSITIONAL ISOMERS OF FATTY ACIDS IN FOOD

Hydrogenated vegetable oils, as well as fats from ruminant animals, may contain as many as 20 *trans*- and *cis*-positional isomers of octadecenoic acid. The distribution of positional isomers in margarine has been studied by several investigators, including Scholfield et al. (1967), Carpenter and Slover (1973), Carpenter et al. (1976), Parodi (1976a), Marchand (1982), Sampugna et al. (1982), Slover et al. (1985), and Caughman et al. (1987). Butter (Parodi, 1976b), milk fat (Hay and Morrison, 1970), and meat (Wood, 1983) have also been analyzed for positional isomers.

In Figure 15.2, the distribution of positional isomers of margarine is compared to that of butter, using data of Sampugna et al. (1982) for margarine and Parodi (1976b) for butter. The predominant *cis*-monoene in both butter and margarine is oleic acid (18:1Δ9c). In butter, this isomer comprises 95% of the total *cis*-octadecenoic acid fraction, and in margarine it typically comprises 60%. The fatty acid with a *cis*-double bond at position 11 (18:1Δ11c) is the second most prevalent *cis*-octadecenoic isomer in butter (Parodi, 1976b) and in meat (Wood, 1983), but in margarine the *cis*-positional isomers are more widely distributed among the Δ8–Δ12 positions (see Figure 15.2). Very small amounts of isomers with double bonds at the other positions are present.

In both butter and margarine, *trans*-octadecenoic acids with double bonds in positions Δ6–Δ16 are present (see Figure 15.2). In butter, the predominant *trans*-isomer is vaccenic acid, or *trans*-11-octadecenoic acid (18:1Δ11t), with 50%–70% of the *trans*-octadecenoic acid having a double bond in the Δ11 position. In a typical margarine, the majority of positional isomers of *trans*-octadecenoic acid have double bonds at Δ9–Δ12, although the exact distribution of positional isomers varies depending on the conditions of hydrogenation (Allen and Johnston, 1960). This variation can be seen in the five brands of margarine analyzed by Sampugna et al. (1982) (see Figure 15.3).

In addition to isomers of octadecenoic acid, *cis*-isomers of 14:1, 16:1, and 17:1 and *trans*-isomers of 16:1 are present in milk fat (Hay and Morrison, 1970; Wolff, 1994). The distribution of double bonds in *cis*- and *trans*-monoenoic fatty acids from bovine milk fat is illustrated in Table 15.1. Somewhat similar distributions of *trans*-positional isomers of octadecenoic acid in milk fats have been reported by other investigators (Parodi, 1976b; Wolff, 1994; Precht and Molkentin, 1995). In hydrogenated soybean oil, the *trans*-monoenes are almost exclusively isomers of octadecenoic acid.

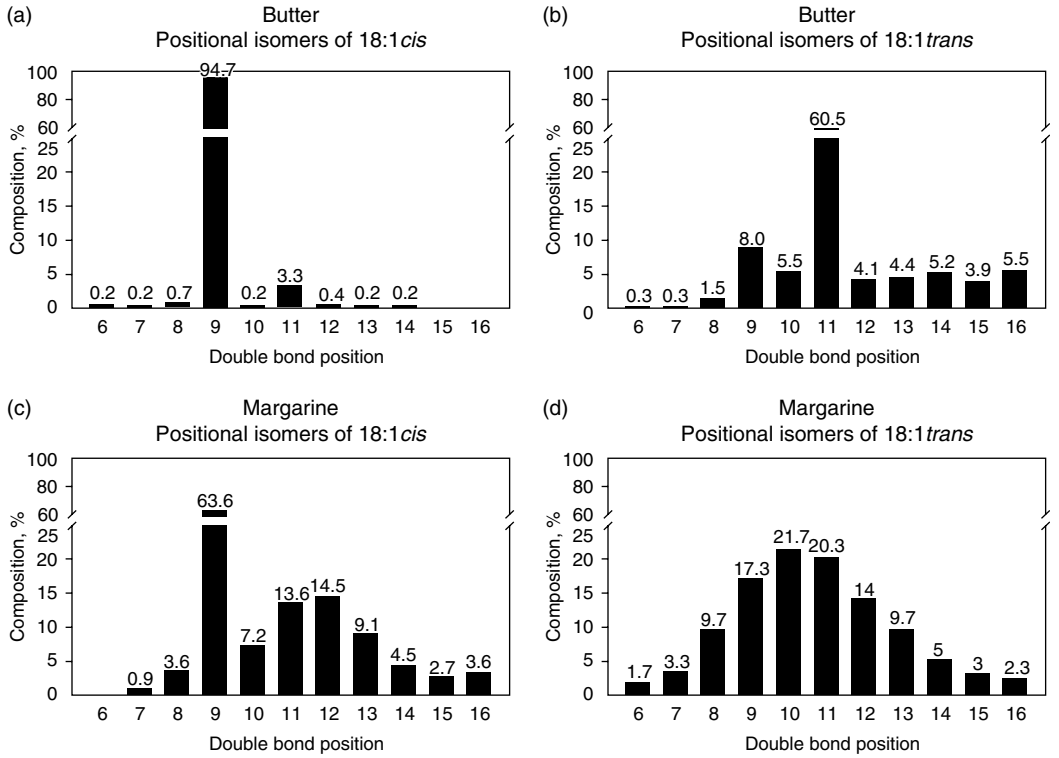

FIGURE 15.2 Distribution of positional isomers of the *cis*- and *trans*-octadecenoate fraction of butter and margarine. (Data for butter from Parodi, P.W. (1976b). *J. Dairy Sci. 59*: 1870–1873; data for margarine from Sampugna, J., et al. (1982). *J. Chromatogr. 249*: 245–255.)

Hydrogenation of vegetable oils to form shortening or margarine results in the conversion of linoleic acid (18:2Δ9c, 12c) to monoenes (Allen and Johnston, 1960). As a result, most of the positional isomers in partially hydrogenated fat are monoenoic fatty acids; however, small amounts of isomeric dienes are also formed. Dienes containing conjugated double bonds are present in hydrogenated fat, and concentrations of these conjugated dienes, ranging from 0.2% to 1.9% of fatty acids in margarine and shortening, have been reported by a number of investigators (Mabrouk and Brown, 1956; Sreenivasan and Brown, 1956; Scholfield et al., 1967; Hølmer and Aaes-Jørgensen, 1969; Carpenter and Slover, 1973; Parodi, 1976a; Smith et al., 1978). Animal fat of ruminant origin also contains about 1%–2% of the fatty acids as conjugated dienes (Parodi, 1977).

The methylene-interrupted diene system in linoleic acid is crucial to its role as an essential fatty acid. Since the double bounds in linoleic acid can be isomerized during hydrogenation in both conformation and position, enzymatic lipoxygenase activity specific for the *cis*,*cis*-methylene-interrupted structure is often used as a measure of "physiologically active polyunsaturated fat" (Smith et al., 1978). In comparing butter and margarine, Smith et al. (1978) reported that butter contained 3%–5% and margarine 15%–42% *cis*,*cis*-methylene-interrupted fatty acids.

IV. *TRANS*-ISOMERS OF FATTY ACIDS IN FOODS

In part because of methodological considerations, data exist primarily for the *trans*-fatty acid isomers rather than the positional isomers of specific food items. In this section, available information on the *trans*-fatty acid content of foods is summarized. The food items are grouped into four broad categories: (1) dairy products, meats, and animal fats; (2) margarines, shortenings, and vegetable oils; (3) fast foods and processed foods; and (4) human milk and infant foods.

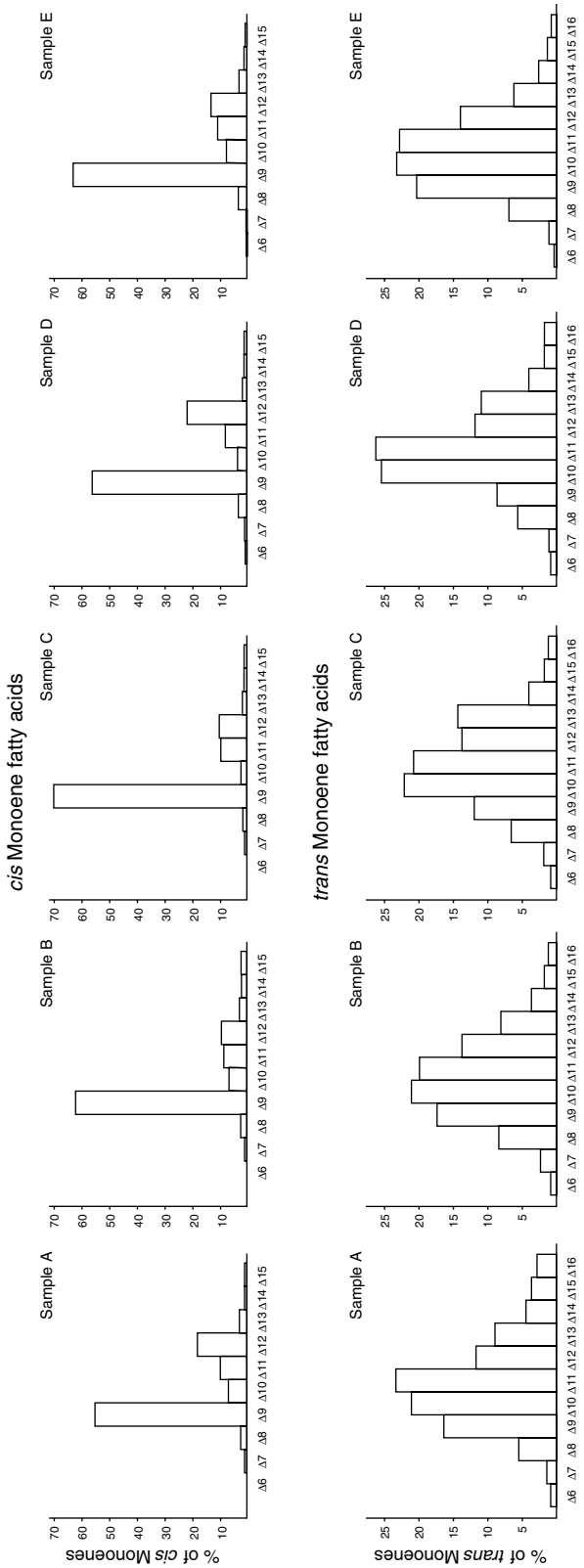

FIGURE 15.3 Distribution of positional isomers of *cis*- and *trans*-monoenes derived from selected margarines and shortenings. (From Sampugna, J., et al., (1982). *J. Chromatogr. 249*: 245–255; reprinted with permission from Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.)

TABLE 15.1
Distribution of Double Bonds in *Cis*- and *Trans*-Monoenoic Fatty Acids from Bovine Milk Fat (Cream)[a]

Position of Double Bond	*Cis*-Isomers				*Trans*-Isomers	
	14:1	16:1	17:1	18:1	16:1	18:1
5	1.0	tr			2.2	
6	0.8	1.3	3.4		7.8	1.0
7	0.9	5.6	2.1		6.7	0.8
8	0.6	tr	20.1	1.7	5.0	3.2
9	96.6	88.7	71.3	95.8	32.8	10.2
10		tr	tr	tr	1.7	10.5
11		2.6	2.9	2.5	10.6	35.7
12		tr	tr		12.9	4.1
13					9.7	10.5
14					10.6	9.0
15						6.8
16						7.5

[a]Values expressed as wt.%. tr = trace.

Source: Adapted from data of Hay, J.D., and Morrison, W.R. (1970). *Biochim. Biophys. Acta 202*: 237–243.

It is important to recognize from the outset the limitations of such data, particularly with regard to incorporation of these values into tables of food composition. First, values of *trans*-fatty acids determined prior to 1970 using older methodology, that is, before capillary gas chromatography, are considered to be less accurate (Brisson, 1981; Lanza and Slover, 1981). Recently, various methods for analyzing *trans*-fatty acids have been compared by Ratnayake (1998). Second, there is tremendous variability in values for any one type of food item. As a result, average values have limited use in food composition tables. Using minimum and maximum values for *trans*-fatty acids within a given food item (Innis et al., 1999) have shown that estimates of the *trans*-fatty acid content of the same diet can vary from a low of 1.4–25.4 g/day. Third, values for the *trans*-fatty acid content of food items presented as percentages of fatty acids without coupling these values to the fat content of food are also of somewhat limited use in food composition tables, because the gram quantity of *trans*-isomers in a serving of food cannot be determined. Fourth, products are constantly being reformulated, making it difficult to build up a reliable database. Industry responds to economic pressures and to public concerns over current health issues, and as a result changes the conditions of processing as well as the type and amount of fat used in various products. This has been particularly true recently as legislation that limits the content of industrially processed *trans*-fatty acids in the food has been passed in countries such as Denmark and as food-labeling regulations requiring *trans*-fat content have been implemented in countries such as the United States (Ratnayake and Zehaluk, 2005; Leth et al, 2006; Moss, 2006). Thus, the composition of the product may have changed by the time values are ready to be incorporated into food composition tables. With these limitations in mind, available data on the *trans*-fatty acid content of various food items are summarized here.

A. *Trans*-Isomers of Fatty Acids in Dairy Products, Meats, and Animal Fats

Foods such as butter, milk, beef, and lamb are derived from ruminant animals. *Trans*-fatty acids are formed as a result of biohydrogenation in the rumen of these animals, and thus dairy products

and meats contain small but significant quantities of isomeric fatty acids (Wolff et al., 1998). Vaccenic acid (18:1Δ11t) is the predominant *trans*-isomer in these foods, although smaller amounts of other *trans*-monoenes are found in the hexadecenoic (16:1) and octadecenoic (18:1) fractions of milk (Hay and Morrison, 1970) and meat (Wood, 1983). In fats of ruminant origin, the *trans*-fatty acids are located in the 1- and 3-positions of the triacylglycerol, with no measurable amounts at the 2-position (Woodrow and deMan, 1968).

The presence of CLA in meat and dairy products is of particular interest because of its reported anticarcinogenic effects as well as other possible beneficial biological effects. These biological effects of CLA and its occurrence in food have been reviewed recently by Banni and Martin (1998). Animal products are the principal dietary sources of CLA, although conjugated dienes are also found in low concentrations in plant oils and partially hydrogenated oils (Chin et al., 1992). The major conjugated diene in fats of ruminant origin is 9-*cis*,11-*trans*-octadecadienoic acid, with this isomer comprising 80%–90% of the total CLA in meat and dairy products (Chin et al., 1992; Parodi, 1994).

Dairy products are the richest dietary sources of CLA. The content of CLA in milk fat varies depending on pasture conditions and may contain up to 30 mg/g fat (Parodi, 1994). Chin et al. (1992) reported the CLA content of a variety of U.S. foods. For homogenized milk, condensed milk, and cultured buttermilk (three samples each), the total CLA content was 5.5 ± 0.30, 7.0 ± 0.29, and 5.4 ± 0.16 mg/g of fat, respectively. Similar values were reported by Lin et al. (1995), with values of CLA for fluid milk products ranging from 3.4 to 6.4 mg/g of fat. Yogurt contains CLA at approximately 4 mg/g of fat (Chin et al., 1992; Lin et al., 1995). Cheeses vary in CLA content, with Lin et al. (1995) reporting 3.59–7.96 and Chin et al. (1992) reporting 2.9–7.1 mg/g of fat. Banni et al. (1996) reported greater values for CLA in Italian dairy products with cow milk at 7.10, sheep milk at 11.72–29.68, yogurt at 7.98, and cheeses at 8.65–24.19 mg/g of fat.

Meats of ruminant origin contain more CLA than meats from nonruminant animals. Chin et al. (1992) reported values of 2.7, 2.9, 3.3, 3.8, 4.3, and 5.6 mg/g of fat for the CLA content of veal, beef round, beef franks, smoked beef sausage, fresh ground beef, and lamb, respectively, in contrast to values for pork (0.6 mg/g of fat) and chicken (0.9 mg/g of fat). Fresh ground turkey contained 0.25 mg/g of fat, whereas seafood contained 0.3–0.6 mg/g.

B. Milk Fat and Butter

Values for the *trans*-fatty acid content of milk fat and butter range from 1.75% (Ellis et al., 2006) to 8.6% (Woodrow and deMan, 1968) of total fatty acids (Table 15.2). In general, butter and milk fat appear to contain approximately 3%–6% *trans*-fatty acids (Table 15.2). The value of 0.65% triglyceride reported by Huang et al. (2006) appears to be much less than values reported by others.

Some of the variations seen in reported values can also be attributed to seasonable variation. Butter is reported to contain the lowest amounts of *trans*-fatty acids (4.3%–4.9%) in winter and the highest values (6.5%–7.6%) in spring and summer (Parodi and Dunstan, 1971; deMan and deMan, 1983). This seasonal variation is believed to be due to differences in the feedstuffs, with spring and summer pastures containing more polyunsaturated fatty acids than the winter feed supply. Henninger and Ulberth (1994) have reported a detailed analysis of the *trans*-fatty acid content of butter samples collected from Austrian dairies at monthly intervals. Consistent with earlier reports, greater values for *trans*-fatty acids were observed for samples collected during pasture feeding in the summer compared to samples collected during winter months. The values for 18:1t were greater if argentation thin-layer chromatography combined with gas chromatography was used rather than direct gas chromatography; however, the variation due to methodology was not as great as that due to seasonal variation. Similar variation in French butters collected at various times of the year has been reported by Wolff et al. (1995).

The *trans*-fatty acid values for cheeses and yogurt are shown in Table 15.2a. Most cheeses contain 3%–6% of total fatty acids as *trans*-fatty acids. As one would expect, the *trans*-content of cheeses, including those made from goat and ewe milk, reflect seasonal variation in the type of feed available (Wolff, 1995).

TABLE 15.2
***Trans*-Fatty Acid Content of Milk Fat and Butter**

Reference	Food Item	*Trans*-Fatty Acid[a] (%)
Woodrow and deMan (1968)	Milk fat	8.6
Hay and Morrison (1970)	Buttermilk fat	6.0
	Cream fat	3.2
Parodi and Dunstan (1971)	Butter (116 Australian samples)	6.0[b]
Smith et al. (1978)	Milk fat[c] (5 U.S. brands)	1.9[d]
deMan and deMan (1983)	Butter (Canadian)	5.7[e]
Enig et al. (1983)	Butter (3 U.S. brands)	3.4
Ball et al. (1993)	Butter, semisoft (New Zealand)	3.2[f]
	Butter, clarified (New Zealand)	1.8[f]
Boatella et al. (1993a)	Butter (15 Spanish samples)	5.3 ± 0.6[g,h]
	Milk fat (34 Spanish samples)	3.5 ± 0.7[g,h]
Pfalzgraf et al. (1993)	Milk fat (15 German samples)	3.64 ± 1.15
	Butter (5 German samples)	4.12 ± 2.37
Henninger and Ulberth (1994)	Butter (Austrian samples)	4.25 ± 1.2[h,i]
Michels and Sacks (1995)	Butter (U.S. samples)	3.4[j]
USDA (1995)	Milk fat (U.S. composite sample, April)	2.94
	(U.S. composite sample, July)	3.39
	(2 U.S. composite samples)	2.75
Wolff et al. (1995)	Butter (12 French samples, January)	2.37 ± 0.27[h,k]
	(12 French samples, May/June)	4.28 ± 0.47[h,k]
Fernandez San Juan (1996)	Milk fat (25 Spanish samples)	3.4 ± 0.4
Precht and Molkentin (1996)	Milk fat (100 German samples)	3.83 ± 1.34[h,k]
	(Belgium sample)	3.19[h,k]
	(4 Danish samples)	4.21 ± 0.60[h,k]
	(10 Spanish samples)	4.04 ± 0.30[h,k]
	(10 French samples)	4.47 ± 0.92[h,k]
	(4 Greek samples)	4.01 ± 0.15[h,k]
	(12 Italian samples)	4.14 ± 1.30[h,k]
	(22 Irish samples)	5.91 ± 0.92[h,k]
	(Luxemburg sample)	3.51[h,k]
	(24 Dutch samples)	4.09 ± 0.91[h,k]
	(23 United Kingdom samples)	4.78 ± 0.91[h,k]
Lake et al. (1996)	Butter (5 New Zealand samples)	6.4 ± 1.02[h]
Richardson et al. (1997)	Butter (New Zealand samples)	6.72
Aro et al. (1998a)[l]	Milk fat (Belgium sample)	4.68
	(Danish sample)	4.07
	(Finland sample)	3.19
	(French sample)	5.09
	(German sample)	3.55
	(Greek sample)	3.90
	(Iceland sample)	5.24
	(Italian sample)	4.37
	(Dutch sample)	4.41
	(Norway sample)	3.72
	(Portugal sample)	4.67
	(Spanish sample)	4.73
	(United Kingdom sample)	3.81
Aro et al. (1998a)[l]	Butter (Belgium sample)	5.43
	(Danish sample)	4.51
	(Finland sample)	4.01

Continued

TABLE 15.2 (Continued)

Reference	Food Item	*Trans*-Fatty Acid[a] (%)
	(French sample)	5.98
	(German sample)	4.04
	(Greek sample)	4.77
	(Iceland sample)	4.36
	(Italian sample)	4.18
	(Dutch sample)	6.15
	(Norway sample)	4.84
	(Swedish sample)	4.43
Collomb et al. (2002)	Milk fat (24 Switzerland Highlands samples)	8.44 ± 0.73[h,m]
Collomb et al. (2002)	Milk fat (12 Switzerland Mountains samples)	6.44 ± 0.48[h,m]
Collomb et al. (2002)	Milk fat (12 Switzerland Lowlands samples)	4.55 ± 0.47 [h,m]
Monge-Rojas et al. (2005)	Butter (Costa Rican samples)	3.49[g,n]
Ratnayake and Zehaluk (2005)	Bovine milk (Canadian sample)	4.3
Ratnayake and Zehaluk (2005)	Butter (Canadian sample)	5.7
Ellis et al. (2006)	Milk fat, conventional (19 United Kingdom samples)	1.75 ± 1.09[h,k]
Ellis et al. (2006)	Milk fat, organic (12 United Kingdom samples)	2.06 ± 0.96[h,k]
Huang et al. (2006)	Butter (U.S. sample)	0.65 ± 0.05[h,o]

[a] *Trans*-fatty acids are expressed as percentage of total fatty acids.
[b] Range = 4.27%–7.64% with minimum values in the winter and maximum values in spring/summer.
[c] Fat content = 81%.
[d] 1.9% by GC/TLC method, 4.0% by AOCS method.
[e] 4.9% in winter; 6.5% in summer.
[f] 18:1t + 18:2t.
[g] 16:1t + 18:1t + 18:2t.
[h] Mean ± SD.
[i] Value includes 16:1t but not conjugated linoleic acid; range = 2.26–6.52 g/100 g milk fat, with high *trans*-fatty acid content reported for pasture feeding in the summer months.
[j] 2.70 g *trans* per 81.11 g fat.
[k] 18:1t only.
[l] Data expressed as percentage of fatty acid methyl esters.
[m] Without CLA t^{12}.
[n] *Trans*-fatty acids expressed as g/100 g food.
[o] Data expressed as percentage of corresponding triglyceride.

C. Meats

Data on the *trans*-fatty acid content of meats are summarized in Table 15.3. The most extensive early isomeric fatty acid analysis of meats was performed in the laboratory of Slover (Slover and Lanza, 1979; Lanza et al., 1980; Lanza and Slover, 1981; Slover, 1985; Slover et al., 1987a,b), although values for *trans*-fatty acids in meats have also been reported by Wood (1983). Since 1990, values for the *trans*-fatty acid content of meats and meat products have been reported in the United States (Litin and Sacks, 1993; USDA, 1995; Huang et al., 2006), Europe (Aro et al., 1998a), Spain (Boatella et al., 1993a; Fernandez San Juan, 1996), Canada (Ratnayake et al., 1993; Innis et al., 1999; Ratnayake and Zehaluk, 2005), France (Wolff, 1995), Costa Rica (Monge-Rojas et al., 2005), and New Zealand (Richardson, 1997), and are in general agreement with earlier values.

TABLE 15.2a
Trans-Fatty Acid Content of Cheese and Yogurt (25)

Reference	Food Item[a]	*Trans*-Fatty Acids (%)[b]	*Trans*-Fatty Acids (g/100 g Food)
Pfalzgraf et al. (1993)	German cheeses		
	Regular (22)	3.01 ± 1.03	
	Goat (2)	3.15 ± 1.06	
	Ewe (3)	5.60 ± 1.66	
Ball et al. (1993)	New Zealand cheeses		
	Cottage cheese	0.90	
	Cream cheese (2)	1.2–2.4	
Wolff (1995)	French cheeses		
	Goat (8)	2.68 ± 0.88	
	Ewe (7)	1.53 ± 1.11	
USDA (1995)	U.S. cheeses		
	Cheddar	2.54	0.59
	Processed (6)	2.41–3.49	0.31–0.57
Banni et al. (1996)	Italian cheeses		
	Pecorino	4.99 ± 2.89[c]	
	Ricotta	9.10 ± 0.33[c]	
	Parmesan	3.22 ± 0.86[c]	
	Swiss cheese	3.70 ± 0.74[c]	
Boulous et al. (1996)	Greek feta cheese		1.40
Pfalzgraf et al. (1993)	German yogurt (1)	3.3	
USDA (1995)	U.S., low-fat yogurt (2)	2.39–3.18	0.01–0.02
Banni et al. (1996)	Italian yogurt	4.34 ± 0.59	
Aro et al. (1998a)[d]	Belgium cheese	5.00	
	Danish cheese	5.24	
	Finland cheese	3.92	
	French cheese	3.85	
	German cheese	3.91	
	Greek cheese	4.65	
	Iceland cheese	3.59	
	Italian cheese	4.45	
	Dutch cheese	4.37	
	Norway cheese	3.83	
	Portugal cheese	5.42	
	Spanish cheese	5.68	
	U.K. cheese	4.52	
Monge-Rojas et al. (2005)[e]	Costa Rican cheese		
	Fresh		1.31
	Cream cheese		1.96
Ratnayake and Zehaluk (2005)	Canadian cheese		
	Cheddar	6.6	
	Cottage	5.5	
	Processed	5.9	
	Yogurt	5.4	

[a]Number of samples analyzed appears in parentheses following food item.
[b]*Trans*-fatty acids are expressed as percent of total fatty acids.
[c]18:1t.
[d]Data expressed as percentage of fatty acid methyl esters.
[e]16:1t + 18:1t + 18:2t.

TABLE 15.3
Trans-Fatty Acid Content of Meats

Reference and Food Item[a]	Fat (wt.%)	*Trans*-Fatty Acids (%)[b]	*Trans*-Fatty Acids (g/100 g Food)
Slover and Lanza (1979)			
Baby food			
Strained beef liver (1)	4.10	1.90	0.05
Strained lamb broth (1)	9.33	7.57	0.33
Lanza et al. (1980)			
Beef, raw (1)	36.57	4.58	1.61[c]
Cooked (1)	37.06	5.00	1.69[c]
Liver lipid (1)	6.15	0.91	0.04
Lanza and Slover (1981)			
Beef, rib roast, separable lean (1)	5.46	4.21	0.23
Wood (1983)[d]			
Beef, lean (3)	3.2	2.9	0.09
Bologna (3 brands)	26.5		0.17
Luncheon meat (3 brands)	23.8		0.002
Hot dogs (3 brands)	27.2		0.55
Vienna sausage (3 brands)	27.3		0.37
Slover (1985)			
Beef, retail cuts, lean portion			
Raw (111)	6.46	3.21	
Cooked (109)	11.03	3.85	
Slover et al. (1987a)[e]			
Beef, 14 retail cuts, lean portion			
Raw (269)	7.37	3.20	
Slover et al. (1987b)			
Pork, 7 retail cuts			
Raw (7)	7.60	0.2	
Cooked (7)	14.23	0.3	
Boatella et al. (1993a)			
Beef (45 Spanish)		8.5 ± 2.7	
Pork (35 Spanish)		0.6 ± 1.0	
Meat products (46 Spanish)		0.5 ± 0.3	
Litin and Sacks (1993)			
Beef (United States)			0.63
Pork (United States)			0.07
Chicken (United States)			0.07
Pfalzgraf et al. (1993)			
Beef (4 German)		2.73 ± 0.56	
Veal (3 German)		1.37 ± 0.42	
Lamb (3 German)		7.53 ± 1.14	
Mutton (3 German)		9.30 ± 1.21	
Pork (German)			
Filet		0.2	
Bacon		0.4	

TABLE 15.3
(Continued)

Reference and Food Item[a]	Fat (wt.%)	*Trans*-Fatty Acids (%)[b]	*Trans*-Fatty Acids (g/100 g Food)
Ham, cooked		0.2	
Ham, smoked		0.5	
Poultry (German)			
Rooster		0.5	
Duck		0.5	
Turkey		1.4	
Wild pigeon		0.2	
Sausages (22 German)		0.68 ± 0.67	
Ratnayake et al. (1993)			
Meat patty (Canadian)	16.5	3.5	
USDA (1995)			
Beef			
Ground, raw (2 United States)	21.57	4.50	0.86
Ground, cooked (2 United States)	19.15	5.10	0.87
Turkey			
Raw (2 United States)	3.59	2.75	0.09
Burger, cooked (2 United States)	17.03	3.63	0.54
Ground, raw (10 United States)	8.57	3.58	0.27
Bologna			
Beef (2 United States)	26.41	5.17	1.30
Pork (2 United States)	28.71	0.67	0.19
Sausages			
Frankfurter (4 United States)	29.38	3.21	0.90
Kielbasa, beef (United States)	29.39	4.55	1.27
Sausage links (United States)	28.90	0.35	0.09
Pork (United States)	28.26	0.41	0.11
Pepperoni (United States)	41.34	0.93	0.36
Wolff (1995)			
Beef (10 French)		1.95 ± 0.94[f]	
Fernandez San Juan (1996)			
Sausages (40 Spanish)	26.5 ± 5.8	0.7 ± 0.5	
Richardson et al. (1997)			
Meat patty (New Zealand)	24.64	4.32	
Luncheon meat (New Zealand)	13.05	4.98	
Aro et al. (1998a)[g]			
Beef (Europe)	3.3–21.5	3.02–9.52	
Lamb/mutton (Europe)	4.4–18.9	4.32–9.19	
Pork (Europe)	2.2–32.0	0.19–0.86	
Chicken (Europe)	1.8–18.8	0.24–1.71	
Turkey (Europe)	1.8–11.6	0.31–1.27	
Sausages (Europe)			
Highest in *trans*	13.8–40.5	0.36–5.30	
Lowest in *trans*	14.7–39.5	0.25–1.01	

Continued

TABLE 15.3
(Continued)

Reference and Food Item[a]	Fat (wt.%)	*Trans*-Fatty Acids (%)[b]	*Trans*-Fatty Acids (g/100 g Food)
Innis et al. (1999)			
Meat patty (4 Canada)	16.4	6.8	1.1
Breaded chicken (8 Canada)	13.4	27.4	3.7
Ratnayake and Zehaluk (2005)			
Beef, steak (Canada)	8.0	3.2	
Beef, roast (Canada)	5.9	1.9	
Beef, ground (Canada)	8.6	3.9	
Veal (Canada)	1.7	2.9	
Lamb (Canada)	6.0	8.1	
Organ meat (Canada)	4.6	3.4	
Pork (Canada)	5.7	0.5	
Chicken (Canada)	2.4	2.5	
Monge-Rojas et al. (2005)[h]			
Red meat (Costa Rica)			0.79
Huang et al. (2006)[i]			
Chicken patties (United States)		0.93 ± 0.4	

[a]Number of samples/brands analyzed appears in parentheses following food item.
[b]Percentage of total fatty acids.
[c]Values presented as g/100 g dry weight.
[d]Calculated from data for neutral lipids and phopholipids.
[e]Larger database than reported in Slover (1985).
[f]Represents total *trans*-18:1 content.
[g]Values presented as range; samples from 14 European countries. Data expressed as percentage of fatty acid methyl esters.
[h]16:1t + 18:1t + 18:2t.
[i]Data expressed as percentage of corresponding triglyceride.

Lipids in the separable lean of raw and cooked beef were analyzed by Slover et al. (1987a). The content of *trans*-monoene ranged from a minimum value of 1.3% in top round steak, choice grade to a maximum value of 4.4% in rib roast, large end, Good (now called Select) grade. Average content of *trans*-fatty acids in all 14 cuts of lean, raw beef was 3.2% of total fatty acids. In addition to *trans*-monoenes, small amounts of *trans*-dienes (18:2Δ9t, 12t and 18:2Δ9c, 12t) were also found, usually in amounts less than 0.2%. Moisture loss and other factors during cooking resulted in a slightly higher concentration of *trans*-fatty acids in the cooked samples than in the raw sample (Slover, 1985). The average value of 3.2% of fatty acids as *trans*-monoenes in lean beef is in relatively good agreement with the value of 2.9% calculated from the data of Wood (1983).

In addition to lean beef, processed meats were analyzed by Wood (1983). In part because of their high fat content, bologna, hot dogs, and Vienna sausage contained more *trans*-fatty acids per 100 g of food than the lean raw beef. On the other hand, beef liver appears to contain much smaller amounts of *trans*-isomers than raw or cooked beef samples (Lanza et al., 1980). Cuts of pork contain 0.2%–0.3% of the fatty acids as *trans*-monoene (Slover et al., 1987b), presumably derived from milk products in diets fed to pigs during the early weeks of life (Sommerfeld, 1983).

Slover and Lanza (1979) reported the *trans*-fatty acid content of baby foods, with strained beef liver containing 1.9% and strained lamb broth 7.6% of total fatty acids as *trans*-isomers.

TABLE 15.4
***Trans*-Fatty Acid Content of Animal Fats**

Reference	Food Item[a]	*Trans*-Fatty Acids (%)[b]	g/100 g Food
Parodi (1976a)	Animal fat (4 Australia)	7.8	
Slover and Lanza (1979)	Beef fat (7 United States)	4.2	
Enig et al. (1983)	Beef fat (1 United States)	1.8	
Slover (1985)	Beef fat		
	70.89% fat, raw (12 United States)	6.55	
	70.32% fat, braised (6 United States)	5.47	
Enig et al. (1983)	Lamb fat (1 United States)	6.6	
Enig et al. (1983)	Lard (1 United States)	0.3	
Slover et al. (1987b)	Pork fat, separable (6 United States)	0.2	
Ball et al. (1993)	Beef fat (New Zealand)	1.7	
Boatella et al. (1993a)	Lard (35 Spanish)	0.7 ± 0.6	
Mansour and Sinclair (1993)	Lard (Australia)	0.73	
	Drippings (Australia)	4.56	
Pfalzgraf et al. (1993)	Beef tallow (Germany)	1.9	
	Lard (Germany)	0.4	
USDA (1995)	Lard (3 United States)	1.00	1.07
	Turkey, visible fat (1 United States)	3.91	2.54
	Chicken broiler fat (1 United States)	1.15	0.75
Wolff (1995)	Beef tallow (2 French)	4.6[c]	
Bayard and Wolff (1996)	Refined beef tallow (10 French)	4.91 ± 0.87[c]	

[a] Number of samples analyzed appears in parentheses following food item.
[b] Percent of total fatty acids.
[c] Value for 18:1t content.

D. Animal Fats

Beef fat is reported to contain 1.8%–6.55% *trans*-fatty acids (Table 15.4). Lamb fat contains more *trans*-fatty acids than beef fat (Enig et al., 1983; Ball et al., 1993; Wolff, 1995; Bayard and Wolff, 1996), whereas lard, because it is derived from a nonruminant source, contains much less *trans*-fatty acid (Enig et al., 1983; Slover et al., 1987b; Boatella et al., 1993a; Mansour and Sinclair, 1993; USDA, 1995). Australian drippings are reported by Mansour and Sinclair (1993) to contain *trans*-fatty acids at 4.56% of total fatty acids. *Trans*-fatty acid values for turkey fat and chicken fat are reported to be 3.91 g/100 g of food and 0.75 g/100 g of food, respectively (USDA, 1995).

E. *Trans*-Isomers of Fatty Acids in Margarines, Shortening, and Vegetable Oils

Hydrogenated vegetable oils are the source of most of the *trans*-fatty acids in the North American diet. The 6–8 billion pounds of hydrogenated vegetable oil produced in the United States per year is used in the production of margarines, shortenings, and salad/cooking oils. The *trans*-fatty acid content of these products varies from small amounts in lightly hydrogenated salad oils—for example, 5% of total fatty acids as reported by Carpenter et al. (1976) (see Table 15.8)—to typical values of 40% or more in commercial frying fats (Smith et al., 1986) (see Table 15.7).

In margarines and other hydrogenated products, *trans*-fatty acids appear to be concentrated in the 2-position of the triglyceride. In some of the 10 margarine samples analyzed by Carpenter and Slover (1973), there was a greater percentage of *trans*-monoene at the 2-position than in the total

fatty acids in the triglyceride. This was probably due to the preferential placement of polyunsaturated fat (e.g., linoleic acid) at the 2-position in the original vegetable oil. In the process of partial hydrogenation, some of these polyunsaturated fatty acids at the 2-position would be converted to *trans*-monoenes. In contrast, as mentioned previously in this chapter, animal fats contain *trans*-fatty acids preferentially at the 1- and 3-positions of the triglyceride (Woodrow and deMan, 1968).

F. Margarines

The *trans*-fatty acid content of stick or hard margarines is greater than that of soft or tub margarines. Both Enig et al. (1983) and Slover et al. (1985) reported that stick margarines, on the average, contained 24% of the fatty acids as *trans*-isomers, with minimum values of 16%–17% and maximum values at 35%–36%. In contrast, tub or soft margarines collected at the same time as the stick margarines generally contained 14%–18% of fatty acids as *trans*-fatty acids with a range of 10%–30% (Table 15.5). Diet margarines analyzed in the 1980s in the United States were similar to soft margarines, containing about 15% *trans*-fatty acids (Enig et al., 1983). The value of 23% used by Enig et al. (1990a) in calculating per capita consumption of *trans*-fatty acids was based on tub margarines comprising 20% of the total margarine market. This value of 23% appears to be a reasonable estimate of the amount of *trans*-fatty acids in U.S. margarines at that time.

Margarines and table spreads, like other foods, have recently undergone reformulation to reduce the *trans*-fatty acid content. For example, this change for German margarines has been documented by Precht and Molkentin (2000) who reported a decrease in the *trans*-fatty acid content of margarine from 22% of total fatty acids in 1994 to 5% in 1999 (Table 15.5). Similarly, Henninger and Ulberth (1996) reported that stick and tub margarines collected in 1995 had decreased to about half of their 1991/1992 values (Table 15.5). Aro et al. (1998b) reported differences in the *trans*-fatty acid content of margarines dependent on the country in which they were collected. Soft table margarines collected in Iceland, Norway, and the United Kingdom had the greatest *trans*-fatty acid content with values ranging from 13.0% to 16.5% of total fatty acid methyl esters. Margarines collected in Italy, Germany, Finland, and Greece had a *trans*-fatty acid content of 5.1%, 4.8%, 3.2%, and 2.9% of total fatty acids, respectively; whereas margarines from other countries (Portugal, The Netherlands, Belgium, Denmark, France, Spain, and Sweden) contained less than 2% of the total fatty acids as *trans*-fatty acids.

Other countries, such as the United States and Canada, have lagged behind Europe in making significant decreases in the *trans*-fatty acid content of margarines (Table 15.5). With the recent advent of *trans*-fat labeling of foods (Moss, 2006), rapid change in the *trans*-fatty acid content of margarines is occurring in the United States. These changes, however, have not been documented in a systematic manner.

Most of the *trans*-fatty acids in margarine and other hydrogenated fats are monoenoic (18:1t) isomers; however, the *trans*-dienes (18:2t,c; 18:2c,t; and 18:2t,t) are found in much smaller amounts. Kinsella et al. (1981) reviewed the literature on possible physiological effects of high levels of *trans*,*trans*-octadecadienoate (18:2t,t) in the diet. Concern was expressed about this particular isomer because of its potential interference with the metabolism of the essential fatty acid linoleic acid (18:2Δ9c, 12c). Currently available data on the composition of margarine (Table 15.6) would indicate that hydrogenated fat contains small amounts of *trans*,*trans*-octadecadienoate and at the same time sufficient quantities of the all-*cis*-isomer (linoleate). Enig et al. (1983) reported maximum values of 5% for the sum of *trans*-dienes (18:2i)* in 40 margarine samples. Linoleic acid composed, on the average 26%–35%, of total fatty acids in these margarine samples (Table 15.6). Total *trans*-isomers of Δ9,12-octadecadienoate (18:2t,c, 18:2c,t, and 18:2t,t) in margarines analyzed by Slover et al. (1985) ranged from 0.17% to 11.6%, with an average of 1.9%. The *trans*,*trans*-Δ9,12-octadecadienoate (18:2t,t) made up only 0.19% of total fatty acids, with a maximal value of 1.5% in these margarine samples. Only four of the margarines tested by Slover et al. (1985) contained

*The term 18:2i or "isomeric" octadecadienoic acid is defined as the sum of 18:2c,t, 18:2t,c, and 18:2t,t.

TABLE 15.5
Trans-Fatty Acid Content of Margarines and Table Spreads

		***Trans*-Fatty Acids**					
		% Fatty Acids			**g/100 g Food**		
Reference	**Food Item[a,b]**	**Min**	**Max**	**Avg ± SD**	**Min**	**Max**	**Avg ± SD**
Carpenter and Slover (1973)	U.S. margarines						
	Stick (6)	—	36.0	25.7[c]			
	Tub (4)	14.0	28.0	21.8			
Weihrauch et al. (1977)	U.S. margarines						
	Stick and brick (17)				9.9	28.7	21.7
	Tub (13)				10.5	21.4	14.2
Smith et al. (1978)	U.S. margarines (5)	13.6	23.3	17.8			
Enig et al. (1983)	U.S. margarines						
	Stick (24)	17.4	36.0	24.2			
	Tub (13)	10.6	21.3	14.4			
	Diet (3)	12.7	16.9	15.3			
Slover et al. (1985)	U.S. margarines						
	Stick (57)	16.1	34.8	23.7			
	Block	—	—	21.7			
	Tub (26)	11.7	29.8	17.7			
	Stick, lard (1)	—	—	3.9			
Enig et al. (1990a)	Weighted average for all U.S. margarine[d]			23.0			
Litin and Sacks (1993)	U.S. margarines						
	Soft				3.0	10.2	5.7
	Stick				7.9	19.8	13.2
Michels and Sacks (1995)	U.S. margarines						
	Stick, corn oil			18.6			
	Tub, corn oil			9.0			
	Blend, light			2.0			
USDA (1995)	U.S. margarines						
	Stick (10)	20.14	31.86	25.96 ± 4.14	13.02	25.06	19.44 ± 3.81
	Tub (8)	7.91	17.52	12.89 ± 3.35	3.05	11.30	7.62 ± 3.29
	Spread (5)	13.56	33.58	23.27 ± 9.32	2.79	25.78	15.55 ± 9.5
	Spread, light (2)	15.24	17.95	16.6 ± 1.92	5.66	9.09	7.38 ± 2.43
Ali et al. (1996)	U.S. fat-free margarine			1.52			0.04
Satchithanandam et al. (2004)	U.S. margarines						
	Soybean oil (2)			15.6			
	Soft, soybean oil (2)			27.7			
	Spreadable sticks, 60% soybean oil (2)			19.0			
	Spread, 65% soybean oil (2)			15.1			
	Spread, 70% soybean oil (2)			16.4			
	Corn oil (2)			14.9			
Huang et al. (2006)[e]	U.S. margarines			19.13 ± 0.93			

Continued

TABLE 15.5 (Continued)

		Trans-Fatty Acids					
		% Fatty Acids			g/100 g Food		
Reference	Food Item[a,b]	Min	Max	Avg ± SD	Min	Max	Avg ± SD
Mozaffarian et al. (2006)	U.S. margarines						
	Hard (stick)	15	23				
	Soft (tub)	5	14				
Baere-Rogers et al. (1979)	Canadian margarines (8)	12.0	64.8	30.3			
Sahasrabudhe and Kurian (1979)	Canadian margarines						
	Stick, vegetable oil (33)	27.2	32.9	30.7			
	Soft, vegetable oil (49)	8.7	28.5	15.0			
	Stick, animal fat (6)[f]	4.2	25.3	18.4			
Marchand (1982)	Canadian margarines						
	Stick (17)	21.5	39.3	30.0			
	Tub (30)	9.4	27.4	15.6			
Ratnayake et al. (1991)	Canadian margarines						
	Stick, soybean oil (3)	30.4	49.9	38.8 ± 10.0			
	Stick, vegetable oil (5)	25.6	40.2	33.42 ± 5.78			
	Stick, may contain palm oil (7)	32.8	40.6	35.2 ± 2.6			
	Stick, corn oil (1)			28.2			
	Stick, veg. oil/animal fat (1)			42.2			
	Stick, unspecified (2)	20.9	28.8	24.9 ± 5.6			
	Tub, soybean oil (13)	12.4	27.3	18.7 ± 3.9			
	Tub, vegetable oil (10)	22.1	35.2	26.4 ± 3.8			
	Tub, may contain palm oil (2)	19.5	21.3	20.40 ± 1.27			
	Tub, corn oil (3)	10.1	25.3	16.8 ± 7.8			
	Tub, sunflower oil (2)	—	14.3	7.15			
	Tub, olive oil (1)			19.4			
Ratnayake and Pelletier (1992)	Canadian margarines						
	Hard, 6 types (19)			34.2[g]			
	Soft, 6 types (31)			21.3[g]			
Innis et al. (1999)	Canadian margarines						
	Hard (14)	31.1	44.6	39.8			
	Soft (14)	1.1	44.4	16.8			
Elias et al. (2002)	Canadian margarines						
	Stick (14)	31.1	44.6	39.8 ± 4.5			
	Soft (16)	1.0	44.4	16.8 ± 11.2			
Monge-Rojas et al. (2005)	Costa Rican margarines						6.32[h]
Leighfield (1986)[i]	English margarines						
	Low, <10% PUFA (4)				3.7	21.4	11.5
	Medium, 10%–30% PUFA (10)				5.5	14.9	11.1
	High, PUFA (9)				4.2	15.7	7.1

TABLE 15.5
(Continued)

		Trans-Fatty Acids					
		% Fatty Acids			g/100 g Food		
Reference	**Food Item[a,b]**	**Min**	**Max**	**Avg ± SD**	**Min**	**Max**	**Avg ± SD**
Michels and Sacks (1995)	United Kingdom margarine "no *trans*" margarine			0.5			
Boatella et al. (1993a)	Spanish margarines (47)			10.8 ± 8.8			
Fernandez San Juan (1996)	Spanish margarines (32)			16.8 ± 5.7			
Larque et al. (2003)	Spanish margarines (12)	0.4	19.2				
Bayard and Wolff (1995)	French margarines						
	Tub, <5% *trans* (5)	0.06	1.68	0.45 ± 0.69			
	Tub, >5% *trans* (7)	7.38	19.02	14.11 ± 3.93			
Ovesen and Leth (1995)	Danish margarines						
	Hard, >20% linoleic	17	23	21	14	19	17
	Soft, >20% linoleic	0	15	8	0	12	7
	Soft, >55% linoleic	0	4	3	0	3	2
Ovesen et al. (1996)	Danish margarines						
	1992, <20% linoleic	1.4	10.6	5.8[h]			
	1992, 20%–40% linoleic	0	22.3	9.8[h]			
	1992, >40% linoleic	0	9.6	1.9[h]			
	1995, <20% linoleic	0	8.2	4.2[h]			
	1995, 20%–40% linoleic	0	5.8	1.2[h]			
	1995, >40% linoleic	0	5.6	1.3[h]			
Ovesen et al. (1998)	Danish margarines						
	<20% linoleic (32)	0	14.2	4.1 ± 3.8[h]			
	20%–40% linoleic (19)	0	5.8	3.1 ± 3.3[h]			
	>40% linoleic (38)	0	1.9	0.4 ± 0.8[h]			
Leth et al. (2003)	Danish margarines						
	1995, <10% linoleic (4)			6.2 ± 2.2			
	1995, 10%–20% linoleic (22)			4.6 ± 2.7			
	1995, 20%–40% linoleic (11)			3.6 ± 4.0			
	1995, >40% linoleic (7)			1.1 ± 2.1			
	1999, <10% linoleic (4)			1.0 ± 1.9			
	1999, 10%–20% linoleic (16)			0.7 ± 2.0			
	1999, 20%–40% linoleic (10)			1.0 ± 3.0			
Heckers and Melcher (1978)	German margarines						
	Regular, 1976 (58)	0.1	30.3	8.2			
	Regular, 1973/1974 (61)	0.1	34.7	8.1			
	Low-calorie, 1976 (9)	0.2	11.2	3.9			
	Low-calorie, 1973/1974 (8)	0.2	5.3	1.9			
Pfalzgraf et al. (1993)	German margarines						
	Undeclared composition (1)			23.5			

Continued

TABLE 15.5 (Continued)

		Trans-Fatty Acids					
		% Fatty Acids			g/100 g Food		
Reference	**Food Item[a,b]**	**Min**	**Max**	**Avg ± SD**	**Min**	**Max**	**Avg ± SD**
	Hydrogenated vegetable oils (6)	6	23.4	9.3 ± 6.9			
	Hydrogenated vegetable oils containing some animal fat (5)	4	18.8	10.1 ± 5.9			
	Diet and reduced fat margarines (4)	0.6	3.6	1.6 ± 1.4			
Michels and Sacks (1995)	German margarines						
	Dietary			1.2			
	Reformatory			1.4			
Pfalzgraf and Steinhart (1995)	German margarines						
	Margarine, vegetable oils (10)	0.7	6.4	4.1 ± 1.9			
	Sunflower (4)	8.6	21	16.1 ± 5.3			
	Diet, reduced fat (4)	0.5	3.1	2.3 ± 1.2			
	Dietary/reformatory (6)	0.4	2.4	1.3 ± 0.7			
Molkentin and Precht (1996)	German margarines						
	Margarine (46)	0.17	25.90	9.32 ± 7.35[h]			
	Dietary/reformatory (31)	0.03	2.94	0.65 ± 0.65[h]			
Fritsche and Steinhart (1997)	German margarines						
	Fat reduced (2)	0.83	1.74				
	Vegetable (5)	0.32	4.07				
	Diet (7)	0.15	0.53				
	Sunflower (3)	3.33	4.88				
Precht and Molkentin (2000)	German sunflower margarines						
	1994 (11)	13.78	26.29	21.77 ± 3.3			
	1999 (9)	1.98	6.15	5.37 ± 1.31			
Henninger and Ulberth (1996)	Austrian margarines						
	1991/1992 stick (75)			21.30 ± 5.29			
	1991/1992 tub (183)			15.72 ± 5.84			
	1991/1992 dietary (124)			0.42 ± 0.20			
	1995 stick (14)			8.65 ± 9.36			
	1995 tub (28)			9.95 ± 9.55			
	1995 dietary (40)			0.82 ± 0.17			
Aro et al. (1998b)	European margarines[j,k]						
	Soft table (typical) (14)	0.13	16.51				
	Soft table (lowest in *trans*) (10)	0.11	2.61				
	Soft table (highest in *trans*) (11)	0.47	16.64				
	Hard household (typical) (12)	0.25	21.21				

TABLE 15.5
(Continued)

		Trans-Fatty Acids					
		% Fatty Acids			g/100 g Food		
Reference	**Food Item[a,b]**	**Min**	**Max**	**Avg ± SD**	**Min**	**Max**	**Avg ± SD**
	Hard household (lowest in *trans*) (7)	0.09	14.29				
	Hard household (highest in *trans*) (8)	6.27	28.07				
	Low-fat spreads (typical) (12)	0.16	13.29				
	Low-fat spreads (lowest in *trans*) (10)	0.13	12.27				
	Low-fat spreads (highest in *trans*) (9)	1.10	15.98				
Marekov et al. (2002)	Bulgarian margarine[k]	<0.2	11.2				
Triantafillou et al. (2002)	Greek margarine (15)	0.1	19.0				
Demirbas and Yilmaz (2000)	Turkish margarine						
	Hard			15.95			
	Soft			5.55			
	Breakfast			6.74			
	Paste and cake			18.32			
Arici et al. (2002)	Turkish margarine[k]						
	Hard (8)	20.1	34.3				
	Soft (8)	0.8	8.9				
Tekin et al. (2002)	Turkish margarine[k]						
	Tub (1)			7.7			
	Stick (7)	18.0	37.8				
Cetin et al. (2003)	Turkish margarine (10)[h,k]	0.2	27.4				
Enig et al. (1984)	Israeli margarines						
	Hard (3)	30.8	34.2	32.8			
	Soft (2)	13.4	14.3	13.9			
Bhanger and Anwar (2004)	Pakistan margarines (11)	1.45	23.09				
Khaloui et al. (1998)	Marocan margarines			33.7 ± 2.5			
Parodi (1976a)	Australian margarines						
	PUFA margarines (40)	12.9	22.1	17.1			
	Margarine (7)	10.8	19.0	14.9			
Wills et al. (1982)	Australian margarines (12)	4	14	11.0 ± 2.5			
Mansour and Sinclair (1993)	Australian margarines (13)	8.01	14.54	12.16 ± 2.28			
	Butter/dairy blends (5)	3.44	4.75	4.26 ± 0.49			
Ball et al. (1993)	New Zealand margarines						
	Margarine (8)	7.6	9.6	8.5 ± 0.7			
	Margarine blend (3)	5.4	6.7	6.1 ± 0.7			
	Fat reduced (4)	7.9	11.8	10.5 ± 1.8			

Continued

TABLE 15.5 (Continued)

		Trans-Fatty Acids					
		% Fatty Acids			g/100 g Food		
Reference	Food Item[a,b]	Min	Max	Avg ± SD	Min	Max	Avg ± SD
Lake et al. (1996)	New Zealand margarines						
	Margarine (7)	12.6	19.7	16.4 ± 2.60			
	Table spreads (5)	14.3	16.9	15.7 ± 1.07			
	Butter/margarine blend (2)	6.1	13.1	9.6 ± 4.96			
Richardson et al. (1997)	New Zealand margarine (2)	14.33	14.66	14.50 ± 0.23			

[a] Number in parentheses following food item indicates number of samples/brands analyzed.
[b] PUFA = polyunsaturated fatty acids.
[c] Average includes one sample containing tropical oils; average if this sample is omitted = 30.8%.
[d] Based on margarine consumption of 20% tub and 80% stick.
[e] Data expressed as percentage of corresponding triglyceride.
[f] Margarines containing various combinations of vegetable oils, animal fat, and marine oil.
[g] Weighted mean.
[h] Represents only *trans*-18:1 content.
[i] Personal communication (1986) from Martin Leighfield, Nuffield Laboratories of Comparative Medicine, Institute of Zoology, Regent's Park, London.
[j] Number of European countries from which samples were analyzed appears in parentheses following food item. Values presented as range.
[k] Data expressed as percentage of methyl esters.

the *trans*,*trans*-isomer in amounts exceeding 1% of the fatty acids. Linoleate (18:2Δ9c, 12c) was found in much greater amounts, ranging from 6.1% to 46.4%. Whether the small amounts of *trans*, *trans*-diene found in margarine would interfere with essential fatty acid and prostaglandin metabolism, particularly if the overall diet provided adequate sources of linoleic acid, is not known.

Depending on the starting material, additional *trans*-isomers may be formed in margarine. For example, if marine oils are hydrogenated, *trans*-isomers of C_{20} and C_{22} fatty acids are present in the final product (Ovesen et al., 1996). Similarly, if the original oil contains significant quantities of 18:3n-3, *trans*-isomers of 18:3 are formed (Ratnayake et al., 1991; Ratnayake and Pelletier, 1992).

G. Shortenings

Values reported for shortenings made from hydrogenated vegetable oils range from 6% to 50% *trans*-fatty acids (Table 15.7). Scholfield et al. (1967) reported that the average *trans*-fatty acid content of four vegetable shortenings was 22.5%. This value agrees with the value of 26% *trans*-fatty acids used by Hunter and Applewhite (1986) to estimate *trans*-fatty acid intake from shortenings in 1960. According to values of the Institute of Shortening and Edible Oils (ISEO), the typical *trans*-fatty acid content of household vegetable shortenings decreased from 26% in 1960 to 17% in 1984 (Hunter and Applewhite, 1986). Enig et al. (1983) analyzed seven brands of shortening, including household brands as well as commercial frying fats (Table 15.7). The *trans*-isomer content (18:1t plus 18:2i) of these shortenings averaged 25.3% of total fatty acids, with values for the *trans*-isomers of octadecadienoic acid (18:2i) ranging from 1.9% to 4.4%. The average value reported by Smith et al. (1986) for *trans*-fatty acid content of commercial frying fats collected in California was 41.5% of total fatty acids. The content of *trans*-fatty acids in these shortenings was reported to decrease during

TABLE 15.6
***Trans*-Octadecenoate and Isomers of Octadecadienoate in Margarine**

Reference	Margarine[a]	18:1t	Isomer of Octadecadienoate (% of Total Fatty Acids) 18:2t,c	18:2c,t	18:2t,t	18:2i[b]	18:2c,c[c]
Enig et al. (1983)	Margarine, U.S.						
	Stick (24)	22.3 (15.9–31.0)	—	—	—	1.9 (0–5.2)	26.1 (8.2–46.5)
	Tub (13)	12.7 (6.8–17.6)	—	—	—	1.7 (0–4.2)	34.6 (8.6–48.4)
	Diet (3)	12.0 (11.3–13.3)	—	—	—	3.3 (1.4–5.0)	32.6 (29.8–37.8)
Slover et al. (1985)[d]	Margarine, U.S.						
	Stick (57)	22.23 (14.82–30.06)	0.61 (0–3.49)	0.69 (0.17–3.76)	0.16 (0–1.28)	1.46 (0.17–8.4)	25.30 (6.06–43.57)
	Tub (26)	14.77 (10.74–18.44)	1.31 (0.10–4.78)	1.34 (0.19–5.31)	0.26 (0–1.47)	2.91 (0.35–11.56)	31.18 (19.46–46.39)
	Overall (83)	19.89 (10.74–30.06)	0.83 (0–4.78)	0.89 (0.17–5.31)	0.19 (0–1.47)	1.92 (0.35–11.56)	27.14 (6.06–46.39)
Ratnayake et al. (1991)	Margarine, Canadian						
	Stick, soybean oil (3)	31.13 (23.8–40.8)	6.27[e] (5.0–7.6)		1.23 (0.3–2.8)		8.3 (2.3–14.6)
	Stick, vegetable oil (5)	30.16 (23.6–30.2)	2.4[e] (2.1–3.1)		0.50 (0–1.2)		8.06 (4.1–11.4)
	Stick, palm oil (7)	32.90 (30.4–40.6)	2.33[e] (1.2–3.2)		0.44 (0.2–0.8)		4.60 (3.6–5.6)
	Stick, corn oil (1)	26.4	1.7[e]		0.2		30
	Stick, vegetable/ animal fat (1)	37.2	3.1[e]		1.4		4.8
	Stick, unspecified (2)	20.60 (17.8–23.4)	3.4[e] (2.0–4.8)		0.60 (0.6–0.6)		3.15 (2.3–4.0)
	Tub, soybean oil (13)	16.36 (11.6–21.4)	1.25[e] (0.2–2.7)		0.20 (0.0–0.4)		33.05 (23.7–39.2)
	Tub, vegetable oil (10)	23.53	1.45[d]				
	Tub, palm oil (2)	17.95 (17.8–18.1)	1.25[e] (0.4–2.1)		0.20 (0.2–0.2)		23.80 (14.8–32.8)
	Tub, corn oil (3)	15.93 (10.1–24.2)	0.97[e] (0–1.7)		—		36.13 (25.6–43.3)
	Tub, sunflower oil (2)	6.65 (0–13.3)	0.45[e] (0–0.9)		0		51.3 (48.1–54.5)
	Tub, olive oil (1)	19.2	0.2[e]		—		9.7

Continued

TABLE 15.6
(Continued)

			Isomer of Octadecadienoate (% of Total Fatty Acids)				
Reference	**Margarine[a]**	**18:1t**	**18:2t,c**	**18:2c,t**	**18:2t,t**	**18:2i[b]**	**18:2c,c[c]**
Bayard and Wolff (1995)	Margarine, French						
	Tub, <5% *trans* (5)	0.31 (0–1.53)				0.14 (0.06–0.27)	46.60 (28.00–52.92)
	Tub, >5% *trans* (7)	13.53 (7.23–17.62)				0.59 (0.15–1.40)	37.26 (21.57–43.34)
Pfalzgraf and Steinhart (1995)	Margarine, German						
	Vegetable oil (10)	3.7 (0.46–6.1)	0.18 (0.1–0.4)	0.17 (0.1–0.4)			22.05 (18.2–26.6)
	Sunflower (4)	15.2 (7.9–20.1)	0.43 (0.3–0.7)	0.5 (0.3–0.8)			38.78 (36.5–42.1)
	Reduced fat (4)	2 (0.1–2.9)	0.15 (0–0.3)	0.2 (0.1–0.3)			29.43 (19.9–54.5)
	Diet/reformatory (6)	0.58 (0–1.7)	0.33 (0.1–0.7)	0.35 (0.1–0.8)			39.83 (30.9–54.2)
Lake et al. (1996)	Margarine, New Zealand						
	Margarine (7)	14.6 (10.9–17.2)	1.2[e] (0.2–2.0)		0.2 (0–0.4)		40.7 (38.7–42.0)
	Table spreads (5)	13.8 (12.5–14.7)	1.1[e] (0.4–2.5)		0.1 (0–0.5)		37.7 (22.4–42.7)
	Butter/margarine blends (2)	7.1 (3.5–10.6)	1.0[e] (1.0–1.1)		—		24.4 (21.7–27.1)
Fritsche and Steinhart (1997)	German margarines						
	Fat reduced (2)	(0.68–1.55)		(0.12–0.17)	(0.0–0.0)		
	Vegetable (5)	(0.19–3.83)		(0.10–0.13)	(0.0–0.13)		
	Diet (7)	(0.05–0.36)		(0.0–0.43)	(0.0–0.03)		
	Sunflower (3)	(2.97–4.35)		(0.18–0.33)	(0.0–0.19)		
Larque et al. (2003)	Spanish margarines (12)	4.6 ± 6.5 (0.1–18.6)	0.1 ± 0.0 (0.1–0.2)	0.1 ± 0.0 (0.1–0.2)			
Cetin et al. (2003)	Turkish margarine (10)	(0.2–27.4)	(0.3–13.7)[e]				
Monge-Rojas et al. (2005)	Costa Rican margarines	6.3			0.5[f]		

[a] Numbers in parentheses indicate number of samples analyzed.
[b] Sum of 18:2t,c + 18:2c,t + 18:2t,t = 18:2i.
[c] 18:2c,c = all-*cis*-linoleic acid.
[d] Data of Slover, H.T., et al. (1985). *J. Am. Oil Chem. Soc. 62*: 775–786. Reported as isomers of Δ9,12-octadecadienoates (normalized wt.%, as triglycerides).
[e] Includes both 18:2t,c and 18:2c,t.
[f] Includes both 18:2c,t and 18:2t,t; data expressed as g/100 g food.

TABLE 15.7
***Trans*-Fatty Acid Content of Shortenings[a]**

		Trans-Fatty Acids					
		(%)[c]			g/100 g Food		
Reference	**Food Item[b]**	**Min**	**Max**	**Avg[d]**	**Min**	**Max**	**Avg[d]**
Scholfield et al. (1967)	U.S. vegetable shortenings (4)	16.6	29.2	22.5[e]			
Slover and Lanza (1979)	U.S. vegetable shortening (1)			10.7			
Lanza and Slover (1981)	U.S. shortenings						
	Vegetable oils only (6)				8.0	23.9	14.5
	Meat fats + vegetable oils (3)				2.8	6.6	4.3
Enig et al. (1983)	U.S. vegetable shortenings (7)	13.0	37.3	25.3			
Smith et al. (1986)	U.S. commercial brands[f]						
	Fresh (9)	40.4	42.4	41.5			
	Used (56)	12.8	41.4	30.7			
Hunter and Applewhite (1986)[g]	U.S. shortenings						
	Vegetable, 1960			26			
	Vegetable, 1970			19			
	Vegetable, 1984			17			
	Animal fats, 1960–1984			10			
Pfalzgraf et al. (1993)	German frying and baking fats (5)	0.1	31.8	12.72 ± 16.14			
Litin and Sacks (1993)	U.S. vegetable shortenings						13.4
Ratnayake et al. (1993)	Canadian shortenings						
	Unhydrogenated fats (3)	2.0	3.2	2.5 ± 0.61			
	Hydrogenated fats (3)	17.4	20.2	18.4 ± 1.5			
Bayard and Wolff (1995)	French shortenings (3)	27.99	63.61	50.29 ± 19.44			
Ovesen and Leth (1995)	Danish shortenings/frying oils						
	Hardened, new	17	36	30			
	Hardened, used	33	41	37			
	Nonhardened, new	0	1	0			
	Nonhardened, used	0	3	2			
USDA (1995)	U.S. shortenings (12)	11.17	34.05	19.64 ± 6.49	10.68	32.55	18.73 ± 6.24

Continued

TABLE 15.7
(Continued)

		Trans-Fatty Acids					
		(%)[c]			g/100 g Food		
Reference	**Food Item[b]**	**Min**	**Max**	**Avg[d]**	**Min**	**Max**	**Avg[d]**
Molkentin and Precht (1995, 1996)	German shortenings/cooking fats (16)	0.04	32.51	9.79 ± 8.51			
Henninger and Ulberth (1996)	Austrian shortenings						
	1991/1992 (86)			13.28 ± 14.68			
	1995 (15)			12.06 ± 14.08			
Ovesen et al. (1996)	Danish shortenings	0	13.7	6.8[h]			
Richardson et al. (1997)	New Zealand shortenings (3) (pastry fat)	5.42	7.02	6.33 ± 0.82			
Ovesen et al. (1998)	Danish shortenings (38)	3.0	13.6	6.7 ± 2.3[h]			
Precht and Molkentin (2000)	German shortenings/cooking fats						
	1994 (16)	0.08	33.63	11.77 ± 8.79			
	1999 (10)	0.43	19.72	5.91 ± 6.47			
Elias et al. (2002)	Canadian shortenings (3)	8.7	23.9	15.7 ± 11.4			
Leth et al. (2003)	Danish shortenings						
	1995, <10% linoleic (17)			8.0 ± 3.4			
	1995, 10%–20% linoleic (36)			6.5 ± 3.3			
	1999, <10% linoleic (15)			6.8 ± 4.4			
	1999, 10%–20% linoleic (24)			5.5 ± 4.5			
Bhanger and Anwar (2004)	Pakistan shortenings (11)	7.34	31.70				
Satchithanandam et al. (2004)	U.S. shortenings soybean and cottonseed oils (4)	12.6	13.2				
Monge-Rojas et al. (2005)	Costa Rican shortening, palm						0.55[h]
Mozaffarian et al. (2006)	U.S. vegetable shortenings			19			

[a] Fat content of shortening = 100%.
[b] Number of samples analyzed appears in parentheses following food item.
[c] Percentage of total fatty acids.
[d] Mean ± SD.
[e] Determined by IR methods.
[f] Frying fats collected from nine restaurants in California.
[g] Data on typical *trans*-fatty acid levels of products obtained from member companies of the Institute of Shortening and Edible Oils (ISEO).
[h] Total represents only *trans*-18:1 content.

commercial deep-fat frying in fast food restaurants. The *trans*-C_{18} monoenes in these commercial fats decreased from over 40% in the fresh fat to as low as 13% in the used product (Table 15.7).

German and Austrian shortenings (Pfalzgraf et al., 1993; Molkentin and Precht, 1995, 1996; Henninger and Ulberth, 1996) appear to be somewhat less in *trans*-fatty acid content compared with those sold in North America. On the other hand, French shortenings were reported by Bayard and Wolff (1995) to contain relatively large amounts of *trans*-fatty acids (28%–64%, with an average of 50%). Ovesen and Leth (1955) reported that Danish shortenings made from hydrogenated fat contained 30%–37% of total fatty acids as *trans*-isomers; however, more recently Ovesen et al. (1996) reported 7% for Danish shortenings. New Zealand shortenings appear to be relatively low in *trans*-fatty acids, with an average value of approximately 6% (Richardson et al., 1997).

Like margarines, shortenings are also being reformulated to contain less *trans*-fat. Precht and Molkentin (2000) reported that the *trans*-content of German shortenings decreased from 12% of total fatty acids in 1994 to 6% in 1999. There is movement worldwide to make shortenings with little or no *trans*-fatty acids available to the baked goods industry.

H. Vegetable Oils

In general, nonhydrogenated vegetable oils contain minimal *trans*-fatty acids (Table 15.8). However, in the past soybean oil sold for use as salad and cooking oil was often lightly hydrogenated to reduce the content of α-linolenic acid (18:3) and thereby reduce the potential for oxidation and rancidity.

TABLE 15.8
***Trans*-Fatty Acid Content of Vegetable Oils**

		***Trans*-Fatty Acids (%)[b]**		
Reference	**Food Item[a]**	**Min**	**Max**	**Avg[c]**
Scholfield et al. (1967)	Vegetable oils (2)	4.9	12.0	8.5[d]
Carpenter et al. (1976)	Vegetable oils			
	"Specially processed" (5)	5.6	13.3	9.7
	Other (9)			0
Enig et al. (1983)	Soybean oil, partially			
	Hydrogenated (4)	11.0	13.4	12.4
	Other vegetable oils[e] (14)		tr	
Hunter and Applewhite (1986)[f]	Salad and cooking oils (hydrogenated)			
	1963–1975			15
	1980			10
	1984			8
Ball et al. (1993)	New Zealand vegetable oils			
	Rapeseed			0.9
	Safflower			0.4
	Soya			0.6
	Corn			1.1
Boatella et al. (1993a)	Spanish vegetable oils			
	Refined olive oil (12)			0.5 ± 0.2
	Seeds oils (12)			2.3 ± 0.6
Litin and Sacks (1993)	U.S. vegetable oil	0	1.06	0.42[g]
Pfalzgraf et al. (1993)	German vegetable oils	0		
	Plant oils (6)		1.5	0.28 ± 0.60
	Almond oil (1)			0.1

Continued

TABLE 15.8 (Continued)

Reference	Food Item[a]	*Trans*-Fatty Acids (%)[b] Min	Max	Avg[c]
	Peanut oil (1)			0.5
	Walnut oil (1)			0.2
USDA (1995)	U.S. vegetable oils			
	Canola oil (2)	0.17	0.23	0.20 ± 0.04
	Sunflower oil (1)			0.5
	Olive oil (1)			0.09
Fernandez San Juan (1996)	Spanish vegetable oil			
	Olive (30)			0.1 ± 0.1
Aro et al. (1998b)	European vegetable oils[h]			
	Olive oil (4)	0	0.11	
	Soybean oil (3)	0.4	0.86	
	Sunflower oil (6)	0.13	0.89	
	Corn oil (4)	0.11	1.91	
	Rapeseed oil (1)			0.39
	Peanut oil (2)	0.04	0.63	
Bhanger and Anwar (2004)	Pakistanian hydrogenated vegetable oils (vegetable ghee)			
	>40% palmitic, <8.5% stearic			14.24
	<40% palmitic, <8.5% stearic			21.68
	<40% palmitic, >8.5% stearic			34.36
Satchithanandam et al. (2004)	U.S. vegetable oils			
	Canola oil (4)	0.1	0.5	
	Soybean oil (4)			0.3
	Safflower oil (2)			0.3
	Olive oil (2)			0.1
	Virgin olive oil (2)			0.0
	Extra virgin olive oil (2)			0.0
	Spanish olive oil (2)			0.0
	Corn oil (2)			0.0
Ratnayake and Zehaluk (2005)	Canadian refined oils			
	Canola oil			2.42
	Soybean oil			1.89
	Corn oil			0.47
	Sunflower oil			0.74
	Safflower oil			0.73
	Rice bran			1.06
	Coconut			0.19
	Extra virgin olive oil			0.05

[a] Number of samples analyzed appears in parentheses following food item.

[b] Percentage of total fatty acids; tr = trace.

[c] Mean ± SD.

[d] Determined by IR methods.

[e] Includes cottonseed oil (2), soybean oil (2), corn oil (3), peanut oil (1), olive oil (1), safflower oil (1), sunflower oil (1), vegetable oils (mixture or unspecified (3)). Trace amounts of *trans*-fatty acids were detected in two samples.

[f] Data on typical *trans*-fatty acid levels of products obtained from member companies of the Institute of Shortening and Edible oils (ISEO).

[g] Data of Litin, L., and Sacks, F. (1993). *N. Engl. J. Med. 329*: 1969–1970 are expressed as g/100 g of food.

[h] Number of European countries from which samples were analyzed appears in parentheses following food item. Data expressed as percentage of fatty acid methyl esters.

Reported values for the *trans*-fatty acid content of hydrogenated salad and cooking oils range from 5% to 15% (Table 15.8), with average values in the 1980s ranging from 8% (ISEO data; Hunter and Applewhite, 1986) to 12% (Enig et al., 1983). One exception to this is the *trans*-fatty acid content of Pakistani-hydrogenated vegetable oils (vegetable ghee/vanaspati) reported by Bhanger and Anwar (2004). These values for the *trans*-fatty acid content of ghee range from 14% to 34% of total fatty acids and are much greater than those reported for vegetable oils in other countries.

The content of *trans*-18:2 dienes (18:2i) in hydrogenated vegetable oils is high in comparison to the *trans*-18:1 content. For example, in one sample of partially hydrogenated soybean oil analyzed by Enig et al. (1983), 18:2i was 5.6% of the total fatty acids, with 18:1t comprising only 7.0%. Linoleate (18:2c,c) was reported to be 36.2% in this product. Thus, lightly hydrogenated products appear to have proportionately more *trans*-dienes and fewer *trans*-monoenes than more heavily hydrogenated products.

Many brands of salad and cooking oils contain oils that are not hydrogenated and thus contain no or only small amounts of *trans*-isomers (Table 15.8). Hunter (1990) has reported that since the mid-1980s, manufacturers have increased their production of household salad and cooking oils made from unhydrogenated oils. Processing of these oils can result in a minimal level of *trans*-fatty acids (Table 15.8). For example, Innis et al. (1999) reported that the content of nonhydrogenated vegetable oils ranged from 0.05 g/100 g food for extra virgin oil to 2.42 g/100 g of food for canola oil. Thus, these products contribute very little *trans*-fatty acids to the current food supply in the United States, Canada, or other countries.

I. Salad Dressings, Mayonnaise, Soups, and Sauces

Several samples of salad dressings, mayonnaise, and other sauces were analyzed for *trans*-fatty acids by a number of investigators (Table 15.9). This category of food contributes only small amounts of *trans*-fatty acids to the diet, and many of the products analyzed contain no *trans*-fatty acids (Table 15.9). For example, two out of the three salad dressings analyzed by Slover et al. (1980) contained small amounts of *trans*-fatty acids (0.24%), but none of the seven brands tested by Enig et al. (1983) contained *trans*-fatty acids. Of the five brands of mayonnaise analyzed by Slover et al. (1980) and Enig et al. (1983), only two contained *trans*-fatty acids. Elias et al. (2002) reported that most salad dressings contained less than 2 g/100 g of food as *trans*-fatty acids. Satchithanandam et al. (2004) found similar values for mayonnaise and salad dressings.

Prepared soups contain significant amounts of *trans*-fatty acids with values ranging from 10% for beef bouillon to 35% for onion cream soup (Pfalzgraf et al., 1993; USDA, 1995; Fernandez San Juan, 1996; Aro et al., 1998c; Innis et al., 1999). Thus, soups can contribute reasonably large amounts of *trans*-fatty acids to the diet if they are consumed routinely.

J. *Trans*-Isomers of Fatty Acids in Fast Foods and Processed Foods

Because commercial shortenings containing relatively large amounts of *trans*-fatty acids (Smith et al., 1986) are used as frying fats in fast food establishments and other restaurants, fast foods have the potential to contain relatively large amounts of *trans*-fatty acids. In addition, hydrogenated fats are used in many processed foods because of the increased shelf life, as well as the dependable consistency, the hydrogenation process affords the products. Therefore, many processed foods including cookies and other baked goods would be expected to contain significant amounts of *trans*-isomers. In fact, several investigators including Elias and Innis (2002) in Canada and Lemaitre et al. (1998) in the United States report that the greatest source of *trans*-fatty acids in the North American diet is baked goods.

Values for the *trans*-fatty acid content of processed foods and fast foods vary widely, depending on the type of fat used in processing. For this reason, some investigators prefer to present their data as ranges rather than as means. Values for the *trans*-fatty acid content of fast food items and processed foods are summarized in the remainder of this section.

TABLE 15.9
***Trans*-Fatty Acid Content of Salad Dressings, Mayonnaise, Soups, and Sauces**

		Trans-Fatty Acids	
Reference	**Food Item[a]**	**(%)[b]**	**(g/100 g Food)**
Slover et al. (1980)	U.S. salad dressings (2)	0.24	
	U.S. salad dressings (1)	n.d.	
	U.S. mayonnaise (1)	0.34	
	U.S. tarter sauce (1)	0.37	
	U.S. burger sauce (2)	0.14	
Enig et al. (1983)	U.S. salad dressings (7)	n.d.	
	U.S. mayonnaise (1)	4.5	
	U.S. mayonnaise (3)	n.d.	
	U.S. burger sauce (1)	4.6	
Litin and Sacks (1993)	U.S. mayonnaise, reduced calorie		0.21
Pfalzgraf et al. (1993)[c]	German soups		
	Clear soup	28.3	
	Beef bouillon	9.9	
	Onion cream soup	34.9	
	German sauces		
	Curry sauce	25.4	
	Instant tomato sauce	2.9	
	Mayonnaise	0.4	
USDA (1995)	U.S. salad dressings		
	French (2)	0.64 ± 0.11	0.24 ± 0.04
	Italian (2)	0.94 ± 0.64	0.40 ± 0.21
	Low calorie (1)	0.91	0.19
	Ranch (1)	8.95	3.71
	Low calorie (2)	13.17 ± 0.89	1.57 ± 1.79
	U.S. mayonnaise (2)	2.38 ± 2.94	1.82 ± 2.24
	U.S. soups		
	Beef bouillon cubes (3)	19.54 ± 13.75	1.25 ± 1.69
	Chicken bouillon cubes (3)	20.33 ± 20.49	1.41 ± 2.12
Ali et al. (1996)	U.S. salad dressing, blue cheese (1)	5.47	1.21
Fernandez San Juan (1996)[c]	Spanish soups, dehydrated (42)	15.4 ± 9.4	
Aro et al. (1998c)[c]	European soups (4)	6.57–30.13	
	European sauces (6)	0.20–38.63	
Innis et al. (1999)	Canadian soups (11)	22.4 (1.1–51.6)	2.6 (0–9.1)
Elias et al. (2002)	Canadian salad dressing, oil/vinegar (8)	1.4 ± 0.3	1.4 ± 0.3
	Canadian salad dressing, creamy (10)	1.3 ± 0.4	1.3 ± 0.4
Satchithanandam et al. (2004)	U.S. mayonnaise (6)	0.2–0.5	
	U.S. light mayonnaise (2)	0.0	
	U.S. salad dressings		
	Ranch (4)	0.1	
	Creamy ranch (2)	0.6	
	Fat-free ranch (2)	0.0	
	Italian dressing (8)	0.2–2.2	
	Olive oil and vinegar (2)	0.2	
	Reduced-fat dressing (2)	0.0	

[a] Number of samples analyzed appears in parentheses following food item.
[b] Percentage of total fatty acids; n.d. = not detected.
[c] Number of European countries from which samples were analyzed appears in parentheses following food item. Values presented as range. Data expressed as percentage of fatty acid methyl esters.

K. Fast Foods

Fast food items can be a significant source of *trans*-fatty acids in the diet. In the lunch/dinner items analyzed (Table 15.10), the *trans*-fatty acid content varied from 0.04 g/100 g food for milk shakes to 1.38 g/100 g food for fried pies and turnovers to 3.0 g/100 g of food for French fries. Hamburgers, as well as items such as fried fish and fried chicken, are all sources of *trans*-fatty acids. Based on the data of Lanza and Slover (1981), a typical fast food meal could provide 3–4 g of *trans*-fatty acids (Table 15.11), and even more if the USDA (1995) data for French fries are used.

Recently, Stender et al. (2006) reported that a fast food meal of French fries and chicken nuggets contained over 10 g of industrially produced *trans*-fatty acids if purchased at McDonald's in New York City, USA, or almost 25 g if purchased at Kentucky Fried Chicken in Hungary. Breakfast items would also provide *trans*-fatty acids, with pastries providing relatively high amounts (Table 15.10).

The values for *trans*-fatty acids in fast foods, however, must be interpreted with caution, because many fast food restaurants have changed the type of fat used for frying since the analyses were done by Slover et al. (1980). On the other hand, if the data of Smith et al. (1986) on commercial frying fats and that of Pfaltzgraf et al. (1993), Fernandez San Juan (1996), USDA (1995) are typical of products used in the 1980s and 1990s, then fast food items in the United States and some countries in Europe contained significant amounts of *trans*-isomers at that time. In other countries, such as New Zealand (Lake et al., 1996), the *trans*-fatty acid content of commercial frying fats used in fast food restaurants appears to be less than in the Untied States; thus, the *trans*-content of a fast food meal in these countries would be expected to be less.

The report by Stender et al. (2006) on the *trans*-fatty acid content of chicken nuggets and French fries purchased at McDonald's or Kentucky Fried Chicken gives an interesting cross-country comparison. For a fast food meal at McDonald's, 5–10 g of industrially produced *trans*-fatty acids would be obtained if the meal was purchased in the United States, Peru, United Kingdom, South Africa, Poland, Finland, France, Italy, Norway, Spain, Sweden, Germany, or Hungary. The same meal of chicken nuggets and French fries would contain 1–5 g of *trans*-fat only if it were purchased in Austria, Portugal, the Netherlands, Russia, Czech Republic, or Spain. Only if the meal were purchased in Denmark would contain less than 1 g of *trans*-fatty acids. A meal of French fries and chicken from Kentucky Fried Chicken would contain 10–25 g of *trans*-fatty acids if purchased in Hungary, Poland, Peru, or Czech Republic; 5–10 g in the Bahamas, South Africa, or USA; 2–5 g in Germany (Hamburg), France, United Kingdom (London or Glasgow), Spain, or Portugal; and less than 2 g in United Kingdom (Aberdeen), Denmark, Russia, or Germany (Wiesbaden). Thus, the *trans*-fatty acid content of fast food can vary widely depending on the country and even the city.

L. Processed Foods

Several investigators have reported values for the *trans*-fatty acid content of snack items (Table 15.12). The *trans*-isomer content of these food items is highly variable, with some brands providing negligible amounts and others containing as much as 30%–50% of the fatty acids as *trans*-isomers. Enig et al. (1990b) contend that the *trans*-content of many of these snack items increased when food analyzed in 1978 were compared with comparable items analyzed a decade later. However, when labeling laws were implemented in 2006 in the United States, many snack items were reformulated and advertised as containing "0 *trans*."

The most comprehensive early analysis of processed food items is that published by Enig et al. (1983). In this study, the fat in 220 samples from 35 food types was analyzed for individual fatty acids including *trans*-octadecenoic acid (Table 15.13). None of the samples of peanut butter contained *trans*-fatty acids. Maximum values in the other categories ranged from 24% to 39% of the fatty acids as *trans*-isomers. Mean values for the *trans*-content of samples in which any amount of *trans*-fatty acids was detected ranged from 11% to 28%.

TABLE 15.10
Trans-Fatty Acid Content of Fast Foods

Reference	Food Item[a]	*Trans*-Fatty Acid Content						Fat Content (wt.%)
		% of Fatty Acids			g/100 g Food			
		Min	Max	Avg	Min	Max	Avg	
Lunch/dinner items								
Slover et al. (1980)	Hamburgers (10)	3.00	5.16	3.73				
Lanza and Slover (1981)	Hamburger (10)				0.38	0.79	0.55	15.4
Fernandez San Juan (1996)[b]	Hamburgers, beef (40)			3.8				10.2
	Hamburgers, burger (50)			4.1				11.6
Elias et al. (2002)	Hamburgers (4)	5.6	9.6	6.8 ± 1.9				
	Chicken burgers (8)	12.8	57.9	27.1 ± 15.8				
	Fish burgers fillets (6)	17.7	41.8	29.3 ± 10.4				
Monge-Rojas et al. (2005)	Hamburger						1.03[c]	
Slover et al. (1980)	Cheeseburgers (9)	2.66	4.38	3.44				
Lanza and Slover (1981)	Cheeseburgers (9)				0.45	0.69	0.59	17.3
Monge-Rojas et al. (2005)	Cheeseburger						0.54[c]	
Ratnayake et al. (1993)[d]	Buns, hamburger							
	Unhydrogenated fat (2)	0.4	2.6	1.5				4.0
	Hydrogenated fat (1)			26.3				5.5
Slover et al. (1980)	Ham/cheese sandwiches (1)			1.62				
Lanza and Slover (1981)	Ham/cheese sandwiches (1)						0.19	13.1
Slover et al. (1980)	Fish sandwiches (4)	1.39	3.58	2.75				
Lanza and Slover (1981)	Fish sandwiches (4)				0.22	0.43	0.36	15.4
Smith et al. (1985)	Fried fish pieces (4)	5.8	29.9					10.1
Enig et al. (1983)	Fried fish (3)	0	15.2					
Enig et al. (1990a)	Fried fish (4)	22.9	33.5					
Mozaffarian et al. (2006)	Breaded fish burger			28				
Slover et al. (1980)	Fish platter (1)			4.59				
Lanza and Slover (1981)	Fish platter (1)						0.76	18.6
Slover et al. (1980)	Beef platter (1)			3.54				
Lanza and Slover (1981)	Beef platter (1)						0.69	20.4
Smith et al. (1985)	Fried chicken pieces, thighs (5)	7.7	16.4					13.8

Enig et al. (1990a)	Fried chicken (1)			27.8				
Elias et al. (2002)	Chicken nuggets (3)	29.5	56.7	42.3 ± 13.0				
Huang et al. (2006)[e,f]	Chicken nuggets	2.08 ± 0.5	3.33 ± 0.6					
Huang et al. (2006)[e,f]	Chicken strips			4.86 ± 0.8				
Stender et al. (2006)	Chicken nuggets (24)							
	McDonald's	1	14					
	Chicken nuggets (19)							
	Kentucky Fried Chicken	0.4	38					
Mozaffarian et al. (2006)	Breaded chicken nuggets			25				
Elias et al. (2002)	Potato nuggets (2)	44.5	46.3	45.4				
Slover et al. (1980)	French fries (3)	3.87	4.13	4.02				
Lanza and Slover (1981)	French fries (3)				0.52	0.72	0.60	18.1
Enig et al. (1983)[g]	French fries (3)	4.6	5.1	4.8				
	French fries (4)	6.2	37.4	17.8				
Smith et al. (1985)	French fries (6)	6.3	34.1					13.5
Enig et al. (1990a)	French fries (4)	3.2	25.8					
Litin and Sacks (1993)	French fries (2)				2.12	3.02	2.6	
Pfalzgraf et al. (1993)[b]	French fries (2)	22.5	32.8	27.7				
Ratnayake et al. (1993)[d]	French fries (1)			n.d.[h]				17.2
USDA (1995)	French fries (7)	7.3	34.2	20.6	1.0	5.2	3.0	16.0
Fernandez San Juan (1996)[b]	French fries (15)			20.9				20.4
Lake et al. (1996)[i]	French fries (2)	5.4	5.8	5.6				
Aro et al. (1998c)[j]	French fries (11)	0.45	34.84					
Innis et al. (1999)	French fries (16)	4.9	56.9	37.7				5.8
Elias et al. (2002)	French fries (15)	4.9	56.9	39.6 ± 15.3				
Huang et al. (2006)[e,f]	French fries	4.67 ± 0.6	5.63 ± 0.9					
Stender et al. (2006)	French fries (24)							
	McDonald's	1	24					
	French fries (19)							
	Kentucky Fried Chicken	1	42					
Mozaffarian et al. (2006)	French fries	28	36					
	French fries, frozen			30				
Satchithanandam et al. (2004)	Frozen potato product (12)	24.7	38.2					
Huang et al. (2006)[e,f]	Potato wedges			6.39 ± 0.8				
Slover et al. (1980)	Onion rings (1)			3.78				

Continued

TABLE 15.10
(Continued)

Reference	Food Item[a]	*Trans*-Fatty Acid Content						Fat Content (wt.%)
		% of Fatty Acids			g/100 g Food			
		Min	Max	Avg	Min	Max	Avg	
Lanza and Slover (1981)	Onion rings (1)						1.26	16.7
Slover et al. (1980)	Milk shakes (9)	1.76	3.1	2.2				
Lanza and Slover (1981)	Milk shakes (9)				0.05	0.10	0.07	3.4
USDA (1995)	Milk shakes (4)	3.0	4.9	3.7	0.01	0.07	0.04	1.2
Slover et al. (1980)	Pies and turnovers (4)	6.33	16.63	11.71				
Lanza and Slover (1981)	Pies and turnovers (4)				0.68	2.02	1.38	16.5
Slover et al. (1980)	Cookies (1)			3.17				
Lanza and Slover (1981)	Cookies (1)						0.49	14.7
Elias et al. (2002)[d]	Cookies (19)	1.3	45.6	23.0 ± 13.4				
Breakfast items								
Slover et al. (1980)	Egg muffin (1)			0.87				
Lanza and Slover (1981)	Egg muffin (1)						0.08	11.6
Slover et al. (1980)	English muffin with butter (1)			2.27				
Lanza and Slover (1981)	English muffin with butter (1)						0.19	10.0
Slover et al. (1980)	Scrambled eggs (1)			1.16				
Lanza and Slover (1981)	Scrambled eggs (1)						0.21	19.9
Slover et al. (1980)	Hot cakes with butter (1)			8.02				
Lanza and Slover (1981)	Hot cakes with butter (1)						0.52	6.8
Slover et al. (1980)	Sausage biscuit (1)			0.27				
Lanza and Slover (1981)	Sausage biscuit (1)						0.04	16.8
Enig et al. (1983)	Apple-cheese pastry (1)			12.0				
	Cheese danish (1)			34.6				
Innis et al. (1999)[d]	Muffins (7)	1.7	36.2	11.2				9.4
	Granola bars (7)	5.1	21.7	11.3				11.5
	Chocolate bars (9)	0.1	35.9	9.16				23.6
	Cereals (11)	0.2	24.3	4.2				3.0

Elias et al. (2002)[d]	Muffins (4)	1.7	3.1	2.4 ± 0.7
	English muffin (2)	12.2	16.2	14.2
	Pancakes, waffles (7)	0.8	55.1	30.5 ± 16.8
	Granola (3)	0.6	24.3	8.7 ± 13.6
	Cereals (8)	0.2	9.4	2.6 ± 3.5
Satchithanandam et al. (2004)[e]	Cereals (4)	0.0	2.0	
	Cereal bars (4)	21.0	48.8	
	Cereal bars with strawberry			26.0
	Fig bars			27.8
	Apple cobbler bars			25.4
	Oatmeal bars			22.4
	Granola bar with brown sugar			0.0
	Granola bar with chocolate chips			7.6
	Granola bar with peanut butter, chocolate chunks			7.1
Mozaffarian et al. (2006)	Granola bar			18
	Chocolate bar			2
	Breakfast bar			15
	Pancakes			21
	Muffin			14

[a]Number in parentheses indicates number of samples analyzed. Food items are U.S. samples unless otherwise noted.
[b]Spanish samples.
[c]Total represents only *trans*-18:1 content.
[d]Canadian samples.
[e]U.S. samples.
[f]Data expressed as percentage of corresponding triglyceride.
[g]In Enig et al. (1983). *J. Am. Oil Chem. Soc. 60*: 1788–1795, french fries from seven national fast food chains were analyzed. The first three given are from the same chains as reported by Slover et al. (1980). *J. Food Sci. 45*: 1583–1591; values for the other four are from different chains.
[h]n.d. = not detected.
[i]French fries (labeled as potato chips in New Zealand) are from nationally (New Zealand) distributed retail takeaway chains.
[j]Number of European countries from which samples were analyzed appears in parentheses following food item. Data expressed as percentage of methyl esters.
[k]German samples.

TABLE 15.11
***Trans*-Fatty Acid Content of a Typical Fast Food Meal**

	Weight[a] (g)	*Trans*-Fatty Acids[b] (g/100 g)	Total *Trans*-Fatty Acids (g)
Cheeseburger	215	0.59	1.27
Fries	106	0.60	0.64
Milkshake	290	0.07	0.20
Turnover	125	1.38	1.73
			3.84

[a] Data from Table of Food Composition (Hamilton, E.M.N., et al. (1988). *Nutrition: Concepts and Controversies*, 4th ed., West, New York).
[b] Data from Lanza, E., and Slover, H.T. (1981). *Lipids 16*: 260–267. As summarized in Table 15.10.

M. *Trans*-Isomers of Fatty Acids in Human Milk and Infant Foods

Whereas isomeric fatty acids may have no effects on an adult, in theory infants may be particularly vulnerable to the effects of factors that interfere with essential fatty acid metabolism and normal membrane structure (ILSI, 1997; Craig-Schmidt, 2001). Thus, it is important to know the *trans*-fatty acid content of foods consumed during this period of rapid development.

Trans-fatty acids comprise 1%–7% of total fatty acids in human milk (Table 15.14). The amount of *trans*-octadecenoic acid (18:1t) appearing in the milk reflects the *trans*-content of the maternal diet consumed on the previous day (Aitchison et al., 1977; Craig-Schmidt et al., 1984). Friesen and Innis (2006) have found that the *trans*-fatty acid content of human milk has decreased from 7.1% in 1998 to 4.6% in 2005/2006.

Infant formulas contain variable amounts of *trans*-fatty acids, with values of 0.1%–4.5% of total fatty acids reported (Picciano and Perkins, 1977; Hanson and Kinsella, 1981; Permanyer et al., 1990; O'Keefe et al., 1994; Jorgensen et al., 1995; Ali et al., 1996; Chardigny et al., 1996; Fernandez San Juan, 1996; Ratnayake et al., 1997) (Table 15.14a). One brand of infant formula analyzed by Hanson and Kinsella (1981) contained 15.7% of the fatty acids as *trans*-isomers, but all the others contained less than 2%.

Three types of baby foods were analyzed for *trans*-fatty acids by Slover and Lanza (1979) and eight brands of dry infant cereals by Hanson and Kinsella (1981). All these products except the lamb broth (Slover and Lanza, 1979) contained less than 2% *trans*-fatty acids. More recent analysis (USDA, 1995) revealed that some U.S. infant foods contained greater than 5% *trans*-fatty acids.

Thus, foods normally consumed by infants contain relatively low levels of *trans*-fatty acids. In most cases, *trans*-isomeric fat in these products is no greater than that found in cow's milk (see Table 15.2).

V. ESTIMATES OF ISOMERIC FATTY ACIDS IN THE DIET

Several approaches can be taken in estimating isomeric fatty acids in the diet: (1) laboratory analysis of composite diets; (2) dietary analysis of diet records using food composition data such as those summarized above; (3) estimates based on *trans*-fatty acid content of biological tissues; and (4) estimates based on "disappearance" or market share data. All these approaches have been used although most estimates are based on disappearance data. These methods and their use worldwide have been reviewed recently by Craig-Schmidt (1998, 2006). The reader is referred to these references for an update on estimates of isomeric fatty acids in the diet throughout the world.

TABLE 15.12
***Trans*-Fatty Acid Content of Snack Items**

Reference	Country	Food Item[a]	*Trans*-Fatty Acids (%)[b]			Fat (g/100 g)
			Min	Max	Avg	
Enig et al. (1983)[c]	United States	Potato chips (6)	0	27.4[c]	4.6	
Smith et al. (1985)[d]	United States	Potato chips (9)	0	1.6	1.3	35.3–44.5
USDA (1995)	United States	Potato chips (11)	0	29.7	5.4 ± 9.1	29.0–39.2
Satchithanandam et al. (2004)	United States	Potato chips (8)	0.2	9.3		
Huang et al. (2006)[e]	United States	Potato chips			0.0	
Ratnayake et al. (1993)	Canada	Potato chips				
		Unhydrogenated (2)	0.4	2.0	1.2	30.1–41.0
		Hydrogenated (3)	29.7	39.7	33.9	32.3–40.0
Innis et al. (1999)[d]	Canada	Potato chips (6)	0.4	25.3	5.9	25.1
Elias et al. (2002)[d]	Canada	Potato chips (9)	0.4	26.9	8.9 ± 12.0	
Pfalzgraf et al. (1993)	Germany	Potato chips				
		Unhydrogenated (1)			0.7	
		Hydrogenated (2)	17.2	19.9	18.6	
Ovesen and Leth (1995)	Denmark	Potato chips (1)			0	29
Fernandez San Juan (1996)	Spain	Potato chips (40)			0.6 ± 1.4	35.2 ± 4.3
Lake et al. (1996)	New Zealand	Potato chips (3)	0.3	0.8	0.5 ± 0.3	30–35
Enig et al. (1983)[f]	United States	Corn snacks (5)	0.8	22.0		
Smith et al. (1985)	United States	Corn snacks (7)	0.8	22.0		22.9–37.6
Enig et al. (1990a)	United States	Corn snacks (3)	0.8	20.6		
Pfalzgraf et al. (1993)	Germany	Corn snack (1)			19.4	
Ratnayake et al. (1993)	Canada	Corn snacks				
		Unhydrogenated (2)	0.9	1.6	1.3	17.5–33.5
		Hydrogenated (3)	29.9	33.9	32.3	25.0–34.2
Elias et al. (2002)[d]	Canada	Corn chips (5)	0.7	33.6	9.9 ± 15.8	
USDA (1995)	United States	Tortilla chips (1)			17.5	25.6
Satchithanandam et al. (2004)	United States	Tortilla chips (8)	0.0	17.1		
Mozaffarian et al. (2006)	United States	Tortilla chips			22	
Enig et al. (1983)[f]	United States	Cheese snacks (1)			33.4	
Smith et al. (1985)[d]	United States	Cheese snacks (6)	1.0	28.1		22.3–46.0

Continued

TABLE 15.12
(Continued)

Reference	Country	Food Item[a]	*Trans*-Fatty Acids (%)[b]			Fat (g/100 g)
			Min	Max	Avg	
Enig et al. (1990a)	United States	Cheese snacks (4)	23.5	53.9		
USDA (1995)	United States	Cheese snacks (2)	10.0	25.8	17.9 ± 11.2	31.3–32.2
Elias et al. (2002)[d]	Canada	Cheese snacks (2)	35.8	48.0	41.9	
Enig et al. (1983)[f]	United States	Donuts (1)			3.0	
Smith et al. (1985)[d]	United States	Donuts, cake (4)	10.1	34.3		9.1–31.4
Enig et al. (1990a)	United States	Donuts (2)	32.5	36.7		
USDA (1995)	United States	Donuts, cake (5)	3.8	29.2	18.5 ± 10.0	15.1–25.2
	United States	Donuts, yeast (4)	2.3	31.2	13.4 ± 12.7	19.5–32.4
Ratnayake et al. (1993)	Canada	Donuts (5)	25.8	32.7	29.1 ± 2.9	16.6–29.6
Innis et al. (1999)[d]	Canada	Donuts (13)	3.9	42.7	29.6	13.5
Elias et al. (2002)[d]	Canada	Donuts (13)	3.5	42.7	29.7 ± 14.2	
Pfalzgraf et al. (1993)	Germany	Crackers (2)	trace	5.6	2.8	
Ratnayake et al. (1993)	Canada	Crackers				
		Unhydrogenated (1)			—	17.9
		Hydrogenated (7)	13.8	35.4	25.8	9.2–33.0
Elias et al. (2002)[d]	Canada	Crackers (13)	23.5	51.3	40.3 ± 8.5	
USDA (1995)	United States	Crackers (9)	11.6	39.9	32.6 ± 9.6	11.0–24.5
Ali et al. (1996)	United States	Crackers (3)	12.8	42.9	23.3	
		Peanut butter sandwich (1)			16.5	29.5 ± 0.6

Huang et al. (2006)[e]	United States	Crackers			0.66 ± 0.22	
Lake et al. (1996)	New Zealand	Crackers (5)	1.2	3.9	2.0 ± 1.1	8–25
Richardson et al. (1997)	New Zealand	Crackers (1)			0.71	23.8
Monge-Rojas et al. (2005)[g]	Costa Rica	Crackers			0.24	
Pfalszgraf et al. (1993)	Germany	Popcorn (1)			0.9	
USDA (1995)	United States	Popcorn (5)	26.9	35.2	31.3 ± 3.0	11.0–37.2
Mozaffarian et al. (2006)	United States	Popcorn, microwave			11	
Aro et al. (1998c)[h]	Europe	Popcorn (5)	0.04	34.82		
Elias et al. (2002)[d]	Canada	Popcorn (2)	44.1	47.3	45.7	
Pfalzgraf et al. (1993)	Germany	Other snacks				
		Unhydrogenated (4)	0.4	1.2	0.8	
		Hydrogenated (2)	9.9	20.2	15.1	
Fernandez San Juan (1996)	Spain	Other snacks (20)			0.1 ± 0.1	36.9 ± 2.9

[a]Number of samples analyzed appears in parentheses following food item.

[b]Percentage of total fatty acids.

[c]Only one brand contained significant amounts of *trans*-fatty acids. One other brand contained 0.3%; the other four contained negligible amounts.

[d]Data expressed as g/100 g of fat.

[e]Data expressed as percentage of corresponding triglyceride.

[f]Enig et al. (1983). *J. Am. Oil Chem. Soc. 60*: 1788–1795. Data expressed as percentage of methyl esters.

[g]Data expressed as g/100 g of food.

[h]Number of European countries from which samples were analyzed appears in parentheses following food item. Values presented as range. Data expressed as percentage of methyl esters.

TABLE 15.13
***Trans*-Fatty Acid Content of Bread, Cakes, Sweets, and Miscellaneous Processed Foods**

			Trans-Fatty Acids (%)		
Reference	**Country**	**Food Item[a]**	**Min**	**Max**	**Avg**
Enig et al. (1983)[b]	United States	Breads and rolls (10/9)	0/0.2	27.9	10.1/11.2
		Breading mixes, fried crusts (8/5)	0/12.1	33.5	12.9/20.6
		Cakes (4)	0.1	24.0	13.9
		Candy and frostings (9/7)	0/3.2	38.6	15.8/20.4
		Cream substitutes, cereals, puddings (8/7)	0/0.4	36.1	10.7/12.2
		Cookies (25/21)	0/2.5	37.4	18.7/22.3
		Crackers (20/17)	0/2.3	31.6	12.2/14.3
		Snack chips (13/9)	0/0.3	33.4	11.9/17.2
		Pastry and pastry crusts (18/16)	0/0.6	34.6	10.3/11.5
		Peanut butters (6)	0	0	0
		Pizza crusts and pretzels (6/3)	0/14.4	31.4	10.1/20.2
		Fried potatoes (4/2)[c]	0/19.4	36.2	13.9/27.8
Laryea et al. (1988)	Germany	Nut–nougat creams (12)	0.35	12.35	7.2 ± 3.9
Won and Ahn (1990)[d]	Korea	Cookies and cakes			1.3
		Animal food, fried and sauteed			1.0
		Vegetables, fried and sauteed			0.7
		Fried foods			1.2
Boatella et al. (1993a)	Spain	Assorted bakery products (83)			1.6 ± 1.6
Ratnayake et al. (1993)	Canada	Bread			
		Unhydrogenated fat (6)	0	2.9	1.4 ± 1.3
		Hydrogenated fat (1)			15.7
		Muffins			
		Unhydrogenated fat (2)	0.5	1.3	0.9 ± 0.6
		Hydrogenated fat (2)	16.5	24.2	20.4 ± 5.4
		Pie crusts, unhydrogenated fat (2)	0.9	1.2	1.1 ± 0.2
		Pizza crusts			
		Unhydrogenated fat (3)	0	1.7	0.6 ± 1.0
		Hydrogenated fat (3)	22.1	28.8	25.5 ± 4.7
		Cereals, breakfast			
		Unhydrogenated fat (6)	0	1.6	0.5 ± 0.7
		Hydrogenated fat (5)	9.2	33.7	16.5 ± 10.0
		Cakes			
		Unhydrogenated fat (2)	2.4	3.0	2.7 ± 0.4
		Hydrogenated fat (6)	10.1	25.7	16.3 ± 6.7
		Cookies			
		Unhydrogenated fat (3)	2.6	6.4	4.3 ± 1.9
		Hydrogenated fat (13)	7.6	38.7	22.1 ± 11.2
		Candies/chocolates			
		Unhydrogenated fat (18)			0
		Hydrogenated fat (1)			11.1
		French fries[c]			
		Unhydrogenated fat (1)			0
		Hydrogenated fat (3)	32.8	42.8	36.6 ± 5.4
Pfalzgraf et al. (1993)	Germany	Bakery products (13)	0	27.9	7.4 ± 8.3
		Sweets			
		Nut/nougat creams (5)	0.5	15.2	6.6 ± 6.6
		Other (15)	0.2	15.7	3.2 ± 4.9

TABLE 15.13
(Continued)

Reference	Country	Food Item[a]	*Trans*-Fatty Acids (%) Min	Max	Avg
Ovesen and Leth (1995)[d]	Denmark	Pastry	8	14	10
		Croissants	8	14	11
		Cookies	3	17	8
		Pizza	0	8	4
USDA (1995)	United States	Bread (6)	1.5	25.5	12.8 ± 9.2
		Biscuits (2)	22.9	36.5	29.7 ± 9.6
		Muffins (1)			31.9
		Rolls (4)	2.2	25.6	10.0 ± 10.6
		Taco shells (1)			31.5
		Tortillas (1)			16.6
		Cereals, breakfast (6)	4.2	40.3	20.7 ± 13.2
		Cakes (6)	3.9	28.3	18.5 ± 9.5
		Snack cakes (1)			21.6
		Sweet rolls (1)			14.3
		Danish pastry (1)			8.5
		Cookies (7)	18.7	37.7	29.4 ± 7.2
		Candies (3)	0.3	29.1	12.9 ± 14.7
		Frostings (6)	19.7	24.7	21.7 ± 2.1
		French fries (7)	7.3	34.2	20.6 ± 10.4
Ali et al. (1996)	United States	Peanut butter cookies			1.7
		Biscotti			3.1
		Cereal with raisins			15.0
		Chicken pie			25.5
		Turkey with gravy dressing			13.9
		Beef ravioli with sauce			5.0
		Chili macaroni			3.8
		Breaded fish fillets			16.6
		Taco dinner			29.7
		Chili without beans			5.9
De Greyt et al. (1996)	Belgium	Assorted bakery products			
		<5% *trans* (12)	n.d.	4.1	1.5 ± 1.5
		>5% *trans* (15)	5.1	18.8	9.6 ± 3.8
Demmelmair et al. (1996)	Germany	Chocolate spreads (6)	0.7	11.1	6.2 ± 3.6
		Peanut butter (3)	0	0.3	0.1 ± 0.1
		Vegetarian spreads (6)	0.1	0.4	0.2 ± 0.1
Fernandez San Juan (1996)	Spain	Assorted bakery products (30)			9.4 ± 8.9
		Cookies (42)			1.1 ± 1.1
		Cakes (50)			3.1 ± 2.8
		Creams (15)			1.8 ± 0.4
		Pizzas (20)			3.1 ± 4.0
Lake et al. (1996)	New Zealand	Pastry (5)	3.6	7.5	6.2 ± 1.8
		Cakes (5)	2.6	8.4	5.2 ± 2.1
		Cookies and crackers (5)	1.1	3.5	2.0 ± 1.0
Richardson et al. (1997)[e]	New Zealand	White bread			2.2
		Pastry			5.6
		Chocolate coated cookies			1.7
		Plain sweet cookies			4.5
		Meat pies			3.9

Continued

TABLE 15.13
(Coninued)

Reference	Country	Food Item[a]	*Trans*-Fatty Acids (%)		
			Min	Max	Avg
van Erp-baart et al. (1998)[f]	Europe	Bread (11)	0.05	17.35	
		Cookies and biscuits			
		lowest in *trans* (14)	0.12	12.63	
		highest in *trans* (13)	1.45	27.96	
		Cake and sweet pastry			
		lowest in *trans* (14)	0.39	16.63	
		highest in *trans* (13)	3.66	33.32	
		Croissants (11)	3.03	14.55	
		Cream crackers (4)	9.10	29.08	
		Doughnuts (8)	1.12	31.76	
Parcerisa et al. (1999)[g]	Spain	Bakery products, commercial (15)	0.60	11.85	
Innis et al. (1999)[h]	Canada	White bread (8)	1.3	34.9	18.5
		Whole wheat bread (8)	1.0	36.3	15.6
		Croissants (3)	5.5	40.9	18.1
		Crackers (14)	23.5	51.3	40.3
		Croutons (3)	22.9	51.6	41.9
		Cake mixes (3)	28.7	30.1	29.6
		Cookies (19)	1.4	45.7	23.0
		Pie shells (6)	1.9	45.6	25.8
Elias et al. (2002)[h]	Canada	Bread (12)	1.0	36.3	18.0 ± 15.5
		Rolls (14)	0.3	35.3	8.9 ± 10.4
		Bagels (3)	1.2	2.5	2.0 ± 0.7
		Croissants (3)	5.5	40.9	18.1 ± 19.7
		Danish pastry (2)	41.7	59.2	50.5
		Cake mix (5)	24.9	36.2	30.0 ± 4.1
		Tortilla, flour (2)	27.3	32.7	30.0
		Pastry shells (5)	1.9	45.6	23.6 ± 21.2
		Granola bars (4)	0.7	17.0	8.7 ± 6.7
		Pretzels (2)	0.9	23.2	12.0
		Peanut butter (2)	1.6	6.7	4.2
Vicario et al. (2003)	Spain	Chocolate coated cookies (4)			0.38 ± 0.26
		Butter cookies (3)			1.41 ± 0.23
		Wholemeal cracker (3)			35.54 ± 1.48
		Tea pastry (2)			1.01 ± 0.57
		Croissant (4)			5.94 ± 0.69
		Sponge cakes (2)			2.10 ± 0.08
		Swiss roll (1)			0.15
		Chocolate cake (5)			4.10 ± 1.50
Satchithanandam et al. (2004)	United States	Dinner rolls			16.6
		Crescent rolls, reduced fat			19.3
		Biscuits, butter-flavored	32.7	34.4	
		Flaky biscuits			20.8
		Buttermilk biscuits			34.1
		Biscuits, Texas-style			26.2
		Cake rolls, iced and filled			27.4
		Cake, iced and filled	15.3	34.5	

TABLE 15.13
(Continued)

Reference	Country	Food Item[a]	*Trans*-Fatty Acids (%)		
			Min	Max	Avg
		Cake, chocolate covered			6.6
		Cake, peanut butter covered			0.9
		Sponge cake, filled	0.0	20.8	
		Chocolate cookie cake			3.8
		Mini cake snacks, iced			10.9
		Cornbread			32.1
		Tortillas			19.5
		Tortillas, fat-free			9.8
		Flour tortillas	1.9	18.0	
		Corn tortillas			0.0
		Taco dinner kit	16.3	24.9	
		Pizza kit			22.8
Monge-Rojas et al. (2005)[d,i]	Costa Rica	White bread			0.041
		Cookies			0.046
		Crème-filled cookies			1.027
		Chocolate cookies			1.161
Huang et al. (2006)[e]	United States	Crackers, grocery			0.66 ± 0.22
		Muffin, grocery			0.0
		Biscuit mix, grocery			1.82 ± 0.11
		Cookies, grocery			0.51 ± 0.01
		Cookies, fast food	2.30 ± 0.34	3.18 ± 0.10	
		Biscuits, fast food	8.35 ± 0.29	10.30 ± 1.67	
Mozaffarian et al. (2006)	United States	Pie			28
		Danish or sweet roll			25
		Doughnuts			25
		Cookies			26
		Cake			16
		Brownie			21
		Crackers			34
		Tortillas			25
		Peanut butter			1

[a]Number of total brands analyzed.
[b]Data as classified by the author; minimum and average values for total number of brands analyzed followed by minimum and average values for items excluding those that contained no detectable amounts of *trans*-fatty acids. Values include 18:1t plus isomeric forms of 18:2.
[c]Excluding fast food French fries. See Table 15.10.
[d]*Trans*-18:1 content only.
[e]Data expressed as percentage of corresponding triglyceride.
[f]Number of European countries from which samples were analyzed appears in parentheses following food item. Values presented as range.
[g]Bakery products include 1 Swiss cake, 3 Swiss rolls stuffed with chocolate, 1 sponge cake, 3 sponge cakes filled with chocolate, 2 doughnuts, 1 doughnut filled with chocolate, 1 biscuit coated with chocolate, and 3 cakes filled with chocolate. Values presented as range. Data expressed as percentage of methyl esters.
[h]Data expressed as g/100 g of fat.
[i]Data expressed as g/100 g of food.

TABLE 15.14
***Trans*-Fatty Acid Content of Human Milk**

References	Food Item[a]	*Trans*-Fatty Acids (%)[b] Min	Max	Avg[c]
Aitchison et al. (1977)[c]	U.S., human milk (11)	2.68	5.43	3.88 ± 0.77
Picciano and Perkins (1977)[d]	U.S., human milk (3)	2.1	4.0	3.1
Clark et al. (1980)[e]	U.S., human milk (11)			4.87
Hanson and Kinsella (1981)	U.S., human milk (1)			tr
Hundrieser et al. (1983)[d]	U.S., human milk (10)	1.1	5.7	3.4
Craig-Schmidt et al. (1984)[d]	U.S., human milk (8)			4.76 ± 2.06
Chappell et al. (1985)[f]	Canada, human milk (14)			2.7
Finley et al. (1985)[e]	U.S., human milk (57)			4.24
Koletzko et al. (1988)[c]	Germany, human milk (15)	2.17	6.04	4.40
Koletzko et al. (1991)[c]	Nigeria, human milk (10)	0.79	10.29	1.20
Boatella et al. (1993b)	Spain, human milk (38)			1.2
Chardigny et al. (1995)[g]	France, human milk (10)	1.33	4.4	2.27
Chen et al. (1995)[h]	Canada, human milk (198)			7.19 ± 3.03
Jorgensen et al. (1995)[i]	Denmark, human milk (11)	1.6	4.5	2.5
Laryea et al. (1995)[c]	Sudan, human milk (77)	0.23	1.45	0.61 ± 0.26
Wolff (1995)[d]	France, human milk (10)	1.20	3.17	1.99 ± 0.57[c]
Chen et al. (1997)[c]	China, human milk			
	Chongqing (33)			0.22 ± 0.06
	Hong Kong (51)			0.88 ± 0.61
Genzel-Boroviczeny et al. (1997)[c]	Germany, human milk (38)			1.13 (0.46)[j]
Innis and King (1999)	Canada, human milk (103)			7.1 ± 0.32
Dlouhy et al. (2002)	Czech, human milk (35)	1.84	9.78	4.22 ± 1.87
Mosley et al. (2005)	U.S., human milk (81)	2.5	13.8	7.0 ± 2.3
Mosley et al. (2006)[d]	U.S., human milk (4)			3.0 ± 0.15
Friesen and Innis (2006)	Canada, human milk			
	1998 (103)			7.1 ± 0.32
	November 2004–March 2005 (24)			6.2 ± 0.48
	April–August 2005 (24)			5.3 ± 0.49
	September 2005–January 2006 (39)			4.6 ± 0.32

[a] Number of subject analyzed appears in parentheses following food item.
[b] Percentage of total fatty acids.
[c] Includes *trans*-isomers of 14:1, 18:1, and 18:2.
[d] *Trans*-18:1 content only.
[e] Includes *trans*-isomers of 16:1 and 18:1.
[f] Includes *trans*-isomers of 18:1 and 18:2.
[g] Includes *trans*-isomers of 18:1, 18:2, and 18:3.
[h] Includes *trans*-isomers of 14:1, 16:1, 18:1, 18:2, and 18:3.
[i] Includes *trans*-isomers of 16:1, 18:1, and 18:2.
[j] Median (interquartile ranges).

A. Analysis of Composite Diets

Estimates obtained by analysis of composite diets collected from a given population have the advantage of being based on data obtained by analytical methods in the laboratory. This method suffers from the fact that the diets or populations analyzed may not be representative of the population as a whole. A number of investigators (Aitchison et al., 1977; Åkesson et al., 1981;

TABLE 15.14a
***Trans*-Fatty Acid Content of Infant Formula and Other Infant Foods**

Reference	Food Item[a]	*Trans*-Fatty Acids (%)[b]			Fat (%)
		Min	Max	Avg	
Picciano and Perkins (1977)[c]	U.S. infant formula (3)	0.1	1.3	0.5	
Hanson and Kinsella (1981)	U.S. infant formula (11)	0.8	2.0	1.2	6.0
	U.S. infant formula (1)			15.7	7.0
Permanyer et al. (1990)[d]	Spanish infant formula				
	"Preterm" formula (3)	1.2	2.6	2.1 ± 0.8	
	"0–6 month" formula (10)	1.3	4.5	2.9 ± 1.2	
	"6–12 month" formula (7)	1.0	4.0	2.8 ± 1.0	
	Special formula (8)	0.2	2.6	1.6 ± 0.9	
Koletzko (1991)	German infant formula (28)	0.2	4.6		
O'Keefe et al. (1994)[e]	U.S. infant formula (10)	0.2	1.3	0.8 ± 0.3	
Jorgensen et al. (1995)[f]	Danish infant formula				
	Standard formula (6)	1.4	4.2	2.7 ± 1.1	
	Special formula (7)	0.3	3.8	1.5 ± 1.3	
Chardigny et al. (1996b)[g]	French infant formula				
	Premature formula (2)	1.3	2.5	1.9 ± 0.8	
	"0–5 month" formula (9)	0.4	3.1		
	"6–10 month" formula (8)	0.4	5.0		
	"<10 month" formula (1)			3.0	
Fernandez San Juan (1996)[f]	Spanish infant formula (20)			2.3 ± 1.1	26.5 ± 3.2
Ali et al. (1996)	U.S. toddler formula (1)			1.9	16.5
Ratnayake et al. (1997)[g]	Canadian infant formula				
	Powdered infant formula (14)	0.6	2.5	1.4	
	Liquid infant formula (12)	0.9	3.1	1.9	
Slover and Lanza (1979)	U.S. baby food (3)	0.2	7.6	3.2	5.8
Hanson and Kinsella (1981)	U.S. dry infant cereal (8)	0.3	0.6	0.5	
USDA (1995)[f]	U.S. infant food				
	Vegetable and beef dinner, strained (2)	4.7	5.6	5.1 ± 0.6	2.9 ± 0.2
Holub (1999)	Canadian baby food				
	Baby biscuits		37		
	Infant cereals		23		

[a] Number of brands analyzed appears in parentheses following food item.
[b] Percentage of total fatty acids.
[c] 18:1t only.
[d] Includes *trans*-isomers of 16:1, 18:1, 18:2, and 18:3.
[e] No 18:1t reported; values include *trans*-isomers of 18:2 and 18:3.
[f] Includes *trans*-isomers of 16:1, 18:1, and 18:2.
[g] Includes *trans*-isomers of 18:1, 18:2, and 18:3; does not include conjugated 18:2.

Craig-Schmidt et al., 1984; van den Reek et al., 1986b; Enig et al., 1990a) have used this approach, and the results are summarized in Table 15.15. Relatively, "raw" data are presented to allow the reader to see the variation both between the diets of individuals and within the day-to-day diets of a given individual.

Aitchison et al. (1977) reported that 3-day self-chosen diet collections from 11 adult subjects in the United States contained an average of 5.0% of the fatty acids as *trans*-isomers, with a range of 1.3%–8.3%. The *trans*-fatty acid content of the 33 diet collections ranged from 0.0 to 9.9 g/day,

TABLE 15.15
Trans-Fatty Acids in Diets Determined by Laboratory Analysis of Composite Diets

Reference	No. of Daily Portions	Dietary *Trans*-Fatty Acids	
		Conc. (%)[a]	Intake (g/day)
Aitchison et al. (1977) (self-selected diets of lactating women)			
Subject			
1	3	6.98	0.9–4.2
2	3	6.13	1.8–3.5
3	3	8.27	4.4–5.2
4	3	5.23	2.2–4.8
5	3	1.30	0.0–3.1
6	3	3.54	1.0–5.5
7	3	8.15	0.0–9.9
8	3	5.07	2.7–6.2
9	3	4.40	1.1–4.5
10	3	2.60	1.4–8.7
11	3	3.69	1.4–2.3
Mean		**5.03**	
Åkesson et al. (1981) (Swedish diets)[b]			
1968, men (10)	70	5.0	4.9
1968, women (10)	70	5.0	2.8
1975, women (6)	36	5.1	3.0
1978, vegans (6)	24	1.8	0.9
1980, lactovegetarians (6)	24	3.9	3.0
Craig-Schmidt et al. (1984) (experimental diets)[c]			
Made with hydrogenated fat			
Day 1		12.31	11.04
Day 2		13.15	10.24
Day 3		7.65	6.68
Day 4		8.82	6.91
Day 5		17.11	13.72
5-Day mean		**11.81**	**9.72**
Made with nonhydrogenated fat			
Day 1		0.73	0.64
Day 2		0.87	0.59
Day 3		1.04	0.88
Day 4		1.30	1.17
Day 5		1.07	1.00
5-Day mean		**1.00**	**0.86**
Van den Reek et al. (1986b) (diets of U.S. adolescent girls)			
Subject			
1	7	2.33–7.58	1.13–4.48
2	7	5.01–9.15	1.28–3.93
3	6	1.85–9.05	1.53–7.04
4	7	4.06–17.16	1.06–6.95
5	7	1.98–12.08	0.45–5.72
6	7	2.43–7.48	1.03–6.21

TABLE 15.15
(Continued)

Reference	No. of Daily Portions	Dietary *Trans*-Fatty Acids	
		Conc. (%)[a]	Intake (g/day)
Van den Reek et al. (Continued)			
Subject			
7	7	3.07–7.77	1.20–4.20
8	7	4.04–10.67	2.69–7.97
Mean ± SEM		**6.53 ± 0.42**	**3.14 ± 0.26**
Enig et al. (1990a) (composites of U.S. diets)[d]		6–12	

[a]Expressed as percentage of total fatty acids.
[b]Number of subjects appears in parentheses.
[c]Designed for U.S. lactating women.
[d]Primary data were not given.

with both minimum and maximum values exhibited by the same subject (see Table 15.15). Similar values for Swedish diets were reported by Åkesson et al. (1981). *Trans*-octadecenoic (18:1t) acid was 5.0%, 3.9%, and 1.8% of dietary fatty acids in the normal, lactovegetarian, and vegan diets of Swedish adults. Daily consumption ranged from 0.9 g/day for the vegans to 4.9 g/day for the male subjects. These values are in agreement with those of van den Reek et al. (1986b), who found that the amount of *trans*-octadecenoic acid (18:1t) in the diets of eight adolescent girls collected for a 7-day period ranged from 3.5% to 8.2% of total fatty acids, with an average of 5.3%. The total *trans*-fatty acid content of the diets on 56 individual days in this study ranged from 1.9% to 17.2% of total fatty acids, with an average of 6.5% (see Table 15.15). The average daily consumption of 3.1 g/day (range 0.45–7.97 g/day) is also in agreement with the two earlier studies.

In comparing these studies, it is important to note the large variation both within the diets of the same subject and between subjects. Taken collectively, *trans*-fatty acid intake in these studies ranged from 0.45 g/day in an adolescent girl consuming 500 kcal, with 33% of these calories from fat (van den Reek et al., 1986b), to 9.9 g/day in a lactating woman consuming 2185 kcal, with 42% of calories from fat (Aitchison et al., 1977). Possible extremes in *trans*-fatty acid consumption have been further defined by the analysis of experimental diets in which diets made with hydrogenated sources of fat were compared to diets made with nonhydrogenated sources of fat (see Table 15.15). In the study by Craig-Schmidt et al. (1984), one day's diet made with nonhydrogenated sources of fat provided 0.59 g of *trans*-fatty acids compared to 13.72 g in a diet made with only hydrogenated fat. As percentage of total fatty acids, 18:1t ranged from 0.73% in a "nonhydrogenated" diet to 17.1% in a "hydrogenated" diet (Craig-Schmidt et al., 1984). Because all the diets were designed for lactating women, total calories and other nutrients were kept relatively constant. It is thus easy to see from these studies that extremes can exist in *trans*-fatty acid content of both self-selected diets and experimental diets.

Enig et al. (1990a) reported that recent analyses of total diet composites show a range of 6%–12% of the total fatty acids as *trans*-fatty acids. This range is well within the extremes defined by the experimental diets of Craig-Schmidt et al. (1984).

B. Analysis of Dietary Records Using Food Composition Data

Diet records and dietary recall methods can also be used to estimate *trans*-fatty acid consumption. Using data obtained by a 1-day dietary recall followed by a 2-day dietary record and assuming that the average *trans*-fatty acid content of fats consumed in 1977 was 5.6%, Senti (1985) calculated per capita daily *trans*-fatty acid consumption to be 4.5–6.5 g/day.

From 7-day diet records and published *trans*-fatty acid values in foods, van den Reek et al. (1986a) estimated the dietary levels of *trans*-octadecenoic acid (18:1t) in the diets of eight healthy white adolescent girls to be 5.3% of total fatty acids. This value was the same as that determined by laboratory analysis of diets collected by the duplicate portion technique (van den Reek et al., 1986b).

This value is lower than values reported by Enig et al. (1990a) in which diet history and analytically determined food composition data were used to estimate per capita *trans*-fatty acid intake. Calculations based on data from individual diets developed by the USDA to comply with the Dietary Goals and known *trans*-fatty acid values for individual foods gave a range of 7.2%–20% of the total fat as *trans*-fatty acids. Values for fat intake for participants of the Lipid Research Clinic screening study were used in combination with an estimated dietary *trans*-fatty acid intake (Enig et al., 1990a). For adult males with fat intakes of 40–258 g/person per day, estimates of 2.4–20.6 g/person per day for total *trans*-fatty acid intake were obtained; for adult females consuming 31–179 g fat per day, *trans*-fatty acid intake was estimated to be 1.9–14.3 g/person per day. Ranges of energy intakes for these subjects were not reported by Enig et al. (1990a). *Trans*-fatty acid intake reported per 1000 kcal would be useful comparing results from various studies and in accounting for the extreme variations seen in these *trans*-isomer intake estimations.

C. Estimates Based on *Trans*-Fatty Acid Content of Biological Tissues

Another approach to the estimation of the consumption of dietary isomeric fatty acids is to extrapolate from the concentration found in biological tissues. Craig-Schmidt et al. (1984) estimated that the usual diets of the lactating women in their study contained 7.8% of the fatty acids as the *trans*-isomers of octadecenoic acid (18:1t; Table 15.16). This estimate was based on extrapolation of the relationship between 18:1t in the maternal diet vs. 18:1t in human milk samples. The average content of 18:1t in the milk of the subjects on day 1 of the experiment was believed to reflect the content of the self-chosen diets from the previous day.

A similar approach was taken by Enig et al. (1990a), who used the concentration of *trans*-fatty acids in human adipose tissue to estimate dietary *trans*-fatty acids. Analysis of human adipose tissue provides an indirect method of estimating dietary fatty acid isomer intake, because these isomers are not synthesized by humans. Adipose tissue fatty acids have a long half-life, and therefore the concentrations of *trans*-fatty acids in adipose tissue are believed to reflect the dietary *trans*-fatty acid content. Studies by Heckers et al. (1979) in Germany, Ohlrogge et al. (1981) and Ohlrogge (1983) in the United States, and Thomas et al. (1981) in the Untied Kingdom showed a range of 1.0%–11.6% total *trans*-fatty acids in adipose tissue. However, as Enig et al. (1990a) point out, a 1:1 relationship between dietary and adipose tissue does not necessarily hold true, because some of the *trans*-fatty acids are metabolized, and therefore the concentration of *trans*-fatty acids in adipose tissue would be expected to be lower than the concentration of *trans*-fatty acids in the diet.

TABLE 15.16
Estimates of Dietary *Trans*-Fatty Acids Based on Concentration of *Trans*-Fatty Acids in Human Biological Tissues

References	Tissue	Estimated Dietary *Trans*-Fatty Acid Content (% of Total Fatty Acids)
Craig-Schmidt et al. (1984)	Human milk	7.8
Enig et al. (1990a)	Adipose tissue[a]	2.4–11.1

[a]Data of Ohlrogge, J.B., et al. (1981). *J. Lipid Res. 22*: 955–960. See text for explanation.

Enig et al. (1990a) estimated dietary *trans*-fatty acids using the data of Ohlrogge et al. (1981) on human adipose tissue *trans*-fatty acid levels in combination with an equation expressing the relationship between dietary 18:1t and adipose tissue levels in rats and mice. The animal data were taken from several sources (Alfin-Slater and Aftergood, 1979; Bonaga et al., 1980; Moore et al., 1980; Royce et al., 1984; Selenskas et al., 1984; Ostlund-Lindqvist et al., 1985; Petterson and Opstvedt, 1988). Using a range of 2.0%–5.8% of total fatty acids as 18:1t in adipose tissue (Ohlrogge et al., 1981), Enig et al. (1990a) estimated that U.S. diets contain 2.4%–11.1% of total fatty acids as *trans*-18:1 isomers (see Table 15.16).

D. Estimates Based on "Disappearance" Data

Until recently, the most commonly used method of estimating *trans*-fatty acid consumption has been based on fat disappearance or market size/share data in combination with product composition data. The estimate of Kummerow (1975) that the total *trans*-fatty acid intake from visible fat is approximately 8% was based on 1971 USDA household consumption data and typical *trans*-fatty acid compositional data. In 1971, margarine represented 7%, shortening 13.2% and cooking and salad oils 12.4% of the visible fat intake. These data were combined with typical values for sources of hydrogenated fat, that is, stick margarine containing 25%–35%, tub margarines 15%–25%, shortening 20%–30%, and salad oils 0%–15% *trans*-fatty acids. The small contribution of isomeric fatty acids from meat and dairy products was not included in this estimate.

Several other researchers using similar approaches calculated comparable values for percent of fat as *trans*-fatty acid; for example, 8% (Enig et al., 1978) and 6.8% (Emken, 1981). Elson et al. (1981) stated that data provided by the ISEOs indicate that the dietary fat supply available for human consumption consists of 5%–15% *trans*-fatty acids. Senti (1985) estimated that total per capita consumption of dietary *trans*-fatty acids in the United States is 10.2 g/day (Table 15.17). When Senti (1985) adjusted this value to account for wastage of frying oils and separable fat of retail beef cuts, a value of 8.3 g/day *trans*-fatty acid consumption was obtained. This value is only slightly greater than the value of 7.6 g/day estimated by Hunter and Applewhite (1986). On the other hand, Enig et al. (1990a) contend that Hunter and Applewhite (1986) overestimated wastage in their calculations and estimate that the average person in the United Sates consumes 12.8 g/day of *trans*-fatty acids. The major difference between the estimate of Enig et al. (1990a) and earlier estimates is in the category of shortening, for which both the fat intake as shortening (26.5 g/day) and average percentage of *trans*-isomer concentration are high in comparison to the other estimates in Table 15.17. Estimates for *trans*-fatty acid intake for Canada and the United States are higher than the 4.5–6.5 g/day reported by Heckers et al. (1979) for Germany and 6.5 g/day by Enig et al. (1984) for Israel but lower than the value of 17 g/day reported by Brussaard (1986) for The Netherlands.

In general, estimates of average *trans*-fatty acid consumption using disappearance data are higher as one might expect than estimates obtained by laboratory analysis of composite diets (see Table 15.15). However, extremes in individual intakes, as the data in Table 15.15 show, may extend well beyond these estimates of average intake.

Some investigators (Brisson, 1981; Craig-Schmidt et al., 1984; Senti, 1985; Hunter and Applewhite, 1986; Enig et al., 1990a) have tried to account for the contribution of animal fats as well as that hydrogenated vegetable oils to total *trans*-fatty acid intake (see Table 15.17). Brisson (1981) estimated that about 94% (or 8.5 g of a total of 9.1 g) of *trans*-fatty acids per day is due to hydrogenated vegetable oils, with the remainder being due to fats of ruminant origin. The estimate of Senti (1985) for total *trans*-fatty acid intake (10.2 g/day) is only slightly greater than that of Brisson (1981), but the relative contribution of animal and dairy fats to the total is greater.

The average per capita daily consumption of *trans*-fatty acids of 7.6 g determined by Hunter and Applewhite (1986) is less than the estimates of Brisson (1981) or Senti (1985), which were based on food disappearance data without a wastage factor for discarded fat. The estimate of Hunter and Applewhite (1986) was based primarily on product composition and market share data provided by

TABLE 15.17
Estimated Per Capita Daily Consumption of *Trans*-Fatty Acids from Animal and Dairy Fats and from Vegetable Fats

Reference and Food Category	Fat Intake (g/day)	*Trans*-Fatty Acids			
		Conc. (%)[a]		Intake (g/day)	
		Avg	Max	Avg	Max
Brisson (1981)[b]					
Butter	12.3	1.8	4.0	0.22	0.49
Milk	11.8	1.8	4.0	0.22	0.47
Meat (beef)	23.5	0.5	0.5	0.12	0.12
Total animal and dairy fats				**0.56**	**1.08**
Margarine	15.5	22.4	47.8	3.49	7.41
Shortening and oils	23.0	20.0	37.3	4.60	8.58
Salad oil	10.0	4.5	4.6	0.45	0.46
Total vegetable fats				**8.54**	**16.45**
Total dietary *trans*-fatty acids				**9.10**	**17.53**
Senti (1985)					
Butter	5.1	3.4		0.17	
Milk products	18.3	3.4		0.62	
Meat + edible fat (beef)	18.1	5.8		1.42	
Total animal and dairy fats				**2.21**	
Margarine, hard	6.37	23.9		1.52	
Soft	2.59	16.2		0.42	
Shortening and oils	18.3	16.3		2.98	
Salad oil	30.8	10.0		3.08	
Total vegetable fats				**8.00**	
Total dietary *trans*-fatty acids				**10.21 (8.3)[c]**	
Hunter and Applewhite (1986)					
Total animal and dairy fats				**1.33**	
Margarine				2.73	
Shortening and oils		10–17		0.60	
Salad oil		10		0.35	
Food service fats and oils				1.54	
Industrial fats and oils				1.00	
Total vegetable fats				**6.22**	
Total dietary *trans*-fatty acids				**7.55**	
Enig et al. (1990a)					
Butter	5.0	3.1		0.15	
Dairy	20.0	3.1		0.62	
Meat (beef)	32.0	3.0		0.26	
Edible tallow	2.2	3.0		0.07	
Lard	2.9	0.4		0.01	
Total animal and dairy fats				**1.11**	
Margarine	10.2	23.0		2.35	
Shortening	26.5	25.3		6.70	
Salad oil	26.2	10.2		2.67	
Total vegetable fats				**11.72**	
Total dietary *trans*-fatty acids				**12.83**	

TABLE 15.17
(Continued)

Reference and Food Category	Fat Intake (g/day)	*Trans*-Fatty Acids			
		Conc. (%)[a]		Intake (g/day)	
		Avg	Max	Avg	Max
Craig-Schmidt et al. (1984)[d]					
Total animal and dairy fats		1.00	1.30	**0.86**	1.17
Total vegetable fats		10.81	15.81	**8.86**	12.72
Total dietary *trans*-fatty acids		11.81	17.11	**9.72**	13.89

[a] Typical concentration (%) of *trans*-fatty acids in food category listed in first column.
[b] Calculated for Canada.
[c] Value that takes into account a wastage factor for frying oils and separable fat of beef retail cuts.
[d] Values of Craig-Schmidt et al. (1984) were based on laboratory analysis of experimental diets designed to be adequate for lactating women; all other estimates were based on disappearance data.

the ISEOs, the National Association of Margarine Manufacturers, and A. C. Nielsen Company, and included a 50% wastage factor for deep-frying fat. Thus, estimates of *trans*-fatty acid intake using disappearance data range from 7.6 g/day estimated by Hunter and Applewhite (1986) to 12.8 g/day estimated by Enig et al. (1990a).

The experimental diets used in the study by Craig-Schmidt et al. (1984) also define the relative contribution of biohydrogenation to possible total dietary *trans*-fatty acid consumption. The diets for one experimental period (nonhydrogenated; NH) were made with sources of fat such as butter, corn oil, and lard that had not been subjected to commercial hydrogenation. The diets for the other period were identical to the first but contained hydrogenated (H) products such as margarine, hydrogenated soybean oil, and shortening. The *trans*-fatty acid content of the diets in the NH period average 0.86 g/day (0.64–1.17 g/day) and 1.0% of total fatty acids (0.73%–1.3%). These values would be an estimate of the *trans*-fatty acids appearing in the diet as a result of biohydrogenation in ruminants; that is, the contribution that meat and dairy products would make. The difference between the H and NH diets would then be an estimate of the amount of *trans*-fatty acids in the diet due to commercial hydrogenation. The amount of *trans*-fatty acids from margarines, shortenings, and hydrogenated soybean oil ranged from 6.6% to 16% of total fatty acids (average 10.8%) and from 5.74 to 12.72 g/day (average 8.86 g/day). Using these values, the average contribution of total dietary *trans*-fatty acids due to commercial hydrogenation would be 91%, with only amounts contributed by meat and animal products. This value is in agreement with Emken (1984), who assumed that 90%–95% of the isomeric fat in the adipose tissue is contributed by hydrogenated soybean oil and the rest by butter fat.

Using the consumption data as summarized in Table 15.17, values for the contribution of meat and dairy products to total *trans*-fatty acid intake ranged from 6% (Brisson, 1981) to 22% (Senti, 1985). Heckers et al. (1979) estimated that in West Germany approximately 35%–45% of the 4.5–6.4 g per capita per day *trans*-fatty acid consumption was due to ruminant products. This estimate is high, however, owing to the relatively low consumption of hydrogenated vegetable fat and not to an abnormally high consumption of dairy products.

E. Consumption of Positional Isomers

Almost all the estimates of isomeric fatty acid consumption are for the *trans*-isomers only. Emken (1984), however, used analyses of hydrogenated oil samples and figures for total fat consumption to

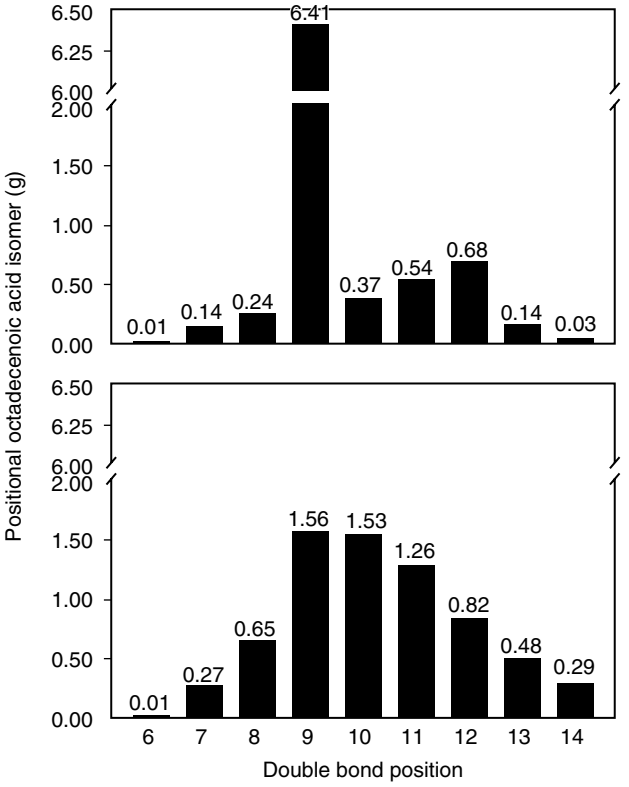

FIGURE 15.4 Daily intake of specific positional 18:1 isomers (top, 18:1c; bottom, 18:1t) as estimated by Emken, E.A. (1984). *Ann. Rev. Nutr. 4*: 339–376.

calculate a daily average intake of individual *cis*- and *trans*-positional monounsaturated isomers. The value for total isomer daily consumption minus oleic acid (18:1Δ9c) is estimated to be 9.0 g, with 2.2 g of this consisting of positional *cis*-isomers. Estimated values for the consumption of *cis*/*trans*-isomers containing double bonds ranging from position 6 to position 14 are shown in Figure 15.4.

VI. CONCLUDING PERSPECTIVE

This chapter has summarized available information on the isomeric fatty acid content (primarily 18:1t) of various food items and has reviewed estimates of per capita daily intake of these fatty acids from a methodological standpoint. To be useful, these data must be put into context.

First, the food composition data must be put into the context of the fat content of the food and overall fatty acid composition. Data on isomeric fatty acid composition should be reported along with the fat content of the food and expressed in grams per 100 g of food. It is crucial to consider the other fatty acids in the food as well. Adequate essential fatty acids (e.g., linoleic, 18:2Δ9c, 12c) must be provided in the diet to minimize possible deleterious effects of isomeric fatty acids. Ackman and Mag (1998) have reviewed the potential for having less *trans*-fat in commercial products.

Second, the desire to increase linoleic acid in the diet must also be balanced against the tendency of polyunsaturated fatty acids to become rancid. When this occurs, the consumer gets a product that is lower not only in organoleptic properties but also in "health-promoting" properties.

Third, the consumption data must be put into the context of the total diet of the consumer. The number of calories consumed, the total fat in the diet, and the percentage of calories provided by fat,

as well as the relative proportions of saturated, monounsaturated, and polyunsaturated fat, must be taken into consideration.

Fourth, the consumption data must be put into the context of studies on the physiological effects of isomeric fatty acids. The amount of dietary isomeric fat used in many human and animal experiments is greater than that normally consumed by the average person. As the possible deleterious effects of isomeric fatty acids are assessed, it is imperative to compare the amounts of these fatty acids in experimental diets with current consumption estimates. Only then can conclusions be drawn about the safety of hydrogenated fat in the diet.

Finally, realization of the detrimental health effects of *trans*-fatty acids has prompted a movement worldwide to eliminate industrially produced *trans*-fatty acids from the food supply. Denmark has been successful in doing this by regulating the amount of industrially produced *trans*-fat allowed in a food item (Astrup, 2006). In an alternate approach, enactment of legislation requiring *trans*-fat values on food labels has brought about rapid changes in the food supply of the United States as industry has responded to consumer pressure. Thus, the *trans*-fatty acid content of the diet may soon be dependent only on the amount of ruminant items it contains.

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16 Genetic Alteration of Food Fats and Oils

Earl G. Hammond

CONTENTS

I. REASONS FOR ALTERATION OF FAT AND OIL COMPOSITION

Interest in changing the composition of food fats and oils is driven by concerns about physical properties, oxidative stability, nutrition, government regulations, and economic advantage (Hammond and Glatz, 1989; Greiner, 1990; Gunstone, 2001). Unfortunately, some of these drivers are not compatible with each other, especially the optimization of nutrition with physical properties or stability. Often changes in fat and oil compositions seek to expand the market for a particular fat or oil commodity by making its properties more desirable than rivals' fats and oils, by reducing processing costs or increasing consumer appeal.

Long-chain saturated fatty acids are needed to produce plastic fats and oils for margarines and shortenings. For nearly a century, hydrogenation has been used to decrease unsaturation in oils and to achieve plasticity. But there has also been interest in producing vegetable oils that naturally contain more saturated stearic and palmitic acid groups to give plasticity while avoiding the cost of hydrogenation (Kok et al., 1999).

There has also been particular interest in increasing the palmitic acid content of vegetable oils that are destined for hydrogenation to plastic fats. Most of the fatty acids in vegetable oils contain 18 carbons (stearic, oleic, linoleic, and linolenic acids), and for plastic fats to have the optimum textural properties, it is desirable that the solid fat crystallizes in the β′-form rather than in the higher melting β-form. Oils with about 15% or more of palmitic acid groups favor crystallization in the β′-form (Erickson and Erickson, 1995; O'Brien, 1995). The unique triglyceride structure of cocoa

butter, in which the outer *sn*-1,3 positions of the glycerol contain saturated palmitic or stearic acid groups and the inner *sn*-2 position contains mostly oleic acid, makes cocoa butter melt sharply at human body temperature and also makes it very appealing for confectionery fats. Cocoa butter's high price has resulted in numerous attempts to find substitutes (Hammond, 2000).

Oils such as coconut and palm kernel oils that are rich in medium-chain length fatty acids have unique stability and melting properties and are sometimes in short supply and expensive. This has led to attempts to introduce saturated fatty acids of chain lengths 8–12 into other vegetable oils (Friedt and Lühs, 1998; Schultz and Ohlrogge, 2002; Scarth and Tang, 2006).

To obtain greater stability to oxidation while still retaining low melting points, there is interest in producing an oil that contains less polyunsaturated linoleic and linolenic acids and more oleic acid, which has more stability to oxidation (Fatemi and Hammond, 1980; Kinney, 1996; Schultz and Ohlrogge, 2002). Linolenic acid groups are particularly prone to producing undesirable flavors at low levels of oxidation, which has generated extensive efforts to reduce the amounts of linolenic acid in soybean and other oils (Hammond and Fehr, 1984; Hammond, 1985). High-oleic oils are particularly desirable for frying operations (Wilson et al., 2002), although if the content of linoleic acid is too low, the fried products may lack typical fried flavor (Warner and Knowlton, 1997).

Nutritional recommendations about the best oil composition have varied with time in many respects but have consistently encouraged the reduction of saturated fatty acids for preventing atherosclerosis. There is a growing consensus that not all saturated acyl groups are equally bad nutritionally (Ulbricht and Southgate, 1991). It may be that only the saturated fatty acids—lauric, myristic, and palmitic are atherogenic, but this is not yet recognized in food labels. Concern about these three atherogenic fatty acids has led to advertising about small differences in the amounts of palmitic acid in various vegetable oils and to attempts to reduce the amount of palmitic acid in soy and canola oils by breeding.

There is also increased appreciation of the nutritional value of linolenic and other fatty acids with their first bond on the third carbon counting from the methyl end of their chain, the so-called n-3 fatty acids (Simopoulos, 2000). These fatty acids have been reported to have various physiological effects, many of which arise from their being precursors of prostaglandin hormones. There is an increasing advocacy for increasing various n-3 fatty acids in food fats and oils. It is also recommended that a certain ratio of n-3 to n-6 fatty acids (such as linoleic acid) be consumed (Hibbeln et al., 2006). There are a limited number of plants that are rich in linolenic acid, and long-chain (20–22 carbon) n-3 fatty acids are mostly found in aquatic animals and fungi. There is also a growing interest in transferring the ability to produce long-chain n-3 fatty acids to oilseeds by genetic engineering. Because of the instability of polyunsaturated n-3 acyl groups to oxidation and the intensity of flavor that their oxidation entails, the incorporation of these materials into food can involve major problems with shelf life.

Recently, the U.S. Government has required food labels to state the amounts of *trans* fatty acids present in a food serving, and consumers have been warned about the importance of restricting intake of *trans* fatty acids (Food and Drug Administration, 2003). This requirement is causing a major change in the fats and oils industry because the food industry has relied on hydrogenation to convert liquid vegetable oils to plastic fats such as spreads and shortenings and to improve the oxidative stability of frying fats. But hydrogenation has also been the chief route by which *trans* fatty acids have found their way into consumers' diets (Craig-Schmidt and Holzer, 2000). A small but significant amount of *trans* fatty acids are also generated by rumen fermentation, and *trans* fatty acids generated in this way are present in ruminant meat and milk products.

The discovery that various conjugated diene and triene isomers of fatty acids have significant physiological effects in experimental animals has generated interest in introducing or increasing the concentration of conjugated fatty acids into various food products (Hunter, 2000). Conjugated dienes are not known in natural fats and oils but can be generated by alkali isomerization of linoleic acid and by hydrogenation. There are a number of sources of conjugated trienes in nature that could be transferred into oilseed by genetic engineering (Liu et al., 1997). Some of these trienes have been shown to have physiological effects similar to conjugated dienes.

II. THE MEANS OF CHANGING PLANT OIL COMPOSITION

Some changes in composition can be achieved rather simply by mixing fats and oils from different sources. This simple solution is sometimes limited by concern about whether a fat is of animal origin, whether an animal has been slaughtered in an acceptable manner or whether the fat or oil is from a species that is considered acceptable for food.

If one wishes to alter the composition of an oilseed crop for such things as oil content or fatty acid composition, traditional plant breeding technology has offered several approaches (Fehr, 1987; Orf, 1988). The technique selected will depend on the characteristics of the particular plant species and the availability of germplasm collections. Generally, the most efficient approach is to screen the germplasm collections of the species for strains that have the desired composition. Often one can find a range of two- or threefold in fatty acid compositions by this approach. Collections of various sizes are available for most commercially important species. In many instances, wild relatives of commercially important species are available that can be crossed with the domesticated species. It is worthwhile to make collections of such wild species and sample their variability as well.

If the desired composition cannot be recovered from germplasm collections or from wild relatives of the domesticated species, generally crossing and selection techniques are attempted. These techniques are good for extending the range of lipid components present in the oilseed, but it would be ineffective in introducing a component not normally present. If, for example, one wishes to decrease the oleic acid content of the seed oil, populations of plants that have the lowest available concentrations of oleic acid in their seed oil are selected and crosses are made. Then the progeny from such crosses are tested for the desired trait. Most of the progeny will have a composition that will fall between those of the two parents, but usually a few of the progeny will have compositions that fall outside the range of parental values. Plants having compositions that exceed the values of the parents in the desired direction are selected, and the cycle is repeated. Often a population can be moved in the desired direction in increments at each cycle of recurrent selection.

Mutation breeding can also be tried as a means of extending the range of compositions beyond that available in germplasm collections. For this approach, usually seeds are treated with mutagenic agents such as ionizing radiation, or soaked in solutions of mutagenic chemicals such as ethyl methanesulfonate, azide, *N*-nitroso-*N*-methylurea, or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Frequently, a mutagen exposure that will reduce the germination of the seed by 50% is used. The resulting viable seeds are germinated and grown to maturity. It is desirable to advance the mutagen-treated population for a generation or two to allow for segregation of the mutated traits before the progeny are tested for the desired trait. This approach has been successful in producing large changes in the fatty acid composition of a number of oilseeds and in discovering the genes that are involved.

Molecular genetic techniques can be used to alter the amounts of the acyl groups normally present in a species or to introduce exotic acyl groups. There has been considerable research activity in these applications in recent years, and there are a number of good reviews of these techniques and their reach, both from the view of molecular geneticists (Liu, 2001; Schultz and Ohlrogge, 2002) and plant breeders (Friedt and Lühs, 1998; Wilson et al., 2002; Scarth and Tang, 2006). Studies of oilseed plant genomes have made it possible to relate certain DNA fragments or genes to various traits (Pantalone et al., 2004). These tests identify particular gene modifications present in a variety and can accelerate breeding experiments. In general, it is possible to use these techniques to extend or contract the amounts of the acyl groups typically present in fats and oils. It has also been possible to introduce significant amounts of shorter-chain acyl groups not normally present in an oilseed.

The introduction of exotic fatty acids into oilseed by genetic engineering is also possible (Hildebrand et al., 2002; Kinney, 2002). There is interest in introducing fatty acids that contain a branch, hydroxy or epoxy group or a positional double-bond isomer into edible oilseeds such as soybean or canola. In instances where the exotic fatty acid is found in a wild plant, it is generally believed that it will be easy to introduce the trait into a domestic oilseed than to domesticate wild

plants that contain the exotic fatty acid (Schultz and Ohlrogge, 2002). Oils with such fatty acids have potential or current industrial uses and may be sold at prices considerably above those of edible vegetable oils because their production entails greater costs. The successful introduction of such exotic fatty acids into soybeans or canola could reduce the cost of such industrial oils and increase their utilization. Some of these altered fats and oils could achieve acceptance as foods, and some could pose a danger if mixed in the food supply. So far it has been difficult to get extensive expression of many of these exotic fatty acids in oilseeds; for example, genes for unusual double-bond isomers can be expressed in a plant but only at the level of a few percent. It may be necessary to incorporate changes in the genes that transfer the exotic fatty acyl groups into triacylglycerols to overcome this problem (Hildebrand et al., 2002; Schultz and Ohlrogge, 2002; Weselake, 2002).

III. BARRIERS TO CHANGING PLANT OIL COMPOSITION

Once the desired seed composition is attained, it is frequently present in a strain that does not have desirable agronomic properties such as high yield, disease resistance, or suitable maturity date. Usually the desired composition can be transferred to material with good agronomic properties by a process known as backcrossing. To do this, the line with the desired composition is crossed with a line with good agronomic properties, and the progeny are tested. The process can be repeated to generate lines with the desired composition and agronomic properties.

The effectiveness of these techniques for change can vary with the species of oilseed and its genetics. Obviously, plants such as oil palm or olive that require several years to mature and come into full oil production are more difficult to work with than annual oilseed crops such as soybean or canola.

Breeding for changes in seed composition often entails some loss in yield. Certain traits have also shown a tendency to reduce oil percentages or germination. These can often be overcome, but the more traits the breeder wishes to optimize in a particular line, the more difficult it becomes to keep them all at the optimum.

A significant number of genetic specialty crops could be produced, and the number that is possible is increasing, but until recently this has not resulted in many oils with genetically modified compositions being on the market. The reasons have been economic. Oilseeds have been handled very efficiently as a single commodity, and there has been resistance to the introduction of a number of kinds of an oilseed. The advantages of new traits introduced into an oil crop must at least pay for the cost involved. If a special variety has a lower yield than is typical for the crop and growing area, farmers will not grow it without a premium to make up for this loss. Often a special variety must be grown on contract, and this often entails a small premium to the grower. Crop handlers will lose flexibility in keeping a special variety segregated and will encounter additional costs. The incoming seed must be tested to make sure it meets specification when it is received by the crop handlers. The oil extractors will often need to modify their processing plants to handle multiple varieties that must be kept segregated. If the oil content is lower than that typical for the crop, oil extraction will run less efficiently.

Often the value of modified crop is uncertain. Its ability to extend shelf life or reduce processing costs is ambiguous, and the impact of flavor changes in a product line is nebulous. Will the improved product increase the number of customers and only leave current customers better pleased? New traits are embraced more readily if they promise an expanded market. Many people fear or are prejudiced against crops modified by direct genetic manipulation or interspecies gene transfer and these attitudes vary geographically.

Oilseed crops with the desired composition may already exist. For example, low-linolenic soybean oil is very much like traditional corn oil, and low-saturate soybean oil is very much like traditional canola. The price difference between oil from the potentially modified crop and the existing oils usually sets the price that a modified oilseed crop can bring. Often this difference is not great

enough to pay the costs of growing a special variety, and this appears to be the primary reason why oilseed crops with altered fatty acids have not been produced on very large scales so far.

As these words are being written we seem to be standing on the threshold of a new era that will see the rapid expansion of oilseed crops with altered fatty acid composition. The new rules about *trans* fatty acids promise to strongly affect what is acceptable to consumers and food manufacturers. It will be difficult to meet all the demands for low-*trans* fats and other traits that are important to consumers with the current technology, especially for frying fats and oils. Seed suppliers are busy trying to furnish seeds with compositions that will meet these needs and find farmers to grow these crops. Contracts with oilseed processors have been made to process the harvest. Some food companies have pledged to use only *trans*-free fats and oils in their products. The next few years should witness interesting changes.

A. Animal Fats

In animals there has been considerable reduction in muscular fat by genetic selection as well as feeding practices in recent years in response to consumer demand (Rhee, 2000). These trends are continuing and being supplemented by molecular genetic tools.

Genetic selection of cows yielding a heart-healthy milk fat composition appears to be feasible and could result in an important decrease in saturated fatty acids such as lauric, myristic, and palmitic that are considered atherogenic (Karijord et al., 1982; Bobe et al., 2003; Chen et al., 2004). Such milk could be segregated and collected from about 5% of the cows in typical herds. Dairy products made from such milk have normal properties and, in the case of butter, improved spreadability. Such products could sell at a premium that will pay for the cost of segregation and collection. The chief technical barrier to such marketing is the availability of an inexpensive, rapid method of testing for cows producing such milk.

IV. CONSTRAINTS ON THE COMPOSITION OF PLANT FATS AND OILS

Fats and oils that are obtained in commercial quantities from plants are generally obtained from the seed or fruit coat. In seeds, the fats and oils usually are present as triacylglycerols and serve as an energy source to fuel the metabolism of the seed during germination until the new plant establishes its photosynthetic ability. In many species, starch is stored as an energy source in the seed as an alternative or in addition to storing fat. The proportion of this oil reserve in seeds varies considerably, from about 5% in cereals to as high as 68% in coconut (Sonntag, 1979). The lipid in fruit coats presumably serves to interest animals in helping with seed dispersal. In animals subcutaneous fat is believed to serve as insulation as well as an energy reserve.

The fatty acid composition of seed oils varies greatly. Generalizing on the analytical data from numerous seed oils, Hilditch (1954) concluded that oleic and linoleic acids together generally accounted for over 80% of the total fatty acids and that most species contained about 7%–8% palmitic acid. In some species, especially those from the tropics, palmitic and stearic acids were more prominent. Other variations on this basic pattern occurred primarily in two ways: (1) A whole family of plants would produce seed oils that contained another fatty acid in addition to the basic oleic–linoleic–palmitic acid mixture. (2) An unusual fatty acid would occur in a single species of seed oil and almost replace the basic mixture usually found.

A possible reason for conservatism in the fatty acid pattern of seed oils may be the necessity to maintain the triglycerides in a liquid state under the conditions in which the plants and animals normally flourish. The importance of liquidity was emphasized by Kartha (1954), but his hypothesis was based on very limited evidence. Many seed oils have low melting points when isolated and obviously are liquid at the temperature at which the plant flourishes. In other instances, isolated seed oils melt in the general vicinity of the growth temperatures, and it is not certain whether the oil is liquid

at all temperatures at which the seed might be growing. It is not easy to observe directly the physical state of the minute droplets of oil dispersed in the seed. The picture is also clouded by crystalline fats that are able to take several different polymorphic forms with different melting points and stabilities (Bailey, 1950). The polymorph with the highest melting point may melt 10°C–20°C above the lower melting polymorphs. The polymorph that separates on cooling depends on the rate of cooling, the presence of seed crystals, and the fatty acid composition. In the microscopic oil droplets of seed, the crystal nuclei that do form cannot grow very far; so much of the oil may not be in equilibrium with its crystalline phases. In addition, phospholipids that may be present in seed oil droplets can act as crystal inhibitors and limit the growth of crystals that do form by adsorbing to their surfaces (O'Brien, 1995). Thus, the oil in a seed might crystallize considerably below the highest melting point observed in isolated bulk oil.

The hypothetical need for liquidity in seed oils is attributed to the inability of various enzymes to exercise their catalytic role on solid fats (Kartha, 1954). The evidence for this was based on observations of the action of lipase on various saturated triglycerides. The work of Willstätter and Waldschmidt-Leitz (1923), who used adsorption on tristearin to purify pancreatic lipase, was cited. Other observers (Collins, 1933; Tofte, 1934; Balls et al., 1937; Schonheyder and Volqvartz, 1944; Wills, 1965) showed that as the chain length of saturated triglycerides increased, the rate of hydrolysis by lipases decreased. For solid triglycerides the rate drops precipitously, but the hypothesis that the rate is zero was not clearly demonstrated by the data.

It is questionable whether the action of enzymes on substrates such as crystalline tristearin is a good model for the fat that might form in a cooled seed droplet. Natural fats and oils usually do not have sharp melting points. As the isolated oil is cooled, the solubility of one of its components is exceeded, and this component crystallizes into a network that holds the remaining liquid oil in its interstices, producing a semisolid, plastic fat. The solid fat in such a mixture has much more surface than chunks of crystalline tristearin, and this may be important in the rate of lipase reactions (Sarda and Desnuelle, 1958).

Table 16.1 shows the melting points of some fatty acids and their simple triglycerides. Triglycerides containing only the long-chain saturated fatty acids obviously have too high a melting point to be liquid at temperatures at which plants normally flourish. Even a small amount of such saturated triglycerides cannot be tolerated if it is necessary to maintain complete liquidity.

TABLE 16.1
Melting Points (°C) of the Highest Melting Polymorph of Some Fatty Acids and the Three Polymorphic Forms of Their Monoacid Triglycerides

Fatty Acid	Fatty Acid Compositions	Fatty Acid m.p.	Triglyceride m.p.		
			β	β′	α
Palmitic	16:0	62.9	65.5	56.0	45
Stearic	18:0	69.6	73.1	65.0	54.5
Oleic	18:1c-ω9	16.25	4.9	–12	–32
Elaidic	18:1t-ω9	43.68	42	37	15.5
Linoleic	18:2c-ω6	–5.0	–13.1	—	–43
Linolenic	18:3c-ω3	–11.0	–24.2	—	–44.6
Arachidic	20:0	75.35	—	—	—
Behenic	22:0	79.95	—	—	—
Erucic	22:1c-ω9	33.4	30	17	6

Source: Bailey, A. E. (195). *Melting and Solidification of Fats*, Interscience, New York.

The solubility of triglycerides in each other is approximated by ideal solution theory (Bailey, 1950; Boatman et al., 1961) so that

$$\ln N = -H(T_m - T)/RTT_m,$$

where N is the mole fraction of high-melting triglycerides in a mixture, T_m is its melting point, T is the temperature of observation in degrees Kelvin, H is the molar heat of fusion of the high-melting triglyceride, and R is the gas constant. From this equation one can determine the amount of high-melting triglyceride soluble in a typical liquid oil at various temperatures as shown in Table 16.2.

Plants do seem to maintain very low levels of long-chain trisaturated triglycerides in their seed oil, and they accomplish this limitation by two strategies: (1) they produce relatively small amounts of long-chain saturated fatty acids or (2) they rigorously exclude long-chain saturated acyl groups from the *sn*-2 position of triglyceride (Mattson and Volpenhein, 1961; Litchfield, 1972). Table 16.2 shows the gains in solubility achieved by the introduction of an oleoyl group into saturated triglycerides. Tropical butters, such as cocoa butter, rely almost entirely on the second strategy, because they consist almost entirely of *sn*-1,3-disaturated 2-oleoyl triglycerides. Isolated cocoa butter melts rather sharply at about 38°C (Bailey, 1950). Seed oils from the temperate zones generally contain saturated acyl groups much below the 63+ mol.% levels of cocoa butter.

Duncan (1984) has summarized the evidence showing that for maximum lowering of the melting point by an unsaturated acyl group, it is important for the double bond to be in the *cis* configuration and near the middle of the chain. Oleic acid meets these criteria. Since most plants flourish at temperatures greater than 5°C, presumably a plant could achieve liquidity of its oil by producing an oil that contained mostly oleic acid (Table 16.1). Additional double bonds in an 18-carbon chain reduce the melting point further, but the effect of additional double bonds is much less than that of the first. Possibly, the presence of linoleic and linolenic groups in plant triglycerides results from the role these fatty acids play in biological membranes more than from their contribution to the liquidity of the storage triglycerides. It is important that the membranes have suitable flexibility (Duncan, 1984; Thompson and Martin, 1984), and unsaturation is important in the attainment of this flexibility. Polyunsaturated acyl groups are often concentrated in biological membranes. Galactosyl diglycerides are often rich in linolenic acid and are important constituents of chloroplasts

TABLE 16.2
Temperatures (°C) Necessary to Dissolve Various Mole Fractions of the β-Form of Various Triglycerides in Liquid Triglycerides according to Ideal Solution Theory

Mole Fraction	Tristearin	Tripalmitin	2-Oleoyldistearin[a]	2-Oleoyldipalmitin[a]
1.00	72.4	65.4	41.6	35.2
0.50	69.0	61.8	38.5	32.0
0.25	65.8	58.2	35.4	28.8
0.12	62.3	54.5	32.3	25.5
0.06	59.2	51.1	29.4	22.4
0.03	56.1	47.7	26.6	19.4
0.01	51.3	42.6	22.1	14.8

[a]The oleoyl group was estimated to reduce the heat of fusion by 4.84 kcal/mol for 2-oleoyldistearin and 2.94 kcal/mol for 2-oleoyldipalmitin (Duncan, 1984).

Source: Based on the data of Bailey, A. E. (1950). *Melting and Solidification of Fats*, Interscience, New York.

(Mudd and Garcia, 1975). Wang et al. (1997) have shown that genetic alteration of the fatty acid composition of soybean oil has a great effect on the fatty acid composition and class proportions of its phospholipid. It is possible that structural features of triglycerides that seem to contribute to their liquidity are simply a consequence of the triglycerides arising from the phospholipids by the Kennedy pathway (Appleqvist, 1975; Slack and Browse, 1984) and have no great significance for the melting characteristics of seed triglycerides. The limitation on total saturated fatty acids and the exclusion of saturated fatty acids from the *sn*-2 position may be strategies only to ensure the flexibility of biological membranes. Even so, the result is that storage triglycerides have similar restraints on their structure.

As Hilditch (1954) noted, individual species or families of plants sometimes depart from the basic pattern present in most species by including additional acyl groups in their seed triglycerides. It is usually not clear what advantage the species or family gains by the unusual pattern, but often the occurrence of the unusual acyl group is limited to the oilseed depots (Appleqvist, 1975; Cahoon and Ohlrogge, 1994; Liu et al., 1997). The seeds of the palm family are rich in short- and medium-chain saturated fatty acids (hexanoic to myristic) and have limited amounts of unsaturation (Rossell et al., 1985). The short-chain fatty acids greatly depress the melting point of the palm kernel oils. This results in oils much more stable to oxidation than the vegetable oils that follow Hilditch's basic pattern, but it is not clear why oxidative stability should be of greater importance in palms than in other plant families. Indeed, some plant families produce oil with considerable amounts of linolenic acid, and many plant oils seem to contain more linolenic acid than is necessary to maintain liquidity (Hammond, 2000). The presence of these methylene-interrupted polyenes greatly decreases the stability of the oil to oxidation (Fatemi and Hammond, 1980).

Defense against attack by predators has often been cited as a reason for the accumulation of various materials in oilseeds. There is some evidence that the short-chain fatty acids found in palm kernels have maximum bactericidal activity (Karabinos and Ferlin, 1954). It may be that the instantaneous production of peroxides and aldehydes by the action of lipoxygenases on polyunsaturated fatty acids when seeds are damaged is a defense mechanism (Galliard, 1975; Shibata and Ohta, 2002). Unusual fatty acids such as ricinoleic in castor beans may serve to make the fat reserves of the plant seed unavailable to many predators because they lack the means of metabolizing the unusual structure. Röbbelen (1975) suggested that such unusual acyl groups are relics of plant evolution.

Auld et al. (1989) examined a number of *Brassica* species and collections and found erucic acid nearly always present. They speculated that the conservation of erucic acid in this family occurred because the longer chain erucic acid allowed plants to generate more energy per triglyceride molecule by β-oxidation. This was thought to be significant in a small-seeded family such as *Brassica*. Erucic acid was limited to 66% by its exclusion from the *sn*-2 position of the triglycerides. Auld et al. (1989) suggested that this was because glyceryl trierucate would have an unacceptably high melting point (Table 16.1).

Although these are reasonable hypotheses, there is little evidence that the removal of lipoxygenases or most large changes in fatty acid composition have exposed plants to noticeable increases in predator attack or diminished vigor or germination under modern field conditions.

One reason why plants are rather conservative in varying their triglyceride composition may be that the enzymes involved in fat metabolism are limited by their specificity. For example, the enzymes involved in producing triacylglycerols from fatty acids may have specificities for certain chain lengths and double-bond position and configuration (Slack and Browse, 1984). Thus, for a new acyl group that does not have the correct characteristics to be incorporated into the seed triglycerides of a plant, not only must there be a mutation for the production of the new acyl group, but there also must be coordinated changes in various enzymes involved in its transfer and metabolism. The probability of such multiple coordinated mutations is very low, and the prevalence of radical changes from the basic pattern of seed triglycerides is relatively rare. This hypothesis has been cited to explain why the introduction of exotic fatty acids into domesticated oilseed plants usually results in their express at very low levels (Hildebrand et al., 2002; Schultz and Ohlrogge, 2002; Weselake, 2002).

V. OIL CONTENT AND FATTY ACID COMPOSITION IN VARIOUS PLANT SPECIES

Table 16.3 gives the range of oil content reported for a number of oil-bearing tissues. The percentage of oil is under genetic control, and there has been success in recent years in increasing the oil content of corn (Weber, 1983) and oats (Frey et al., 1975; Branson and Frey, 1989a,b; Holland et al., 2001). In both these cereals, oil contents as high as about 15% are now possible. In corn, the oil is concentrated in the germ, which may be as high as 45% oil. After dry or wet milling the germ is separated and extracted. In oats the oil is dispersed throughout the endosperm.

In many oilseeds, the meal remaining after extraction of the oil is a rich source of protein that can be used for animal feed. This is particularly true of soybeans, the protein of which is as valuable as the oil, and it has been suggested that breeding for soybean protein at the expense of oil may now be profitable (Greiner, 1990). In soybean, the oil and protein contents tend to be inversely correlated, but some progress has been made in increasing both (Wilcox, 2001). Factors partitioning the products of photosynthesis into carbohydrate, oil, and protein are poorly understood (Hildebrand et al., 2002; Weselake, 2002), although genomic approaches are beginning to identify many gene loci that affect oil concentration in soybean (Pantalone et al., 2004).

Table 16.4 shows the range of fatty acid compositions reported for natural populations of several commercially important oilseeds. Obviously, considerable variation occurs naturally for all

TABLE 16.3
Range of Oil Content for Various Oil-Bearing Plant Tissues

Oil-Bearing Tissue	Tissue Type	Oil Content
Cocoa bean	Oilseed	50–55
Coconut	Oilseed	65–74
Corn	Starchy seed	3–16
Cottonseed	Oilseed	15–24
Hempseed	Oilseed	30–35
Linseed	Oilseed	35–45
Oat	Starchy seed	2–16
Olive	Fruit coat	35–75
Palm	Fruit coat	45–50
Palm kernel	Oilseed	44–58
Peanut	Oilseed	45–55
Poppy seed	Oilseed	36–50
Rapeseed	Oilseed	22–49
Rice bran	Cereal coat	9–22
Safflower	Oilseed	25–37
Sesame	Oilseed	44–54
Soybean	Oilseed	12–30
Sunflower	Oilseed	22–36
Tobacco seed	Oilseed	30–43

Source: Frey, K. J., et al. (1975). *Crop Sci.* 15: 54–59; Schipper, H., and Frey, K. J. (1991). *Crop Sci.* 31: 1505–1510; Sonntag, N. O. V. (1979). Inheritance of oil percentage in interspecific crosses of hexaploid oats. In *Bailey's Industrial Oil and Fat Products*, 4th ed. (D. Swern, ed.), Wiley, New York, pp. 289–477; Weber, E. J. (1983). Lipids in maize technology. In *Lipids in Cereal Technology* (P. J. Barnes, ed.), Academic Press, New York, pp. 353–372.

TABLE 16.4
Range of Fatty Acid Compositions Reported for Natural Populations of Various Kinds of Oilseeds

Oil	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0	16:1	18:1	20:1	22:1	18:2	18:3
Coconut	<1.2	3.4–15	3.2–15	41–56	13–23	4.2–12	1–4.7	<0.2	—	—	—	3.4–12	<0.2	—	0.9–3.7	—
Cottonseed	—	—	—	—	0.5–2.0	17–29	1–4	<0.5	—	—	<1.5	13–44	<0.5	—	33–58	<0.5
Corn	—	—	—	—	<1.0	6–28	0.5–15	<1	—	—	<1	14–64	<0.5	—	10–71	<2
Oats	—	—	—	—	0–5	13–28	0.5–4.0	—	—	—	—	18.7–48	—	—	26–47	0.5–5
Palm	—	—	—	<1.2	0.3–5.9	27–59	1.5–14.7	<1	—	—	<0.6	27–52	—	—	5–16	<1.5
Palm kernel	<0.5	2.4–6.2	2.6–7.0	41–55	14–20	6.5–11	1.3–3.5	<0.3	—	—	—	10–23	<0.5	—	0.7–5.4	<0.7
Peanut	—	—	—	—	—	6–14	2–6.5	1–2	2–4	1–2	<1	40–72	0.5–1.5	—	13–38	<0.5
Rapeseed	—	—	—	—	—	0.5–10	0.5–4	<1.5	<1.5	<2	<1	9–58	5–18	30–60	8–27	3–21
Safflower	—	—	—	—	—	2–10	1–6	<1	<1	—	<0.5	7–40	<0.5	—	55–80	<1
Sesame	—	—	—	—	—	7–12	3.5–6	<1	<1	—	<0.5	35–50	<0.5	—	35–50	<1
Soy	—	—	—	—	—	7–12	2–5.5	<1	<0.5	—	<0.5	20–50	<1	—	35–60	2–13
Sunflower	—	—	—	—	—	4–9	1–6.5	<1	<1	<0.2	<0.5	14–70	<0.5	—	20–75	<0.5

Source: Earle, F. R., et al. (1968). *J. Am. Oil Chem. Soc.* 45: 876–879; O'Connor, R. T., and Herb, S. F. (1970). *J. Am. Oil Chem. Soc.* 47: 186A, 195A, 197A; Hammond, E. G. (1983). Oat lipids. In *Lipids in Cereal Technology* (P. J. Barnes, ed.), Academic Press, New York, pp. 331–352; Weber, E. J. (1983). Biochemistry and biotechnology of triacylglycerol accumulation in plants. In *Lipids in Cereal Technology* (P. J. Barnes, ed.), Academic Press, New York, pp. 353–372; Tan, B. K., et al. (1985). *J. Am. Oil Chem. Soc.* 62: 230–236; Auld, D. L., et al. (1989). *J. Am. Oil Chem. Soc.* 66: 1475–1479.

the major fatty acids in the various species. The table also illustrates the limitations on variation discussed previously. For example, none of the species listed shows any great tendency to produce saturated fatty acids with chain lengths less than 16 carbons except the palm family, and none shows much tendency to produce saturated chain lengths of more than 18 carbons. Of the oils listed in Table 16.4, only rapeseed contains erucic acid. Linolenic acid is present in very small amounts except in soybeans and rapeseed. Palmitic acid content is less than 15% except in the cereal species and oil palms, and in the cereals it never exceeds 30%. The content of stearic acid is less than 15% in these plant species.

Some of these variations are attributable to climatic as well as genetic differences. Fatty acid variations with growth environments has been documented for oats (Hammond, 1983), palm, palm kernel and coconut (Rossell et al., 1985; Tan et al., 1985), and soybean (Hammond and Fehr, 1984; Rennie and Tanner, 1989). In general, low temperatures encourage greater amounts of unsaturated groups and high temperatures favor saturated groups. These are the responses that would maintain the liquidity of membranes and storage fats.

The fatty acid composition of seed also changes considerably during maturation. These data have been summarized by Appleqvist (1975), Hammond and Glatz (1989), and Weselake (2002). They report that in many species of seeds the linolenic and saturated fatty acid content decreased during maturation. In the soybean mutant A6, Graef et al. (1985b) showed that the stearic acid level increased during maturation instead of decreasing while the oleoyl content failed to increase as it normally would. In a high-palmitic soybean mutant, Schnebly et al. (1996) reported that the palmitic acid content started out slightly higher than normal and failed to decrease. In a low-palmitic mutant, the palmitic acid content started out low and decreased even further. In rapeseed, the erucic acid content increases during maturation.

The data in Table 16.4 have been gleaned from the literature, and some of the extreme values may not be reliable. For example, the linolenoyl group content of soybean oil was reported by O'Connor and Herb (1970) to range naturally as low as 2%, but it certainly has not been possible to find values this low in plants grown under normal conditions when modem analytical techniques have been used (Mounts et al., 1988). Probably 4.2% is a more realistic value for the minimum percentage of linolenoyl groups in natural populations of soybeans.

A. Rapeseed and Canola

The modification of fatty acid composition has its oldest and most successful example in canola. This began with the successful identification of rapeseed cultivars that were free of erucic acid groups (Stefansson et al., 1961). Nutritional concerns about the consumption of oils containing erucic acid (Formo, 1979) led to extensive adoption of low-erucic oils, which are now known as canola. Rapeseed belongs to the genera *Brassica* and the family Cruciferae. This family has the advantage that *Arabidopsis thaliana* is also a member and has had its genome sequenced and studied extensively. Plants in this family are easy to transform with exotic genes, and molecular geneticists often prefer to try their ideas first on *Arabidopsis*. The fatty acid composition of *Brassica carinata* can also be determined rapidly by near-infrared reflectance (Velasco et al., 1997).

Efforts to improve canola oil next centered on reducing its linolenic acid content in an effort to increase the oxidative stability of the oil. Mutation breeding with ethyl methanesulfonate (Rakow and McGregor, 1973; Röbbelen and Nitsch, 1975; Röbbelen, 1984) followed by further selection (McVetty and Scarth, 2002) resulted in cultivars with linolenic acid contents of 1.6%–2.8% (Daun, 1998). Such low-linolenic strains have improved oxidative stability in room odor tests (Prevot et al., 1990).

Typical canola contains about 60% oleic acid, and recurrent selection techniques have pushed this up to 85%–90% (Vilkki and Tanhuanpää, 1995). Mutagen treatment of seeds or microspores yielded strains with 80%–86% oleic acid. A molecular engineering approach has yielded similar values of oleic acid (Scarth and Tang, 2006). High-oleic canola with reduced linolenic acid

are being produced commercially as Clear Valley 75® by Cargil, Monola® by Nutrihealth Pty Ltd in Australia, and Natreon® by Dow AgroSciences. Monola® contains 2.5% linolenic and about 70% oleic. Natreon® contains less than 3.5% linolenic and 70% oleic. Clear Valley® is advertised as low-linolenic high-oleic oil. Similar lines are being produced in Europe (Laga et al., 2004).

One of the heavily advertised advantages of canola oil has been its low content of saturated (palmitic and stearic) acids, about 6%–7%. There is interest in lowering the saturated fatty acid content even further. *Brassica* engineered with genes from *Cuphea pullcherrima* and safflower gave 3.4% saturated fatty acids (Scarth and Tang, 2006). There is also interest in increasing the levels of palmitic and stearic acids. The introduction of Cuphea (Jones et al., 1995), elm, or nutmeg (Voelker et al., 1997) genes has raised palmitic acid to 34%. Several strategies have been tried for raising the stearic acid content (Scarth and Tang, 2006), and the highest value reported was 55%–68% for *Brassica napus* transformed with a mutated mangosteen gene (Facciotti et al., 1999).

The Calgene group has developed a high-lauric acid canola by transforming canola with an acyl carrier protein thioesterase from the California bay laurel, which produces short-chain fatty acids (Del Vecchio, 1996; Voelker et al., 1996). Lines with 56 mol.% lauric acid content were produced. When a gene coding for a coconut enzyme that will place short-chain acids on the *sn*-2 position of glycerol was included lines with as much as 67 mol.% lauric acid were obtained.

There is interest in introducing genes for making γ-linolenic acid (all *cis*-6,9,12-octadecatirenoic acid) in oilseeds for human consumption because it lies on the route to arachidonic acid (all *cis*-5,8,11,14-eicosatetraenoic acid), and some believe that this will improve human health. Genetic engineering has allowed γ-linolenic acid to be expressed at levels of 25%–40% in rape (Knutzon et al., 1999; Liu et al., 2001; Hong et al., 2002; Qiu et al., 2003; Huang et al., 2004).

Although erucoyl-free strains are desired for human consumption, erucic acid has a number of industrial uses, and rapeseed with high-erucic acid content has been selected (Calhoun et al., 1975; Röbbelen and Nitsch, 1975). It has been possible to produce strains with 64%–66% erucic acid groups, but the high-erucic cultivars used in Canada are in the 40%–50% range (Daun, 1998). As erucoyl groups normally occur only on the *sn*-1 and -3 positions of *Brassica* species (Ohlson et al., 1975; Zadernowski and Sosulski, 1979), it was believed that the erucoyl content in this plant family could not exceed about 66%. Zou et al. (1997) were able to incorporate erucoyl groups on the *sn*-2 position of the triglycerides of *Brassica napus* by transforming it with a yeast gene that normally places acyl groups on sphingolipids. Molecular engineering approaches have also been tried to increase the chain elongation capacity of rape and to improve the transfer of erucoyl groups to the *sn*-2 position (Friedt and Lühs, 1998). These changes have made it possible to produce glyceryl trierucate in the engineered plants, but have not made it possible to produce much more total erucic acid in the oil. Possibly the expression of erucic acid in the seeds is limited by the melting point of glyceryl trierucate as Auld et al. (1989) suggested.

Genes expressing monounsaturated fatty acids with double bonds in unusual positions as well as epoxy and hydroxy fatty acids have been reported in *Arabidopsis* seed oil at levels between 5% and 15% (Scarth and Tang, 2006).

B. Soybeans

White et al. (1961) attempted to reduce the linolenic acid content of soybean oil by recurrent selection. Their efforts have been continued by others (Hammond et al., 1972; Hammond and Fehr, 1975, 1984; Wilson, 1984; Hammond, 1985). Mutation-breeding techniques have resulted in the production of varieties that will consistently contain about 3.5% linolenoyl groups. Strains with less than 1% linolenic acid have been obtained by crossing certain lines with reduced linolenic content (Fehr and Hammond, 1996a,b). Genetic analyses of low-linolenic crosses indicated that the low-linolenic trait in soybeans is a quantitative character influenced by minor genes and environmental effects (Fehr et al., 1992). Byrum et al. (1997) showed that the low-linolenic acid trait in the variety A5 was caused by a full or partial deletion of the microsomal desaturase gene. Wilson et al. (2002)

have reviewed the recent progress in understanding the genetic relations among the genes controlling linolenic acid content. Because of the recent requirement for putting information about *trans* fatty acids on food labels, there is considerable activity to meet this goal by producing low-linolenic soybean oil that will not require hydrogenation for certain oil applications. Three groups are offering low-linolenic oil: Iowa State with Asoyia containing less than 1%, Monsanto with Vistive and Pioneer Hy-Bred International with Nutrium both containing less than 3% linolenic acid. Asoyia® is free of genetic engineering, but Vistive® and Nutrium® are both Roundup®-tolerant. Pioneer and Monsanto also report that they are working on other modified soybeans that will contain, among other things, elevated oleic acid content.

Besides soybeans with reduced linolenic acid content, mutation breeding with ethyl methanesulfonate, sodium azide, and *N*-nitrosourea has led to changes in the content of other fatty acids. The ranges of palmitic and stearic acids have been expanded so that both have ranges of about 3.5% and 30%, compared with 11% and 3.5%, respectively, in typical strains (Graef et al., 1985a,b; Lundeen et al., 1987; Fehr and Hammond, 1996c–e, 1997a,b). The high-stearic trait appears to be controlled by one genetic locus. High-stearic lines were associated with lower yields, although it appears possible to increase stearoyl content to some extent with no loss in yield (Hartmann et al., 1997). High-stearic lines are also subject to inconsistent germination. The palmitic content of soybeans is controlled by at least four independent loci (Schnebly et al., 1994), and both the reduced- and elevated-palmitoyl traits are associated with lower oil content in the seed (Horejsi et al., 1994; Ndzana et al., 1994; Hartmann et al., 1996). A soybean strain containing 28% stearoyl and 17% palmitoyl chains have been produced, for a total of 45% saturated acyl groups. Such high levels of saturation are very unusual for a temperate zone vegetable oil. Kok et al. (1999) have shown that this oil can make an acceptable margarine by transesterification, which avoids the production of *trans* fatty acids. The genetic control of saturated fatty acids in soybeans has been reviewed by Wilson et al. (2002). Liu et al. (2003) claims that a stable high-stearic acid trait can be introduced into oilseed by suitable genetic engineering.

A group at DuPont has produced a strain with a stable high-oleic percentage of about 85% by multiple expressions of the gene responsible for Δ-12 unsaturation. This "sense suppression" effect turns off both the endogenous and added gene (Kinney, 1996, 1997). Yields were as good as those of the soybean line that was transformed and the oleate content was environmentally stable. There is considerable interest in increasing the oleic content of low-linolenic soybean oil to produce a very stable frying oil, but there seems to be a reluctance to use genetic engineering to achieve this end, probably because of anticipated consumer resistance. The North Caroline breeding program has produced soybeans with low-linolenic and oleic ranging from 29% to 60% (Wilson et al., 2002). However, similar to many oleic mutants in soybeans, the oleic acid content varied considerably with growth environment. Liu et al. (2003) claim that a stable high-oleic acid trait may be introduced into oilseeds by suitable genetic engineering.

Comparisons of typical soybean oil with those containing reduced levels of linolenic and elevated levels of stearic acids have shown that these changes in composition increase oxidative stability (Mounts et al., 1988; White and Miller, 1988; Miller and White, 1988a,b). The high-oleic, low-polyenoic line produced by the DuPont group was tenfold more stable than typical soybean oil (Kinney, 1996).

The gene for making the conjugated triene calendic acid (*trans*-8-, *trans*-10-, *cis*-12-octadecatrienoic acid) has been transferred from *Calenduala officinalis* L. to soybeans, and expressions of 20%–25% calendic acid have been achieved (Cahoon et al., 2001). Oils with this fatty acid may make good surface coating and may also have interesting physiological effects.

C. Linseed

Australian workers have been successful in reducing the level of linolenic chains in linseed oil from typical values of 45%–65% to 1%–2% by mutation breeding and crossing of low-linolenic lines (Green and Marshall, 1984; Green, 1986; Tonnet and Green, 1987). These lines, which are

being grown in Canada, have a linoleic content of about 73% and are finding use as substitutes for sunflower seed oil (Daun, 1998).

D. Safflower and Sunflower

Knowles (1972, 1975) developed cultivars of safflower that yield oils containing up to 85% oleic acid or up to 85% linoleic aicd. Fick (1983) developed sunflower varieties yielding oil with more than 80% oleic acid. These sunflower lines were based on a Russian variety, Pervenet, that had been obtained by mutation breeding with dimethyl sulfate (Purdy, 1986). These varieties have enjoyed considerable commercial production and have commanded premium prices. The high-oleic oils are valued for their stability to oxidation, and the high-linoleic safflower oil has been used to maximize the polyunsaturated acyl content of margarine. The National Sunflower Association has encouraged the development of a sunflower containing 50%–75% oleic acid and 30%–32% linoleic acid as a frying oil. Sunflower is naturally low in linolenic acid. This is being marketed under the name NuSun® (Gupta, 1998, 2001). These changes were brought about by conventional breeding (Vick and Miller, 2002). Cargill has applied for a patent for producing high-oleic sunflower oil by molecular engineering (DeBonte et al., 1998).

E. Peanut

Norden et al. (1987) reported a high-oleic acid variety with a content of about 80% oleic and a much reduced linoleic content in the Florida peanut collection. The high-oleic trait has been shown to be controlled by two recessive genes, one of which occurs commonly in peanut germplasm cultivars (Moore and Knauft, 1989; Knauft et al., 1993; Isleib et al., 1996). High-oleic peanuts had better oxidative stability than common cultivars (Braddock et al., 1995).

F. Corn Oil

Corn oil containing 82% and 89% oleic acid and 10% and 4% linoleic acid, respectively, has been produced by DuPont. They were reported to have enhanced flavor stability compared with typical corn oil by Warner and Knowlton (1997). Dow AgroSciences has applied for a patent on introducing various acyl carrier protein thioesterases in corn that resulted in a decrease in palmitic acid content (Rubin-Wilson et al., 1998).

G. Palm Oil

Targets being pursued for palm oil are converting more of its palmitic acid to oleic acid and increasing its stearic acid and lycopene contents (Parveez et al., 2003; Shah et al., 2003).

VI. GLYCERIDE STRUCTURE VARIATION

The triglyceride composition of vegetable oils can vary considerably as the fatty acid composition changes. Complete triglyceride analyses are available only for the simplest vegetable oils, but stereospecific analyses of vegetable oils indicate that the fatty acid composition of the *sn*-1, -2, and -3 positions of the glycerol differ (Brockerhoff, 1971; Litchfield, 1972). Generally, the *sn*-2 position contains more oleic and linoleic groups and the *sn*-1 and -3 positions contain more long-chain saturated and erucic acid groups. Often the compositions of the *sn*-1 and -3 positions are quite similar. Generally, the amounts of the triglycerides are predicted reasonably well by assuming that the acyl groups on the three positions of the glycerol are carefully controlled, but the biosynthesis at one *sn*-position is oblivious about what is going on at a neighboring *sn*-position. This triglyceride composition can be calculated by the so-called 1-random-2-random-3-random hypothesis (Litchfield, 1972; Fatemi and Hammond, 1977a,b). Deviations from the 1-random-2-random-3-random values can be attributed to changes in the fatty acid proportions available for esterification during oil deposition in the seed.

Christie and Moore (1970) looked for patterns in triglyceride composition by plotting the amounts of particular acyl groups on the *sn*-1, -2, or -3 position vs. the amounts in the whole triglyceride. They found that for such plots, observed values fall on straight lines over the range of fatty acid compositions that were available. Lines of different slopes were obtained for each fatty acid and for each position of the glycerol. These kinds of "stereospecific plots" for soybean oil are shown in Figure 16.1. Fatemi and Hammond (1977a) suggested that if genetic variation in the triglyceride structure of plant oils exists, it could be recognized by finding individual plants that deviate from such typical patterns. Pan and Hammond (1983) performed a stereospecific analysis on the triglycerides from a number

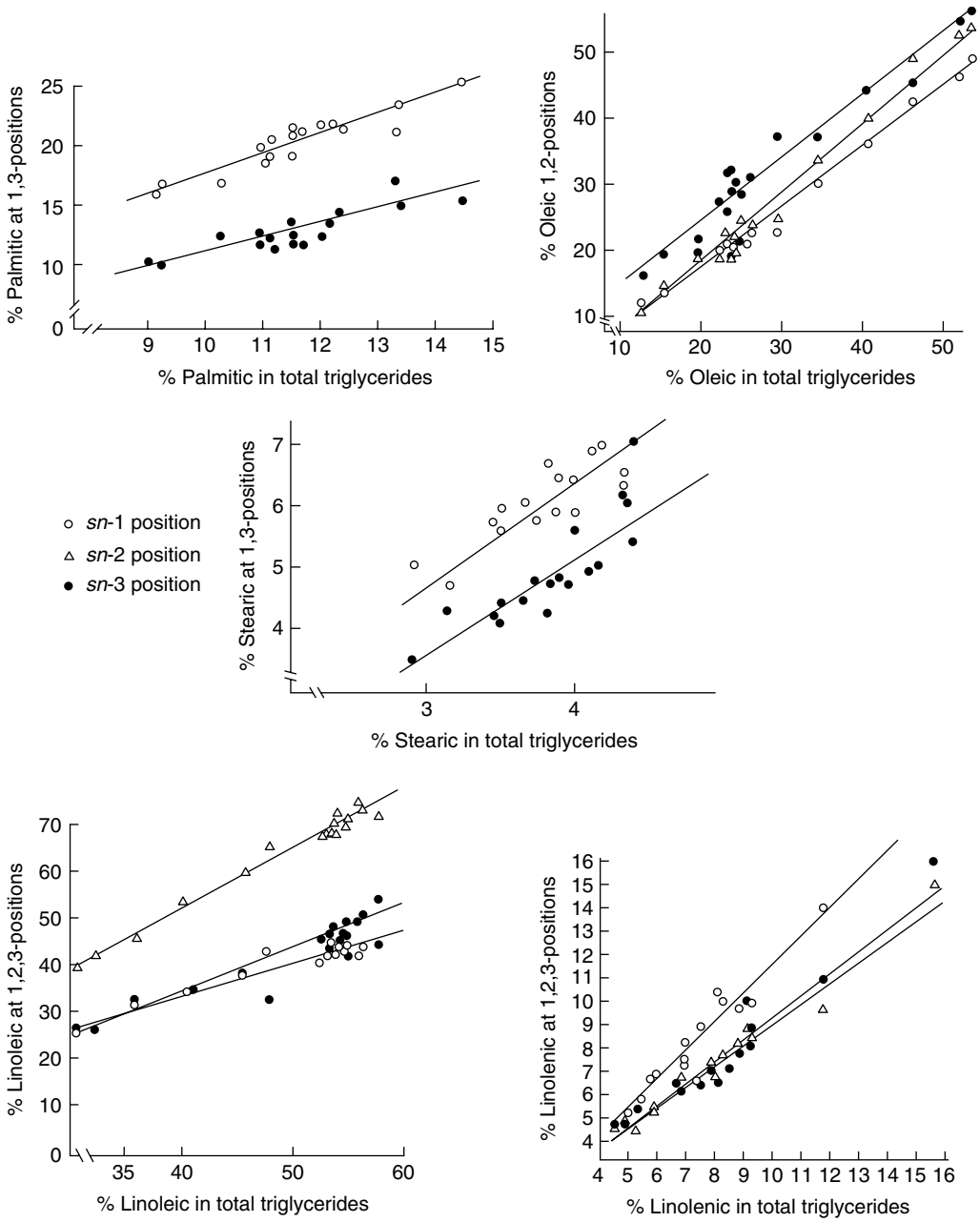

FIGURE 16.1 The amounts of various acyl groups found on the *sn*-1, -2, and -3 positions of glycerol vs. amounts of acyl groups found in the whole triglyceride of soybean lines.

of individual plants of soybean—*Glycine max*, its wild relative *Glycine soja*, as well as oats, *Avena sativa* and its wild relative *Avene sterilis*. Figure 16.1 illustrates the typical deviation from linear plots of individual soybean plants. Ohlson et al. (1975) generated similar data for species of Cruciferae. It has not been verified that the deviation of individual plants from linear plots is reproducible from generation to generation or if such deviations are under genetic control. Lee et al. (1993) fed the oleaginous yeast *Apiotrichum curvatum* (now renamed *Cryptococcus curvatus*) various fatty acid mixtures and found that the stereospecific distribution of fatty acids in the triacylglycerols that were formed could be fit by simple models. One model assumed that the only constraint was the specificity of the enzymes placing fatty acids on the three glycerol positions. Harp and Hammond (1998) obtained stereospecific plots for soybeans in which the stearoyl and palmitoyl content varied over a much wider range than those reported in previous surveys. They found that the plots for linoleic and linolenic acids showed much more scatter and that the direction of the scatter was a function of the amounts of saturated acyl groups. They suggested that this additional scatter did not result from these plants being glyceride-structure mutants but rather that the amounts of saturated acyl groups on the *sn*-1 and -3 positions affected the transfer of the linoleic and linolenic acids from the *sn*-2 position. They could not fit the models of Lee et al. (1993) to these soybeans, suggesting that additional constraints exist in the stereospecific distribution of soybean triglycerides.

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17 Fat-Based Fat Substitutes

Casimir C. Akoh

CONTENTS

I. INTRODUCTION

Fat substitutes are a class of fat replacers. Fat substitutes are often referred to as lipid- or fat-based fat replacers. The other fat replacers are protein- or carbohydrate-based fat mimetics. Fat substitutes are macromolecules that physically, chemically, and functionally resemble triacylglycerols found in fats and oils. In food formulations and nutrition, fat substitutes can be used to substitute for conventional fats and oils with the advantage of being lower in calories, zero calorie, partially absorbed, or for better functionality and nutritional attribute than the fat or oil being replaced. Although fat consumption is declining, probably due to the increased availability of low- and reduced-fat products, fat consumption is greater than the recommended levels, and an increase in the prevalence of the population classified as overweight (Frazao, 1996). It was estimated that 2076 new low- and reduced-fat products were introduced in 1996 (Calorie Control Commentary, 1997). A good fat substitute or replacer must have the taste, mouthfeel, and stability to frying and baking, be able to reduce calories, or provide health benefits to the consumer. Protein- and carbohydrate-based fat replacers can sometimes withstand baking or retort operation, but they are not suitable for deep-fat frying. A variety of fat substitutes can duplicate many of the functional and physical attributes of fats and oils.

Adapted in part from the IFT Scientific Status Summary on *Fat Replacers*, published by Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, Chicago, March 1998.

II. ZERO- AND REDUCED-CALORIE FAT-BASED FAT SUBSTITUTES

A. Olestra/Olean

Sucrose fatty acid polyester (SPE), commonly known as olestra or Olean®, is made up of six to eight fatty acids chemically esterified to the sucrose molecule (Figure 17.1). Olestra was discovered by Procter & Gamble (P&G) (Cincinnati, OH), who obtained the original patent. The synthesis of olestra has evolved from a solvent to a solvent-free process. So far, olestra is synthesized only with chemical catalysis. Olestra was approved by the U.S. Food and Drug Administration (FDA) in January 1996 for replacing up to 100% of the conventional fat in savory snacks (i.e., snacks that are salty or piquant but not sweet, such as potato chips, cheese puffs, and crackers) and not for frying of savory snacks (FDA, 1996). It was estimated that olestra will generate $400 million in annual revenue from its use in savory snacks. In approving olestra, the FDA concluded that olestra is not toxic, carcinogenic, genotoxic, or teratogenic; all safety issues were addressed; and there is reasonable certainty that no harm will result from the use of olestra in savory snacks (FDA, 1996). The Center for Science in the Public Interest (CSPI, Washington, DC), an advocacy and educational organization opposed the FDA approval of olestra on the basis of several allegations, including gastrointestinal (GI) disturbances, and petitioned the FDA to repeal the approval. Olestra passes through the GI tract without being digested or absorbed and, therefore, has the potential to cause GI effects, such as abdominal cramping and stool softening. Olestra can reduce the absorption of fat-soluble vitamins (A, D, E, and K) and nutrients such as carotenoids, which partition into olestra when ingested at the same time. Initially, the FDA required that foods containing olestra be labeled to inform consumers about potential GI effects and the addition of fat-soluble vitamins to compensate for the effects of olestra on their absorption (Table 17.1). Subsequently, the FDA Food Advisory Committee (FAC) reviewed the olestra postmarketing surveillance data that included additional clinical studies under

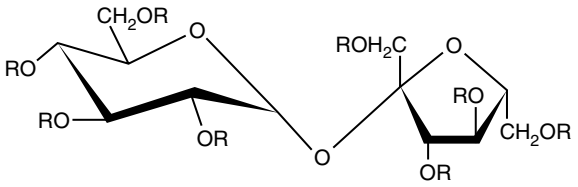

FIGURE 17.1 Structure of SPE (olestra or Olean®). Where R = acyl group of fatty acids.

TABLE 17.1
Compensation Levels of Fat-Soluble Vitamins in Olestra-Containing Snacks

Vitamin	Suggested Supplementation Level
A	51 retinol equivalents/g olestra as retinyl palmitate or retinyl acetate = (170 IU/g olestra or 0.34 × RDA/10 g olestra)
D	12 IU vitamin D/g olestra = (0.3 × RDA/10 g olestra)
E	1.9 mg alpha-tocopherol equivalents/g olestra = (0.94 × RDA/10 g olestra)
K	8 μg vitamin K_1/g olestra = (1.0 × RDA/10 g olestra)

Abbreviations: IU = international unit; RDA = recommended daily allowance.

Note: The suggested levels are to compensate for amounts that are not absorbed the diet because of olestra action.

Source: Adapted from FDA (1996). *Fed. Reg. 61*: 3118–3173.

normal snacking conditions between June 15 and 17, 1998, and reaffirmed their original decision that olestra is safe for salty snacks. The FAC review focused on GI effects, nutritional safety, and the interim label.

A series of studies (Suttie and Ross, 1997) was published in a supplement to the *Journal of Nutrition* to assess the nutritional effects of olestra in pigs and human subjects. In a randomized, double-blind, parallel group (olestra vs. full-fat control), placebo-controlled, single-center study, 1123 human subjects, aged 13–88 years, were given *ad libitum* potato chips made with olestra to assess whether olestra results in different levels of GI symptoms than the consumption of regular full-fat potato chips as placebo (Cheskin et al., 1998). The study concluded that *ad libitum* consumption of olestra potato chips during one sitting was not associated with an increased incidence or severity of GI symptoms, nor does the amount consumed predict who will report GI effects after short-term consumption of either olestra or regular potato chips (Cheskin et al., 1998). The first olestra product was sold by PepsiCo's Frito-Lay units as Wow! Chips in February, 1998. The P&G's Pringles started selling in March 1998. Chips and snacks made with olestra contain about half the calories of regular chips. As of June 1998, more than half a billion servings of olestra snacks were sold with Frito-Lay selling 80-million bags of chips.

Olestra is a completely nonabsorbable zero-calorie fat-based fat substitute that can successfully mimic both the culinary and gastronomic characteristics of fats and oils (Goldman, 1997). Olestra appears to impair the absorption of fat-soluble but not water-soluble nutrients. Highly lipophilic drugs are most likely to interact with olestra (Goldman, 1997).

The physical properties of olestra are controlled by the fatty acids attached to the sucrose molecules. The melting points of triacylglycerols are dependent on the chain length and degree of unsaturation of their fatty acid constituents. Likewise, the melting point of olestra also reflects their fatty acid constituents and degree of esterification. For example, sucrose octaoleate is a liquid and sucrose octastearate and octapalmitate are solids at room temperature (Jandacek and Webb, 1978). It is well known that lipids with high melting points are organoleptically undesirable and leave an unacceptable waxy taste in the mouth. Fat substitutes with a melting point body temperature are preferred for food formulations except in salad dressings where liquid oils are more desirable. Since most foods are formulated with partially hydrogenated vegetable shortening, olestra can be produced with shortening consistency by blending appropriate amounts of liquid oils and fats prior to synthesis (Akoh and Swanson, 1990). Olestra is completely miscible with triacylglycerols. Heating or frying with olestra does not produce any new class of products that are not normally found in heated triacylglycerols. The apparent viscosity of olestra is however higher than that of triacylglycerols with a similar fatty acid composition (Jandacek and Webb, 1978; Akoh and Swanson, 1990). The oxidative stability of liquid olestra is lower than comparable vegetable oils, but the addition of antioxidant results in more stable olestra (Akoh, 1994; Shieh et al., 1996). The density, specific gravity, and refractive indices of olestra approximate those of commercial vegetable oils (Akoh and Swanson, 1990; Akoh, 1994; Shieh et al., 1996). It was suggested that the physical properties of SPEs can be adjusted by varying the degree of unsaturation and chain length of the fatty acid used in the synthesis to produce sucrose polyesters with functional properties appropriate for food use (Akoh and Swanson, 1990).

Olestra can cut calories. Examples of how olestra cuts calories are given in Table 17.2. Possible food applications and functions of some fat-based fat substitutes are shown in Table 17.3. A dialog conference on The Role of Fat-Modified Food in Dietary Change was recently held at Tufts University (1998). Essentially, the conference participants discussed (a) dietary fat consumption and health, (b) physiology of fat replacement and fat reduction, (c) behavioral and social influences on food choice, (d) educating consumers regarding choices for fat reduction, and (e) recommendation on what needs to be done in the future. They concluded that efforts to reduce fat and calories from the diet must be pursued both in the context of the total diet and overall healthful lifestyle that includes adequate regular physical activities. A recent review on fat replacers can be found elsewhere (Akoh, 1998).

TABLE 17.2
How Olestra Cuts Fat and Calories in a 1-Oz Serving of Triacylglycerol and Olestra-Made Snack Foods

Food	Fat Content (g)	Calories (kcal)	Fat Reduction (%)	Calorie Reduction (%)
Potato chips				
With triacylglycerol	10	150		
With olestra	0	60	100	60
Tortilla chips				
With triacylglycerol	7	140		
With olestra	1	90	85	36

TABLE 17.3
Possible Food Applications and Functions of Some Fat-Based Fat Substitutes

Food Application	Fat Substitute Type	Functions
Baked goods	Sucrose esters, mono- and diacylglycerols	Emulsification, cohesiveness, tenderizer, flavor carrier, shortening replacer, antistaling agent, prevent retrogradation of starch, dough conditioner
Frying and cooking	Olestra,[a] SLs	Texturizer, provide flavor, crispiness, heat conduction, calorie reduction[b]
Salad dressing	Olestra, SLs	Emulsification, mouthfeel, hold flavorants, calorie reduction
Frozen desserts	Olestra, SLs	Emulsification, texturizer, calorie reduction
Margarine, shortening, spread, butter	Olestra and olestra-type molecules, SLs	Spreadability, emulsification, provide flavor, plasticity, calorie reduction
Confectionery	SLs, olestra	Emulsification, texturizer
Processed meat products	Sugar esters, SLs	Emulsification, texturizer, provide mouthfeel
Dairy products	Olestra, SLs	Provide flavor, body, mouthfeel, texturizer, stabilizer, increase overrun calorie reduction
Soups, sauces, gravies	SLs, olestra	Provide mouthfeel, lubricity, calorie reduction
Snack products	SLs, olestra	Emulsification, provide flavor, calorie reduction

[a] Means olestra and olestra-type molecules such as sorbitol, raffinose, stachyose, and alkyl glycoside polyesters can be used to achieve the same functionality.
[b] Calorie reduction can be achieved with the products given in "a" above and with some types of SLs such as Benefat and caprenin.

B. Polyol or Carbohydrate Fatty Acid Polyesters

Other polyol or fatty acid polyesters with a potential for use in place of SPEs as zero- or reduced-calorie fats and oils are the monosaccharides (mannitol, sorbitol), disaccharides (lactitol, trehalose), trisaccharide (raffinose), and tetrasaccharide (stachyose) fatty acid polyesters. If the degree of the polyol hydroxyl group substitution with fatty acids is greater than 4, the susceptibility of the polyol fatty acid polyester to lipolysis decreases. Their synthesis, purification, analysis, and physical properties are essentially the same as that of SPEs (Goldman, 1997). None of the molecules listed above is approved for use in foods by the FDA. However, further studies and product developments will be required before petition can be filed. Now that olestra is approved, it is expected that the approval process for new polyol fatty acid polyesters will take less time and these are expected to compete with olestra for the fat-based fat-substitute market.

C. Alkyl Glycosides Fatty Acid Polyesters

Alkyl glycosides suitable for the synthesis of fatty acid polyesters include methyl or ethyl glucose and galactose or octyl-β-glucose. Among these, methyl glucoside fatty acid polyester has received the greatest attention as a possible reduced-calorie replacement of conventional fats and oils in foods. The preparation of alkyl glycoside fatty acid polyesters and their physical properties have been described elsewhere (Meyer et al., 1990; Akoh, 1994). In general, up to four to five of the available hydroxyl groups of the glycosides are esterified with fatty acids. Alkyl glycoside fatty acid polyesters can be used as frying oils and in preparing Italian or white salad dressings. Curtice Burns, Inc. (Rochester, New York) owns the patent on the application of alkyl glycoside fatty acid polyesters (Meyer et al., 1989). No approval petition has been filed with the FDA.

D. Sorbestrin

Sorbestrin was developed by Pfizer, Inc. (now owned by Cultor Food Science, Inc., New York). Sorbestrin is a mixture of tri-, tetra-, and pentaesters of sorbitol and sorbitol anhydrides with fatty acids. Sorbestrin contributes 1.5 kcal/g to diet. It has a bland oil-like taste with a cloud point between 13°C and 15°C. Sorbestrin is mainly in liquid form and can serve as a reduced-calorie fat-based fat substitute. Sorbestrin is thermally stable and can withstand deep-fat-frying temperatures. It is intended for use in frying, baking, and salad dressings. Sorbestrin is not yet commercially available and will require FDA Food Additive approval prior to use. Akoh and Swanson (1989) synthesized sorbitol hexaoleate as a low-calorie fat substitute. Recently, Chung et al. (1996) optimized the synthesis of sorbitol fatty acid polyesters. Procter & Gamble also worked on sorbitol polyesters but did not follow up with further developments as with olestra. Table 17.4 lists the calories available from selected fat-based fat substitutes.

E. Dialkyl Dihexadecylmolonate

Dialkyl Dihexadecylmolonate (DDM) is a noncaloric synthetic fat substitute developed by Frito-Lay, Inc. (Dalls, TX). DDM is synthesized from malonic acid, hexadecane, and fatty acids (Haumann, 1986). It can be used for high-temperature frying operations like potato chips or can be blended with vegetable oils for the same purpose. No toxicity effect has been reported, but anal oil leakage is possible when consumed in large amounts (Calorie Control Commentary, 1996). No formal petition has been filed with the FDA. Usually, approval of a Food Additive Petition (FAP) will require many years. For example, it took Procter & Gamble almost 10 years from the date of the FAP to obtain FDA approval for olestra. This process can be costly and may prevent commercialization of many potential fat substitutes.

TABLE 17.4
Caloric Availability of Selected Fat-Based Fat Substitutes

Product	kcal/g
Olestra	0
Caprenin	5
Benefat	5
MCTs	8.3
Sorbestrin	1.5

F. Esterified Propoxylated Glycerol

Esterified propoxylated glycerol (EPG) differs from triacylglycerols, because it contains an oxypropylene group between the glycerol and fatty acids. Glycerol is first propoxylated with propylene oxide to form a polyether polyol that can be subsequently esterified with fatty acids. EPG can be used to replace conventional triacylglycerols in most foods, because EPG resembles fats and oils; examples include frozen desserts, salad dressings, baked goods, and spread. EPG is heat stable and can be used for cooking and frying operations. It has similar physical properties to triacylglycerols, but EPG is not hydrolyzed (Gershoff, 1995) by lipases owing to steric hindrance and therefore is not absorbed by the body. EPG becomes resistant to lipases when the propoxylation number exceeds 4. EPG contributes zero to few calories to the diet. It was developed by the ARCO Chemical Company (Wilmington, DE) and CPC International/Best Foods (Englewood Cliffs, NJ). EPG is not commercially available and no FAP has been filed with the FDA to date.

G. Polycarboxylic Acid Compounds

Trialkoxytricarballylate (TATCA), trialkoxycitrate (TAC), and trialkoxyglyceryl ether (TGE) are polycarboxylic acid esters and ethers proposed by Hamm (1984) as possible low-calorie replacements for edible oils. Owing to the nature of their structures (some with ester group reversed), they are not susceptible to complete hydrolysis by lipases. Their synthesis and properties have been reported (Hamm, 1984). Consumption of polycarboxylic acid esters and ethers can lead to anal leakage, weakness, depression, and death in animals. No FAP has been filed for TATCA, TAC, or TGE, and it appears no active research is ongoing with these molecules.

III. EMULSIFIERS

A. Sucrose Fatty Acid Esters

Sugar fatty acid esters have a lower degree of substitution or esterification of the sucrose hydroxyl groups with fatty acids (Ds, 1–3) compared to olestra (Ds, 6–8). Sucrose fatty acid esters (SFEs) are easily hydrolyzed by the digestive lipases and contribute reduced calories to the diet. They are made by transesterification reactions essentially like the SPEs (Osipow et al., 1956) except that the ratio of the reactants and reaction time are used to control the degree of esterification. The product contains five to seven hydroxyl groups of sucrose unesterified with fatty acids. Free hydroxyl groups together with the esterified fatty acids impart both hydrophilic and lipophilic character to SFEs and thus are excellent emulsifier surfactants. SFEs were approved for use in the United States as emulsifiers in baked goods and baking mixes in dairy product analogs, and as components of protective coatings for fresh apples (Drake et al., 1987), bananas, pears, pineapples, avocados, plantains, limes, melons, papaya, peaches, and plums to retard ripening and spoilage. In addition, SFEs are useful as antimicrobials (Marshall and Bullerman, 1994) and can be used for food, cosmetic, and pharmaceutical applications. As emulsifiers, SFEs function by lowering interfacial and surface tension of emulsions (Gupta et al., 1983).

B. Other Emulsifiers

Polyglycerol esters and other emulsifiers such as mono- and diacylglycerols, sodium stearoyl-2-lactylate (SSL), and lecithin can be used as fat substitutions where their main function is emulsification. They possess both hydrophilic and lipophilic properties that enable them to stabilize the interface between fat and water droplets through hydrogen bonding. Emulsifiers play an important role in fat reduction and in reduced-calorie food formulations involving fat mimetics (protein- and carbohydrate-based fat replacers). Emulsifiers work best with other ingredients as part of a fat-reduction system (Calorie Control Commentary, 1996). The addition of mono- and diacylglycerols

to cake shortenings is known to allow a reduction in fat content. Emulsifiers can also increase the perception of lubricity by emulsifying the fat in the mouth (Calorie Control Commentary, 1996). Emulsifiers can be combined, by experimentation, to achieve superior performance in a food system (full- or low-fat product).

The synthesis of polyglycerol esters has been described (Babayan, 1985, 1986). They have a wide hydrophilic–lipophilic balance (HLB) as found in the sugar fatty acid esters. The more unesterified hydroxyl groups available, the more hydrophilic the polyglycerol ester becomes. Polyglycerol esters, lecithin, SSLs, SFEs, and mono- and diacylglycerols can be used in margarines, baked goods, frozen desserts, dairy products, spreads and shortenings, and confectioneries. Many emulsifiers serve as tenderizers in baked products. Polyglycerol esters serve as aeration agents in food containing fat replacers. The functions of emulsifiers in low-fat products include serving as shortening and starch-complexing agents, syneresis control, air entrapment, foam stabilizer, flavor carrier, and as rheology control agents.

IV. STRUCTURED LIPIDS

Structured lipids (SLs) are triacylglycerols containing short-chain fatty acids (SCFAs) and/or medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) preferably on the same glycerol molecule (Akoh, 1995). SLs can be prepared as specialty lipids for nutritional, pharmaceutical, or medical applications (Jensen and Jensen, 1992) or as reduced-calorie lipids (Akoh, 1995). SLs containing MCFAs and LCFAs were developed to meet the energy requirements of specific individuals (Heird et al., 1986). Pure medium-chain triacylglycerol (MCT) emulsions do not provide essential fatty acids to the diet but provide readily absorbed nonglucose energy. A physical mixture of MCT and long-chain triacylglycerols (LCTs) can meet both the nutritional and energy requirements of hospitalized patients. However, SLs with modified absorption rate is a preferred alternative to physical mixtures (Bell et al., 1991; Kennedy, 1991). SLs can improve fat utilization in patients with cystic fibrosis, pancreatic insufficiency, and a fat-absorption disorder.

SLs are prepared by chemical and/or enzymatic transesterification reactions (Heird et al., 1986; Kennedy, 1991). When two triacylglycerols are the substrates, SLs are synthesized by ester interchange reactions (Akoh, 1995). Application of lipases in the synthesis of SLs offers advantages over conventional chemical catalysts such as synthesis of positional-specific SL molecules and stereoisomers under mild reaction conditions. The type of fatty acid at the 2-position of the glycerol moiety is critical to fat metabolism. Generally, the absorption of fatty acids (including poorly absorbed fatty acids such as palmitic and stearic acids) or linoleic at the 2-position of glycerol backbone are enhanced. Akoh (1995) reviewed the strategies for enzymatic synthesis of SLs and discussed their potential health benefits. The use of enzymes to synthesize reduced-calorie SLs with the potential to lower cholesterol, modulate the immune response, and reduce the caloric content of foods are being explored (Akoh, 1995). The general structure of SLs or structured triacylglycerols is shown in Figure 17.2.

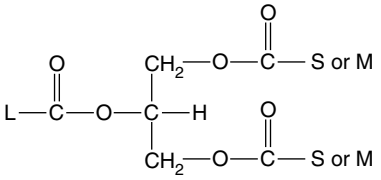

FIGURE 17.2 SLs or structured triacylglycerols. S, M, and L are for SCFA, MCFA, and LCFA, respectively. The position of S, M, or L is interchangeable.

A. Caprenin

Caprenin is a reduced-calorie SL providing 5 kcal/g compared to 9 kcal/g for conventional fats and oils. Caprenin consists of saturated caprylic (C8:0), capric (C10:0), and behenic acids (C22:0) esterified to a glycerol molecule. Behenic acid is poorly absorbed and contributes less calories than other fully absorbable unsaturated LCFAs found in fats and oils. Caprenin was originally produced by Procter & Gamble from coconut and palm kernel oils, and it was intended for use in soft candies and as a confectionery fat to replace cocoa butter. Procter & Gamble filed an FDA Generally Recognized As Safe (GRAS) affirmation petition to use caprenin as a confectionery fat in soft candy and confectionery coatings (Calorie Control Commentary, 1996). Caprenin was used by M&M Mars (Hackettstown, NJ or Elizabethtown, PA) and Hershey Foods Corp., Hershey, PA) in reduced-calorie chocolate bars such as Milky Way II, but was not commercially successful, because it proved difficult to temper. These products are no longer in the market, and caprenin is not currently available commercially. Procter & Gamble does not seem to be aggressively pursuing further research on caprenin at this time.

B. Benefat

Benefat, originally known as salatrim (*s*hort *a*nd *l*ong *a*cyl *tri*acylglycerol *m*olecules), is also a reduced-calorie SL with a caloric content of 5 kcal/g. It is composed of a mixture of SCFAs (C2:0, C3:0, or C4:0 fatty acids) and at least one LCFA (predominantly C18:0, stearic acid) randomly attached to the glycerol molecule. In making salatrim, SCFAs are randomly transesterified with vegetable oils such as highly hydrogenated canola or soybean oil using a chemical catalyst. Salatrim, a family of structured triacylglycerols, was developed by Nabisco Foods Group, Parsippany, NJ (Smith et al., 1994), and licensed to Cultor Food Science (New York, NY) under the brand name, Benefat. It has the taste, texture, and functional properties of conventional fats and oils. Stearic acid is partially absorbed resulting in salatrim only 55% or 5/9 of the value of conventional fats and oils (Smith et al., 1994), to the overall molecule. Benefat I was developed primarily for cocoa butter replacement. Benefat cannot be used for deep-fat frying, because the SCFA components will volatilize. Benefat can be produced to have select functional and physical properties, for example, a range of melting points, hardness, and appearance by adjusting the amounts of SCFAs and LCFAs used in their chemical synthesis. Products on the market that contain Benefat are the reduced-fat chocolate-flavored baking chips introduced in 1995 by the Hershey Foods Corporation and Snack Well's fudge-dipped granola bars. Benefits can also be used as a cocoa butter substitute. The Nabisco Foods Group intends to introduce Benefat-containing granola bars under SnackWell's brand. Benefat is intended for use in chocolate-flavored coatings, deposited chips, caramels, fillings for confectionery and baked goods, margarines and spreads, savory dressings, dips, and sauces and in dairy products (Kosmark, 1996). FDA accepted for filing in 1994 a GRAS affirmation petition submitted by the Nabisco Foods Group (Calorie Control Commentary, 1996).

C. Neobee and Captex Series

The Neobee series of SLs are manufactured chemically by interesterification and marketed by the Stepan Company (Maywood, NJ). These SLs are tailor-made for customers and for specific applications such as in medicine. For example, Neobee-1814 is made by random interesterification of MCTs and butter oil (50:50, w/w). Other products from the Stepan Company are SL-110 (interesterified MCT and soybean oil), SL-120 (MCT and menhaden oil), SL-130 (MCT and sunflower oil), SL-140 (MCT, menhaden, soybean oils, and tributyrin), SL-210 (coconut and soybean oil), SL-220 (coconut, menhaden, and canola oils), SL-230 (coconut, menhaden, and soybean oils and tributyrin), SL-310 (MCT and menhaden oil), and SL-410 (MCT, butter, and sunflower oil). Abitec Corporation (Columbus, OH) makes and markets the synthetic Captex series of SLs. Examples include Captex-350 (transesterified coconut oil with caprylic and capric acids) and 810D (contains

caprylic to linoleic acids). The main MCFAs in the Neobee and Captex products are caprylic, capric, and lauric acids, whereas the LCFAs include oleic, linoleic, eicosapentaenoic, and docosahexaenoic acids. Uses include nutritional supplements and clinical and consumer products. Their use in food applications has not received adequate attention. However, Akoh et al. (1998) recently showed that the consumption of a Captex 810D diet resulted in increased heat production and altered energy metabolism in obese Zucker rats. This study compared the effects of Captex 810D and Simplesse, a protein-based fat replacer, on energy metabolism, body weight, and serum lipids in lean and obese Zucker rats.

V. MEDIUM-CHAIN TRIACYLGLYCEROLS

MCTs contain saturated fatty acids of chain length C6:0–C12:0, predominantly C8:0–C10:0 fatty acids with traces of C6:0 and C12:0. A typical fatty acid composition for an MCT is C6:0 1–2%, C8:0 65–75%, C10:0 25–35%, and C12:0 2% (35). MCTs are different from the conventional fats and oils. Babassu, coconut, cohune, palm kernel, and tocum oils are the main sources of the fatty acids required for MCT synthesis after fractionation of the oils (Babayan, 1987; Megremis, 1991; Bach et al., 1996). MCTs are prepared by hydrolysis of the oils followed by fractionation of the resulting fatty acids to concentrate MCFA and reesterification with glycerol to form triacylglycerols (Babayan, 1987). Clinically, MCTs have been used since the 1950s in enteral and parenteral diets for the treatment of lipid absorption, digestion, or transport disorders. They provide 8.3 kcal/g and are commercially available on the basis of GRAS self-determination. They are more soluble in water than LCTs, and they can be used as a solvent for flavors and drugs and to carry vitamins. Because MCTs are not incorporated into chylomicrons, they are less likely than LCTs to be stored in adipose tissue. They are readily metabolized for energy. MCTs bind to serum albumin and are transported to the liver via the portal system rather than the lymphatic system. Infants and hospital patients, body builders, and fitness-conscious individuals may obtain a dense source of readily metabolizable energy from MCT consumption. MCTs can be incorporated into reduced-calorie foods because of their reduced-calorie content. They can be used in baked goods, beverages, frostings, dairy products, frozen desserts, snack foods, soft candies, and confectioneries. MCTs can be used in enteral and parenteral diets for people who cannot digest, absorb, or transport LCTs. Those that contain saturated fatty acids are oxidatively stable and have been used in food for over 30 years. Recent research on the metabolism of MCTs is available (Bach et al., 1996). MCTs are available from the Abitec Corporation (Columbus, OH) and the Stepan Company (Maywood, NJ).

VI. CONCLUSION

The desire by the American public to maintain good health while enjoying tasty foods has resulted in the development of a number of fat-based fat substitutes. The availability of a wide variety of fat replacers or fat substitutes is a step in the right direction toward providing consumers with many choices as they look for ways of reducing total fat calories. However, some fat is needed, because fat supplies essential fatty acids required for growth, proper development, and physiological functions. Fat substitutes are no "magic bullet" and there is no simple solution to fat replacement, but proper nutrition (choice of food groups), a healthy lifestyle, regular exercise, and a choice of appropriate fat-substitute-containing diets may lead to better nutrition and good health. Partial replacement and reduction in the amount of total fat consumed in the diet may be prudent. Research on structured fats with desired specific functionality, physical properties, and healthy appeal are being performed at various food industries and in academia. The future of fat replacement may well depend on the structure–function relationship of the molecules. In other words, fat replacers that possess healthy appeal, good taste, and desirable functionality will be acceptable to majority of consumers and could mean a sizeable share of the fat-replacer market.

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18 Commercial Applications of Fats in Foods

Ronald Jandacek

CONTENTS

I. INTRODUCTION

Since the previous edition of this book, the high level of interest in dietary fat has continued to grow. There have been advancements in the understanding of the reasons why fat in foods appeals to many people. Effective pharmaceutical treatments of elevated blood lipids that are risk factors for the development of atherosclerosis have appeared and have made it relatively easy to reduce these risk factors when dietary approaches have failed. The understanding of the metabolism of ω-3 fatty acids has contributed to recommendations for their inclusion in the diet for optimum nutrition. Labels requiring the level of *trans* acids are now required to appear on packaged foods.

The discussion of commercial applications of fatty acid-based food components that is presented in this chapter will discuss both broad applications and niche markets. This chapter will address the production and composition of dietary fats and oils, the physical properties of solid fats, margarines and spreads, triacylglycerol substitutes, triacylglycerols with specific structures, and fatty acid derivatives with beneficial physical or metabolic functions.

II. TRIACYLGLYCEROLS—PRODUCTION AND COMPOSITION

In terms of economic impact and total dietary consumption, triacylglycerols (triglycerides) in vegetable oils and animal fats are commercially the most important forms of fatty acids in foods. Although diet composition discussions often cite the "fatty acid composition" of the diet, essentially all of ingested fat is composed of esterified fatty acids in the form of triacylglycerols. The production and sale of edible oils and fats on an international scale is enormous, with projected 2005/2006 world production forecast of 145 million metric tons (Anonymous, 2005a). Approximately 85% of all edible oil produced is used in foods. On the basis of world population (6.5 billion people), edible fat production could potentially supply 470 kcal/person daily.

In 2004–2005, the principal sources of food oils were soybeans and palm, with 27.7 and 26 million metric tons produced, respectively (Gunstone, 2005). Canola oil was the third largest product, with 12.9 million metric tons produced in 2004–2005.

The method of production of edible vegetable oils varies with the source. Soybeans and canola seed are generally extracted with hexane. Purification steps include removal of residual lecithin (degumming) and removal of color pigments with bleaching earth. Steam deodorization removes materials that can give undesirable flavors to the oils. Olive oil is obtained by pressing. Palm oil is obtained from the fruit primarily by pressing a steamed digest mash of the palm fruit.

The fatty acid composition of the world's principal edible oils has become a major and evolving focus in the areas of health and nutrition. The positive correlation between elevated blood levels of low-density lipoprotein (LDL) cholesterol and the risk of atherosclerosis has become generally accepted. In addition, it has been generally accepted that blood cholesterol levels are affected by the type of dietary fatty acids, with saturated fatty acids increasing LDL cholesterol and polyunsaturated and monounsaturated fatty acids decreasing LDL cholesterol (Kris-Etherton and Yu, 1997). A significant exception to this generalization is that the long-chain saturated stearic (octadecanoic) acid, does not contribute to the elevation of LDL cholesterol. The fatty acid composition of widely consumed fats and oils is presented in Table 18.1 (Technical Committee, 2006).

As noted below and elsewhere in this book, high dietary levels of the geometric isomers of the unsaturated fatty acids in the *trans* form have been demonstrated to cause unfavorable increases in LDL cholesterol and decreases in high-density lipoprotein cholesterol (HDL). Since HDL levels are negatively correlated with the risk of the development of atherosclerosis, reduction in dietary *trans* fatty acids has become a focus in nutritional recommendations. Effective January 1, 2006, food label requirements in the United States require the inclusion of the level of *trans* acids or a statement that notes that the food is not a significant source of *trans* acids.

Dietary *trans* fatty acids primarily enter our diet in fats containing unsaturated fatty acids that have been partially hydrogenated. Although this process is generally viewed as a product of an industrial metal-catalyzed reaction of unsaturated fatty acids at elevated temperatures and pressures, it has in fact been with us since ruminant animals began grazing. Butter and dairy products contain small amounts of *trans* acids resulting from the biohydrogenation of fatty acids catalyzed by bacterial enzymes in the rumen of the cow.

The major portion of *trans* fatty acids in the diet has, however, been the result of partial hydrogenation of vegetable oils. Hydrogenation has been carried out to achieve two purposes. First, the production of higher melting fatty acids by saturating the double bonds of lower melting fatty acids yields higher melting triacylglycerols with physical properties that may be desirable for baking (semisolid shortening) or in spreads (stick margarine). Second, the saturation of double bonds in oxidatively unstable unsaturated fatty acids, particularly the triunsaturated α-linolenic acid, reduces the rate of development of rancid off flavors.

In response to the negative attributes of *trans* acids, food producers have sought alternative means to optimize physical and oxidative properties. Hunter (2005) recently reviewed the approaches that have been taken to produce alternatives to *trans* fatty acids in foods.

TABLE 18.1
Fatty Acid Composition of Commercial Edible Oils

Oil or Fat	Carbons: Double Bonds	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Pentadecanoic	Palmitic	Margaric	Stearic	Arachidic	Behenic	Lignoceric	Myristoleic	Palmitoleic	Oleic	Margaroleic	Gadoleic	Linoleic	α-Linolenic
		4:0	6:0	8:0	10:0	12:0	14:0	15:0	16:0	17:0	18:0	20:0	22:0	24:0	14:1	16:1	18:1	17:1	20:1	18:2	18:3
Beef tallow							3	1	24	2	19				1	4	43	1		3	1
Butterfat		4	2	1	3	3	11	2	27	1	12					2	29			2	1
Canola									4		2						62			22	10
Cocoa butter									26		34	1					34			3	
Coconut			1	8	6	47	18		9		3						6			2	
Corn									11		2						28			58	1
Cottonseed							1		22		3					1	19			54	
High oleic canola									4		2						75			17	2
High oleic safflower									7		2						78			13	
High oleic sunflower									4		5						79			11	
Lard								2	26		14					3	44		1	10	
Mid oleic sunflower									4		5						65			26	
Olive									13		3	1				1	71			10	1
Palm kernel				3	4	48	16		8		3						15			2	
Palm							1		45		4						40			10	
Peanut									11		2	1	3	2			48		2	32	
Safflower									7		2						13			78	
Soybean									11		4						24			54	7
Sunflower									7		5						19			68	1

Source: From Institute of Shortening and Edible Oils. With permission.

Approaches to reducing *trans* acids include modification of the hydrogenation process. Selection of catalyst and process conditions can affect the production of *trans* acids, although minimizing the production of saturated fatty acid production is a constraint that is important (Beers and Mangnus, 2004). Another approach is to use fats and oils that are naturally high in solids, such as cottonseed oil, although saturated fatty acid content must also be considered (O'Brien and Wakelyn, 2005). The development of oilseeds that are low in α-linolenic acid has been and is being actively pursued. By reducing the level of this oxidatively unstable fatty acid, a stable oil can result without the need to reduce α-linolenic concentration by hydrogenation (Ross et al., 2000). This development has been achieved by conventional seed development methods to give a commercial oil with 3% α-linolenic acid (Monsanto, Vistive™), compared with a typical level of 8% in soybean oil. Planting of this cultivar is expanding to meet the demands for a fat low in α-linolenic acid. Finally, semisolid fat-oil compositions appropriate for shortenings can be produced by interesterification of fats to favorably alter the solid content and crystalline phase behavior (Cowan and Husum, 2004). This interesterification is being performed by standard chemical methods, and increasingly, by enzymatically catalyzed processes.

Although taste, texture, cost, and consistency appear to be the principal driving forces for desirability of foods, the understanding of the relationship between diet and health continues to generate recommendations for individuals to optimize their diets for better health. These recommendations have had a commercial impact, as seen in the move to reduce dietary *trans* fats.

Extensive studies have examined the relationship of dietary triacylglycerol fats to obesity, heart disease, health development, and cancer. There have been controversies about these roles, but there are dietary recommendations for the amount and type of dietary fat that are prudent in health. In the publication, "Dietary Guidelines for Americans, 2005," published jointly by the U.S. Department of Health and Human Services and the U.S. Department of Agriculture, there are several recommendations related to dietary fat (Anonymous, 2005d). These guidelines recommend a total fat intake of 30%–35% of calories while reducing *trans* fatty acids and minimizing dietary cholesterol. They also recommend saturated fatty acid intake below 10% of calories, and mono- and polyunsaturated fatty acid from 20% to 35% of calories (including sources of ω-3 fatty acids).

Although these recommendations are somewhat general, they do provide guidance to the consumer. Moreover, the food industry has shown sensitivity to nutritional needs by directing efforts toward the development of products that are consistent with these guidelines. Marketing and advertisement of these products may emphasize conformance with dietary guidelines to enhance sales, but these efforts can also serve as a source of nutritional education for consumers.

In the United States, the fatty acid composition of cooking oils and oil-based products such as margarine and spreads is readily available to the consumer on the required labels. As noted above, the label now includes the level of *trans* acids as well as total fat and total saturated fatty acids. If there is less than 0.5 g of *trans* or saturated acids in a serving, there is no requirement for the amount to be included on the label. The amount of polyunsaturated and monounsaturated fatty acids in the food is indicated by subtracting label values for saturated and *trans* fat from total fat.

It is also now generally recognized that ω-3 long-chain polyunsaturated fatty acids are desirable in a healthy diet. This requirement is most important in newborns since the ω-3 acid, docosahexaenoic acid (DHA), a principal component of the brain and retina, is needed for normal neurological development. Sources of ω-3 fatty acids include fish oil (DHA and eicosapentaenoic acid, EPA); soybean, canola, and flax oils (α-linolenic acid); and single cell systems grown to maximize DHA production. The oxidative instability of oils with high concentrations of these fatty acids is a challenge that has limited their use in foods. Low levels of oils high in DHA are included in infant formulas and in gelatin-encapsulated supplements.

Oils from vegetable sources account for most of the "visible" forms of dietary fat and oils. Dietary fat from animal sources accounts for a significant portion of the diet. With the exception of butter, most animal fat is "hidden" as part of the meat, fowl, fish, and dairy products.

III. FUNCTIONALITY PROVIDED BY SOLID FATS

For several decades, most discussion of dietary triacylglycerols has emphasized the value of the lower melting, unsaturated fatty acids. The nutritional essentiality and LDL-lowering property of linoleic acid, and the healthfulness of oleic acid in Mediterranean-like diets have been foci from a nutritional viewpoint. In addition, the health benefits of the long-chain polyunsaturated ω-3 fatty acids have become a major topic for research and recommendations.

Higher melting saturated and *trans* fatty acids, however, continue to play a major role providing foods with texture and stability appropriate for specific food uses. The functional benefits of high-melting fats are due to their phase behavior. Many studies of this phase behavior have contributed to improving the use of high-melting fats in foods, and some of this understanding of the properties of lard and shortening is given below to provide historic perspective.

Lard, fat rendered from swine, was for many decades the standard ingredient in many baking and frying applications because of its ready availability. Although its oxidative stability is relatively low, its performance in baked goods was the time-tested standard. Its commercial importance has greatly decreased because of the nutritional concerns about animal fats described above. Nutritional recommendations for reductions in saturated fat intake emphasize the replacement of animal-based fats with fats from vegetable sources. As a result, the vegetable oil shortenings essentially displaced lard from a position of commercial importance. From 1970 to 1995 lard consumption in the United States decreased from 4.6 to 1.7 lb per capita per year (Putnam and Allshouse, 1997). Interestingly from 1996 to 2003, the per capita consumption increased to 2.4 lb per capita (Anonymous, 2005b).

A discussion of the composition and properties of lard is important, because cooking experience with lard once set the standards against which shortening performance was compared. The fatty acid composition and the structure of the principal triglyceride in lard are related to the properties of foods prepared with lard.

A feature of lard that distinguishes it from the vegetable oils and hydrogenated vegetable oils is the high level of triacylglycerols of the class with unsaturated fatty acids esterified to the 1- and 3-positions of glycerol and saturated fatty acids (principally palmitic acid) esterified to the 2-position. This dominant structure in lard triglycerides contrasts with those in vegetable oils, in which virtually all of the saturated fatty acids are on the 1- and 3-positions. It is of interest that this high level of palmitic acid in the 2-position of the triglyceride, although rare in natural fats, is also seen in human milk triglycerides (as discussed later in this chapter).

The melting range of lard is 91°F–115°F (33°C–46°C). Lard contains crystalline triacylglycerols at room and lower temperatures. The principal stable crystalline phase (or polymorph) of lard is the β-form, the most ordered and stable polymorph of triglyceride solid phases. β-Triglycerides are the highest melting of the phases. β-Phase triglycerides in lard and in shortening tend to grow readily to form large crystals within the liquid oil phase.

Although most solid triglycerides are stable in the highly ordered β-phase, some are stable in a lower melting β′-solid phase generally tend to form very small crystals. As discussed below, this limitation on crystalline size is important in the formulation of shelf-stable, homogeneous shortenings. In addition, the β′-phase fat crystals are preferred as the solid fat components in icings.

The stability of lard in the β-phase is the result of the high level of 2-palmitoyl triacylglycerols described above. It was found that the rearrangement of the fatty acids of lard to form a high level of triacylglycerol with essentially random positional distribution of fatty acids results in an improvement in melting behavior. Although rearrangement (interesterification) improves the performance of lard in some baking applications, this process is seldom used today, presumably because vegetable oil shortenings dominate this market segment.

Substitution of shortenings for lard came from the trend toward replacement of animal fats with vegetable fats over several decades. While the use of lard in foods in the United States declined from 1970 to 1995, shortening use increased from 17.3 to 22.5 lb/person per year during the same period (Putnam and Allshouse, 1997). This replacement presumably reflected the emphasis on nutrition

that directed consumers to select fats and vegetable oils without cholesterol and with minimal levels of saturated fatty acids. The recent increase in consumption of lard noted above may reflect concern about *trans* fatty acids in shortening.

The term *shortening* originates from the addition of fat to pastry dough to make it tender and flaky, or to "shorten" it. The principal objective for shortening formulation has therefore been to provide performance in baked goods. Shortenings used as ingredients in icing as well as baked products are called *all-purpose* shortenings. Shortenings used commercially and in households are also often used as frying fat. In addition to baking performance, the formulation of shortening has also been directed toward shelf stability and nutritional effects. The relationship of composition to these product requirements is discussed below.

Shortenings are mixtures of triacylglycerol components and phases that combine to provide the levels of solid and liquid fat that are appropriate for baking. Shortenings are predominantly liquid oil that is stabilized by a relatively low level of a crystalline fat matrix. The amount of solids in the shortening depends on temperature, and it decreases to zero at the complete melting point of all the components.

The solids content of the shortening has traditionally been measured by dilatometry. Dilatometry measures the volume of a mass of fat as a function of temperature. With the use of a predetermined calibration curve for the different changes in volume (density) for solid and liquid fats, the percentage of solid fat in a solid–liquid mixture can be determined. Dilatometry is a time-consuming procedure, and an alternative method has replaced it in many laboratories. This methodology is pulsed nuclear magnetic resonance, which makes use of the difference in relaxation times for hydrocarbon chains that are rapidly moving in the liquid phase or are relatively static in the solid (Madison and Hill, 1978).

The solids content of the shortening over a range of temperatures is an important determinant of the performance of a shortening in baking or in the preparation of icings and fillings. The shortening performance depends on the range in which it remains plastic, that is, between essentially completely liquid and a low-temperature hard form. At room temperature, the level of solids (solid fat index, SFI) typically ranges from 15% to 30%.

In consideration of the nutritional effects of shortenings, it is important to note that the principal component of vegetable shortenings is liquid oil. A relatively low level of triacylglycerol crystals dispersed in a liquid oil can result in a plastic mixture that appears solid. A common misconception is that this solid appearance reflects a composition of the shortening that is virtually entirely saturated fatty acids. In fact, commercial shortenings can be formulated with approximately equivalent levels of saturated and polyunsaturated fatty acids. The fatty acid composition of shortenings can vary considerably. For example, the composition of a current household shortening without *trans* fatty acids is 23% saturated fatty acids, 37% monounsaturated fatty acids, and 34% polyunsaturated fatty acids, and that of an industrial partially hydrogenated shortening is 19% saturated fatty acids, 31% *cis* monounsaturated fatty acids, 40% *trans* monounsaturated fatty acids, and 5% polyunsaturated fatty acids of which approximately half are *cis*, *cis* (Anonymous, 2005c). Not only is the level of crystalline solids in a shortening a requirement for its performance, but also the phase of its fat crystals may be more important. Solid components that crystallize in the β′-phase can be used in all-purpose shortenings that are used in baking and frying. The β′-crystal-based shortenings perform best in icings and in cakes.

β′-Crystals control the liquid triacylglycerol of a shortening for very long periods. This stability contrasts with that due to β-crystals, which grow, aggregate, and result in an inhomogeneous shortening that separates into liquid and solid regions, giving a "soupy" appearance. This distinguishing property of the β′-crystalline polymorph apparently results from the small needle-like crystals that form in this phase. These small crystals maximize the surface area for the adsorption of the liquid phase and minimize the separation of the oil and solid phases. β-Crystals, on the other hand, tend to grow in a crystal "ripening" process as small crystals dissolve and reprecipitate as contributors to the growth of larger crystals. β-Crystalline shortenings therefore may not be desirable as all-purpose shortenings that will be stored on shelves or used in preparations of icings or other creaming applications.

Most solid triacylglycerol fats will eventually crystallize in the β-polymorphic phase. The β-phase is highly ordered and is the highest melting triglyceride phase for most fats. An exception is the triacylglycerol 1,3-dipalmitoyl-2-stearoyl glycerol (PSP). The β′-phase of this triglyceride is its stable, equilibrium phase. Early shortening formulations included a high-melting fraction of cottonseed oil, which provided a significant level of PSP. Later shortenings used completely hydrogenated palm oil to provide the required amount of PSP to direct the solids of the shortening to the desired β′-crystals. The emphasis on the reduction of *trans* fatty acids has once again suggested the suitability of cottonseed oil in shortening and oils (O'Brien and Wakelyn, 2005). Shortenings currently available to consumers also include a fraction of triacylglycerol with an intermediate melting range that contributes to the β′-crystallization. The high-melting β′-phase constituent of a shortening typically has accounted for approximately 7% of the total composition.

Pourable fluid shortenings are also used by the food industry. These shortenings are suspensions of high-melting β-fat crystals in a continuous liquid oil phase. The crystals are formed by a variety of processing techniques to produce crystals of the right size to remain suspended and provide performance. The amount of undissolved, suspended solid fat in a pourable shortening may range from 2% to 10% of the total mixture.

An advantage to the pourable shortening is the convenience of measurement and transfer of the shortening in food preparation. Another approach to these properties is the use of powdered shortenings. Powdered shortenings are composed of the appropriate fat and an encapsulating material that is soluble in water. Encapsulating materials have included milk proteins and other added emulsifiers.

Emulsifiers can be added to shortenings by plasticizing them with the semisolid fat matrix. Monoacylglycerols (monoglycerides) or mixtures of monoacylglycerols and diacylglycerols (diglycerides) have been the most frequently used emulsifiers. The addition of emulsifiers contributes to the performance of the shortening in cake and icing products by stabilizing the mixture of the hydrophilic and lipophilic phases.

Some shortenings are used exclusively for frying. Based on the definition of a shortening, a "frying shortening" is an apparent contradiction in terms. Nevertheless, frying shortenings include a range of formulations from the solid–liquid mixtures described above to completely liquid oils. The totally liquid shortenings may not differ from cooking oils except in terminology.

IV. MARGARINES AND SPREADS

The standard of identity of margarine as defined by the Food and Drug Administration (FDA) requires a minimum of 80% fat. This requirement is part of the goal of providing the same nutrient levels contained in butter, for which margarine is considered to be a substitute. For this same reason, margarine must include at least 15,000 I.U. of vitamin A per pound. The remaining 20% of margarine consists of the aqueous phase and components that are compatible with the aqueous phase. Milk-derived proteins, emulsifiers, preservatives, and coloring agents are included.

The emphasis on the nutritional properties of fat-based foods has also encouraged the use of bread spreads that are substitutes for margarine, which is required to contain 80% fat. Spreads are typically formulated with 55%–60% fat by substituting water and emulsifiers for triacylglycerols. The appearance and packaging of these spreads are similar to those of margarine. The high level of water in spreads does, however, alter their performance in cooking and melted applications. They generally cannot be substituted in baking and perform poorly in frying.

Further efforts to develop low-fat spreads continue with various means of stabilizing the water phase of the spread and providing the lubricity of the higher fat spreads and margarines. Gelatin and other proteins have been used in combination with fat, water, and emulsifiers. Spreads with as little as 20% fat have been introduced to the market.

In terms of consumer usage, margarine (and spread) usage remained relatively constant from 1970 to 1995, ranging from 10.3 to 11.9 lb/person per year (Putnam and Allshouse, 1997). Prior to that period,

margarine had replaced a significant portion of butter usage, which declined from 5.4 to 4.5 lb/person per year from 1970 to 1995 (Putnam and Allshouse, 1997). In 1995, margarine per capita consumption was estimated to be 9.2 lb/person per year (Putnam and Allshouse, 1997). Although cost certainly has an influence, the emphasis on the elimination of animal fats, saturated fats, and cholesterol from the diet undoubtedly contributed to the use of margarine in place of butter. Since 1995, there has been a marked decrease in per capita consumption of margarine and spreads, falling one-third to 6.2 lb/person per year (Anonymous, 2005b). It would seem reasonable to attribute this decline to consumer concerns about solid and *trans* fats, and margarine and spreads are readily recognized visible fats.

Margarines are emulsions with an oil continuous phase. The high ratio of oil to water, the emulsification system, and the processing produce a dispersion of water droplets in the oil, a water-in-oil emulsion. Because of the high level of fat and the presence of relatively high-melting fats, margarine is used frequently as a shortening in baking. Frying with margarine is limited to low temperatures because of the rapid darkening of the protein constituents.

The fatty acid composition of the triacylglycerols in margarine and spreads varies greatly from product to product. Oils that have been hydrogenated to varying degrees have been used in virtually every product. The saturated and *trans* unsaturated fatty acids produced by hydrogenation provide texture and melting behavior appropriate for the product. The melting range, solids content, and emulsion properties can be controlled by the hydrogenation process.

As with other fat-based products, nutritional concerns have directed the formulation of margarines and spreads and the selection of triacylglycerols that are their principal components. Efforts have been made to reduce total saturated fatty acid levels and increase unsaturated fatty acids. Margarines and spreads with greatly reduced or essentially zero levels of *trans* fatty acids have also been developed and are marketed (Berger and Idris, 2005; List et al., 1995). One spread, "Smart Balance," is reported to have 0.71 g *trans* acids per 100 g, compared with 19 g in some other spreads (Anonymous, 2005c).

The structure and physical form of the margarine product are determined by the physical properties of the triacylglycerol component. Margarine produced as traditional "sticks" requires a relatively high-melting triacylglycerol to allow the molded product to retain its shape. As a result, most stick margarines have somewhat higher levels of high-melting fatty acids (including saturated and *trans* unsaturated acids) than margarines sold in tubs (Slover et al., 1985). These margarines do not require this rigidity and can be produced with lower levels of the high-melting fatty acids. Liquid margarines with fatty acid compositions in the range of the lowest saturate and highest unsaturate are also on the market (Reeves and Weirauch, 1979; Anonymous, 2005c).

V. TRIACYLGLYCEROL SUBSTITUTES

Dietary fats and oils provide functional properties to foods, and they also provide the hedonic properties of desirable texture and lubricity. The high-caloric density of fats (at 9 kcal/g, more than twice that of carbohydrates and proteins) is a benefit in situations where food is scarce. However, high-caloric density is associated with health risks when caloric intake is in excess of energy expenditure. It seems reasonable that there is a link between the hedonic properties and the caloric density of triacylglycerols. In the early development of *Homo sapiens*, individuals who consumed fat when available presumably had a survival advantage through the storage energy in adipose tissue for availability of energy during lean times.

With modern agriculture, however, food has become readily available for much of the population in many societies. However, this development over the several decades has not had an effect on the feeding-satiety signal system that developed over millennia to deal with periods of food scarcity. As a result, much of the population has responded with a positive energy balance and the gain of excess body weight and fat. This excess energy storage that can provide a benefit in times of famine has instead resulted in detrimental effects on health. There have been numerous reports that relate excess body fat to the risk of heart disease, diabetes, and joint problems.

Given the high-caloric density of fat, it has become a frequent target for nutritional recommendations designed to optimize health. However, recommendations to reduce dietary fat intake run headlong into its hedonic property, which is an often insurmountable driving force for excessive fat consumption even in the light of nutritional advice.

Several approaches have arisen to address this conflict between the desirable hedonic properties and undesirable caloric density of dietary triacylglycerols. Discussed below are three of these approaches: olestra, diacylglycerol oils, and "structured" lipids.

A. Olestra

Olestra is the generic name given to the class of compounds made by the interesterification of sucrose with fatty acid methyl esters to yield sucrose with 6–8 long-chain fatty acids bonded by ester links (Figure 18.1). The synthesis is a base catalyzed reaction that requires initial steps to provide miscibility of the hydrophilic and lipophilic reactants. The reaction is driven with an excess of the fatty acid methyl esters.

Olestra has two important characteristics that have driven its entry into the edible fat market. First, its physical and organoleptic properties are essentially identical to those of triacylglycerol fats with the same fatty acid composition. For example, olestra made from soybean oil fatty acid methyl esters has a melting point and an oil–water interfacial tension that is similar to soybean oil. The viscosity of this olestra is slightly higher than that of soybean oil, presumably because of the size of the molecule.

The second and equally important characteristic of olestra is its inertness in the presence of pancreatic lipase. Unlike triacylglycerols, which are readily hydrolyzed by the action of pancreatic lipase at the oil–water interface in the intestine, olestra remains intact during gastrointestinal transit. It is of interest that studies performed during the early development of olestra clearly demonstrated that lipase-catalyzed hydrolysis of fats is obligatory for the absorption of dietary fat, thereby putting to rest a controversy about whether or not triacylglycerol oil droplets might be absorbed without digestion (Mattson and Nolen, 1972a; Mattson and Volpenhein, 1972b). This understanding about the requirement of hydrolysis explains why this second characteristic of resistance to hydrolysis is important. It was also established that ingested olestra, because it is not hydrolyzed, is excreted intact and therefore does not provide energy that can be used by the body. The studies that led to this understanding have been reviewed previously (Jandacek, 1991).

Although olestra is not hydrolyzed by pancreatic enzymes, it is metabolized by microorganisms in activated sludge (Lee and Ventullo, 1996). It is therefore degraded in municipal sewage treatment facilities.

The combination of organoleptic desirability with the absence of absorption resulted in this "zero calorie" fat with texture, mouth feel, and cooking properties of normal triacylglycerol fats. Olestra became the subject of a food additive petition to the U.S. FDA, and it was approved for use in the commercial preparation of savory snacks in January 1996.

The physical and hedonic properties of olestra are essentially identical to those of triacylglycerol fats with the same fatty acid composition. Its behavior as a frying fat is also similar to that of triacylglycerol fats. Olestra therefore meets both the biological and the physical requirements of a fat

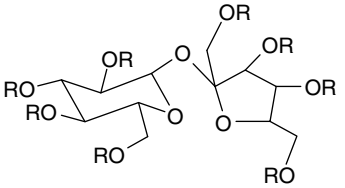


FIGURE 18.1 Olestra is the mixture of long-chain fatty acid esters of sucrose, with 6–8 ester bonds.

substitute. It does not provide calories that the body can use, and it provides the lubricity and texture of triacylglycerol fats.

The selection of the fatty acids that are used in the preparation of olestra was the result of a balance of two considerations. First, olestra that is used in food preparation must provide a desirable nonwaxy mouth feel. Second, olestra that enters the large intestine must not readily separate from fecal matter during gastrointestinal transit. These two requirements led to a blend of olestra made from soybean oil fatty acids along with olestra made with the high-melting fatty acid, behenic (docosanoic) acid. This blend evolved to provide satisfactory hedonic properties and to minimize the separation of oil in the large intestine that can cause undesirable gastrointestinal effects (Jandacek et al., 1999).

The approval of olestra required that a label informing the consumer about the potential for gastrointestinal effects be printed on packages of snack foods made with olestra. As of August, 2003, the FDA decided that this label requirement was unnecessary based on the commercial consumer experience with the products containing olestra.

Because olestra provides the small intestine with a lipid "sink" that can dissolve lipophilic substances, it interferes with the absorption of lipophilic compounds. Cholesterol, chlorinated hydrocarbon pollutants, and fat-soluble vitamins are affected in this manner, and their absorption from the intestine is reduced. The approval of olestra therefore required that the fat-soluble vitamins, A, D, E, and K, are supplemented with the olestra to compensate for the reduction in their absorption.

B. Diacylglycerols

Another fatty acid-based substitute for dietary triacylglycerol fat has been successfully marketed in Japan for several years and is currently being introduced as an edible oil product in the United States. The development of this substitute, 1,3-diacylglycerol (DAG), was based on the understanding of the digestion/absorption of typical triacylglycerol fats. As noted above, triacylglycerol digestion and absorption requires the lipase-catalyzed hydrolysis of emulsified fat droplets in the intestine to give fatty acids and 2-monoacylglycerols. The reesterification of these fatty acids and 2-monoacylglycerol then takes place in the enterocyte, the newly synthesized triacylglycerol is packaged into lipid-rich chylomicron particles that are transported in lymph.

Important to the rationale for the benefit of DAG is the transport of fat in chylomicrons to peripheral tissues after their entry into the blood circulation from the lymphatic circulation. A significant portion of recently absorbed dietary fat is thereby delivered to peripheral tissues prior to entry into the liver.

The digestion, absorption, and distribution of DAG can differ from that of triacylglycerols. Dietary DAG can be hydrolyzed by pancreatic lipase in the manner in which triacylglycerols are hydrolyzed, with splitting of ester links of the primary alcohol groups of glycerol. With DAG, the resulting products are fatty acids and glycerol (Figure 18.2). The rationale for the benefit of DAG is that the resynthesis of fatty acids and glycerol into triacylglycerol is inefficient in the enterocyte due to the absence of 2-monoacylglycerol. This inefficiency may then result in a portion of the fatty acids being absorbed via the portal vein rather than in chylomicrons, in the manner in which medium-chain fatty acids are absorbed.

This redirection of fatty acid absorption to the portal vein is reported to have benefits based on the liver's ability to metabolize the fatty acid prior to its storage in adipose tissue (Nagao et al., 2000). This hepatic metabolism is reported to reduce the deposition of fat in the visceral adipose tissue, with a resulting reduction in the risk to health associated with excess visceral fat accumulation.

Investigators comparing the effects of dietary triacylglycerol and DAG on visceral fat loss in humans in caloric deficit reported greater loss of visceral fat in the DAG group after weeks. A 12-month clinical trial comparing triacylglycerol and DAG in a reduced energy diet resulted in a 3.6% weight loss in the DAG group compared with 2.5% in the triacylglycerol control group (Maki et al., 2002). Fat mass decreases were 8.3% and 5.6% for the DAG and triacylglycerol groups, respectively. Clinical trials and metabolic studies of DAG were recently reviewed (Rudkowska et al., 2005).

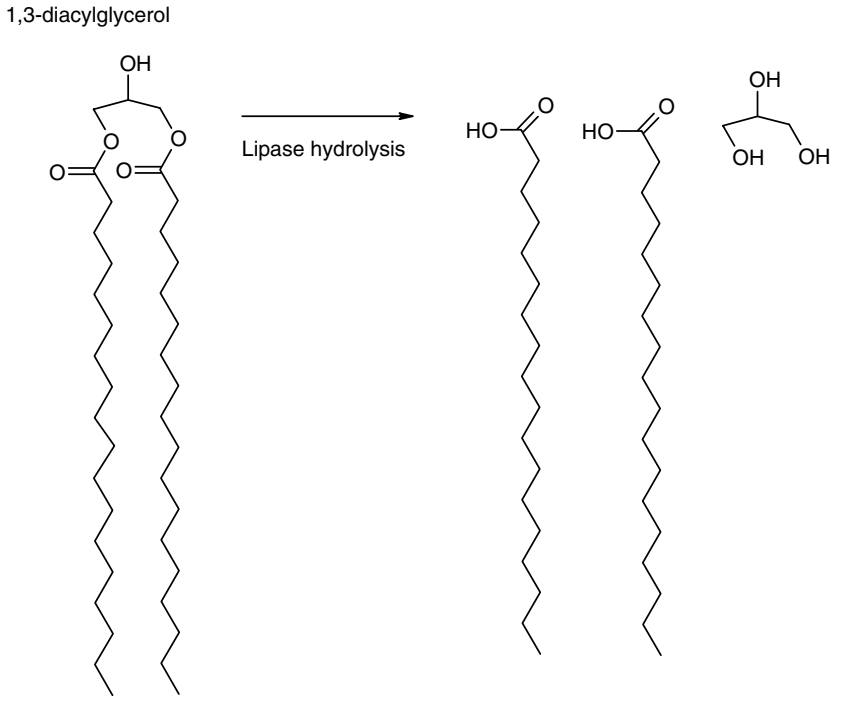


FIGURE 18.2 The lipase catalyzed hydrolysis of 1,3-diacylglycerols results in free fatty acids and glycerol.

The DAG is synthesized by enzymatic direction of fatty acids to the 1- and 3-positions of glycerol. The resulting oil contains <20% triacylglycerol and 80% diacylglycerol. The ratio of 1,3-diacylglycerol to 1,2-diacylglycerol is 7:3 (Watanabe et al., 2004). Because diacylglycerols melt at higher temperatures than triacylglycerols with the same fatty acid composition, the marketed DAG contains very low levels of saturated fatty acids to minimize crystallization of the oil. The flashpoint of DAG is 30°C–40°C lower than that of analogous triacylglycerols (Nakajima et al., 2004).

DAG has been commercially successful in Japan, with sales of 28 billion yen announced by Kao for 2003. These sales reflect the markedly higher price of DAG relative to typical triacylglycerol cooking/salad oil. As noted above, the marketing of DAG is in an early stage in the United States.

C. Triacylglycerols with Specific Structures

a. Medium-Chain Triacylglycerols and Structured Lipids

As discussed above, essentially all dietary fat is in the form of long-chain triacylglycerols. The metabolism of these triacylglycerols follows a sequence of hydrolytic, absorption, and resynthesis steps. Gastric lipase acts in the stomach to remove a fatty acid from some triacylglycerol molecules to form the 1,2-diacylglycerol. A mixture of triacylglycerol and diacylglycerol then enters the duodenum where it is emulsified by bile acids and phospholipid to form small droplets with a large oil–water interfacial surface. Pancreatic lipase/colipase acts at the interface to hydrolyze fatty acids in the 1- and 3-positions of the triacylglycerol to yield free fatty acids and 2-monoacylglycerols. These relatively polar products are transported to the enterocyte membrane via mixed micelles containing bile salts. In the enterocyte, the fatty acid and 2-monoacylglycerol are synthesized into triacylglycerol and packaged with apolipoproteins into chylomicrons for transport into the lymph.

This process is dependent on the chain length of the fatty acids. When the chain length is less than 12 carbon atoms, the lipase-catalyzed hydrolysis is more rapid than that seen for fatty acids

with longer chain length. In addition, it is clear that hexanoic, octanoic, and decanoic acids are primarily transported via the portal vein rather than in chylomicrons in lymph. Dodecanoic acid (lauric acid) can be transported both in lymph and in the portal vein blood.

Absorption in chylomicrons results in fatty acids being delivered to peripheral tissues after hydrolysis of triacylglycerol by lipoprotein lipase. Fatty acids absorbed via the portal vein encounter the liver and its metabolic processes during the first pass, before delivery to peripheral tissues. These fatty acids can therefore be oxidized before delivery to peripheral tissues.

The digestion process of triacylglycerols containing primarily hexanoic, octanoic, and decanoic acids has provided a means to provide dietary lipid to patients with insufficient pancreatic lipase secretion resulting from cystic fibrosis and other disorders. Triacylglycerols have been synthesized from hexanoic, octanoic, and decanoic acids and have been termed medium-chain triacylglycerols (MCT). These fatty acids are available from coconut oil, in which they account for approximately 12% of the total fatty acids. They are available as by-products when the coconut oil is used as a source for dodecanoic acid for industrial uses in surfactant synthesis and other products.

MCT oil was originally produced as a dietary fat for patients who were unable to digest and absorb long-chain triacylglycerol fat. It is still used for this purpose, however, it is well recognized that MCT oil does not provide essential fatty acids that are necessary for health (linoleic acid, α-linolenic acid). MCT oil has more recently been marketed as a health-oriented oil because of its metabolic pathway. Its postprandial metabolism in the liver has been reported to increase dietary thermogenesis relative to long-chain triacylglycerols. This effect suggests that MCT will result in less deposition of dietary fat into peripheral tissues, including adipose tissue, and thereby may provide a benefit to those seeking to reduce body fat. It may also be noted that the caloric content of MCT is approximately 10% less than that of the 9 kcal/g typical of long-chain fats.

The use of MCT in cooking is possible, however, it is recognized that the low molecular weight of the triacylglycerol and its fatty acids results in a flash point that is lower than that of long-chain fats. Frying with MCT oil therefore is a safety concern.

The absence of essential fatty acids in MCT has been addressed by the production of triacylglycerols made from blends of medium-chain fatty acids and long-chain fatty acids. These triacylglycerols may be made by transesterification reactions to yield a random positional distribution of fatty acids on the glycerol, so that medium-chain fatty acids are esterified both in the 2- and in the 1(3)-positions. They may also be made by enzymatic processes or by positionally selective synthesis to give triacylglycerols with medium-chain fatty acids predominantly in the 1(3)-positions. Both the random and the positionally selective mixed chain length triacylglycerols have been termed "structured lipids" (Figure 18.3). The properties of MCT and structured lipids were reviewed by Babayan, who was responsible for much of their development (Babayan, 1987).

Structured lipids can provide some benefits. For example, it has been shown that triacylglycerols with octanoic acid in the 1- and 3-positions are hydrolyzed as rapidly as trioctanoin. This observation indicates that it is possible to provide long-chain essential fatty acids in the 2-position of the triacylglycerol and still allow digestive hydrolysis to occur even in cases of low levels of pancreatic lipase.

It has also been reported that structured lipids may have advantages in fat-containing formulas that provide energy in intravenous feeding (Bellatone et al., 1999; Rubin et al., 2000). This effect may result from optimizing the rate of hydrolysis of intravenously administered fat, so that peripheral tissues may readily utilize the fat component of the diet.

Subsets of the class of structured lipids were developed to offer reduced calorie fats as substitutes for long-chain triacylglycerol fats. Two fats, salatrim and caprenin, resulted from an approach to reduce the utilizable caloric density of fats by synthesis of a triacylglycerol with a composition that included a poorly absorbed long-chain fatty acid along with short- or medium-chain fatty acids. The fatty acids of salatrim included stearic acid and short-chain fatty acids that included acetic,

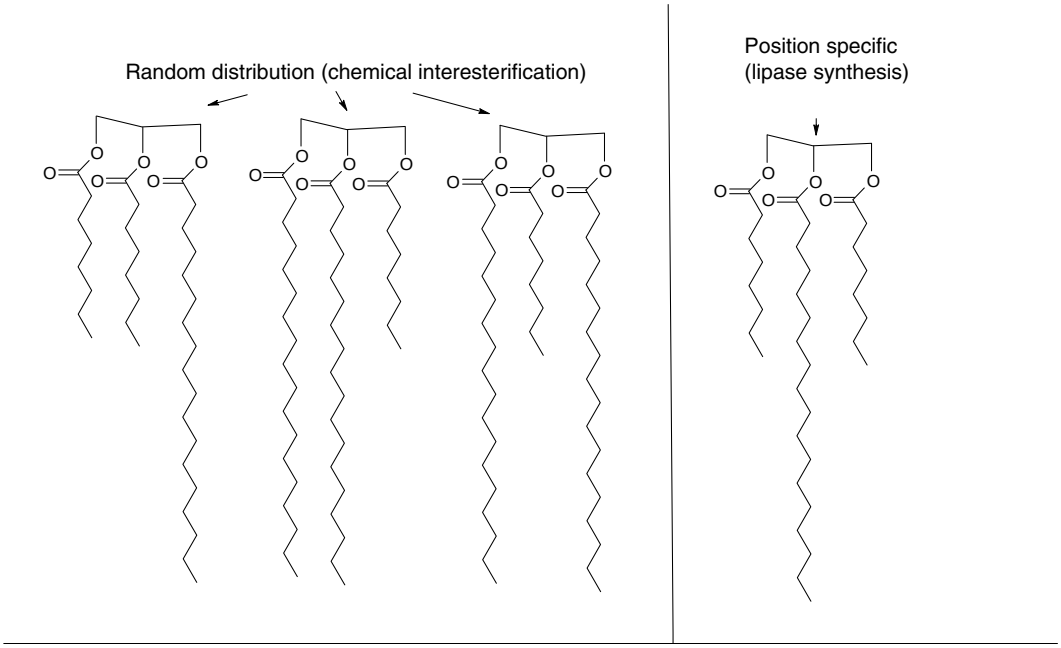


FIGURE 18.3 Structured lipids are triacylglycerols that include both medium- and long-chain fatty acids. They may be of random (chemical interesterification) or specific (lipase directed) structures.

propionic, and butyric acids. The fatty acid composition of caprenin comprised behenic acid (docosanoic acid) with medium-chain fatty acids including hexanoic, octanoic, and decanoic acids. Selected ratios of the long-chain to short/medium-chain acids resulted in fats with appropriate food qualities. The lipase-catalyzed hydrolysis of these fats in the intestine produced readily absorbed short- and medium-chain fatty acids (and/or monoacylglycerols) that are readily absorbed from the intestine. This hydrolysis also yielded the long-chain, saturated fatty acids, stearic acid (from salatrim), and behenic acid (from caprenin). These fatty acids are poorly absorbed from the intestine, so that salatrim and caprenin provide less than 9 kcal/g of energy. At the time of this writing, caprenin is not marketed, and salatrim is sold as Benefat®.

b. Human Milk Fat Mimic

Human milk fat is relatively unique in nature. It provides a relatively large amount of palmitic acid in the 2-position of the triacylglycerol. This positional specificity contributes to two nutritional benefits, improved fat absorption and improved calcium absorption (Kennedy et al., 1999). These benefits result because of the preferential production of 2-monopalmitin relative to palmitic acid during the digestion of the triacylglycerol. Since free palmitic acid can precipitate as the calcium soap in the intestine, the absorption of both the fatty acid and calcium are reduced. The 2-monopalmitin, however, does not precipitate as the calcium soap, and both the calcium and the fatty acid of the monoacylglycerol are available for absorption. Fat that is produced by enzymatic interestification for use in infant formulas has been included in European infant formulas, and its Generally Recognized as Safe (GRAS) approval in the United States was granted in 2004. It contains approximately 20% palmitic acid, of which approximately half is in the 2-position. The specificity of fatty acid position in this fat seems to make it more appropriately termed as a structured lipid than the random structures of mixed medium- and long-chain fatty acid triacylglycerols.

VI. FUNCTIONAL FATTY ACID DERIVATIVES

In terms of mass consumption of foods containing fatty acids, triacylglycerols as vegetable oils and animal fat account for essentially all of human consumption. The substitutes for triacylglycerols replace a small fraction of dietary vegetable oils and animal fats.

There are, however, food additives that are derivatives of fatty acids that provide important functions as minor components of foods. The functions of these derivatives may be categorized as physical and biological (health oriented) as presented in the discussion below.

A. Fatty Acid Derivatives Providing Textural Benefits

The lipophilic carbon chain and the functional carboxyl head group of fatty acids have allowed them to be combined with hydrophilic moieties via ester links to give compounds with surface active properties. The combination of hydrophilic compounds that are normally found in foods in combination with edible fatty acids has resulted in a variety of compounds that are safe food emulsifiers.

Although emulsifiers are added to most foods in trace quantities, their use is widespread. The market projected for fatty acid-based emulsifiers was 280 million pounds in the United States in 1995 (Dziezak,1988) and worldwide was estimated to be 660–880 million pound tons per year according to the report of a presentation at the 2005 International Palm Oil Congress.

Virtually all emulsifiers that are added to foods or are intrinsic to foods include one or more fatty acids as part of the emulsifier molecule. Emulsifiers are surface-active molecules with affinity for both a polar (aqueous) and a nonpolar (oil) phase. The hydrocarbon chain of the fatty acid provides the lipophilic portion of food emulsifiers, whereas the hydrophilic portion may be one of a wide range of polar groups including anionic (e.g., carboxylate), nonionic (e.g., ethoxylate groups), or zwitterionic (as in lecithin). An example of an emulsifier frequently used in foods is a monoglyceride. The two hydroxyl groups of glycerol are compatible with an aqueous phase, whereas the hydrocarbon chain of the fatty acid will dissolve in an oil phase.

The principal function of emulsifiers in foods is to stabilize oil–aqueous mixtures. In addition, emulsifiers stabilize aerated systems and foams, form a complex with starch in foods, and modify the crystal and polymorphic formation of high-melting fats.

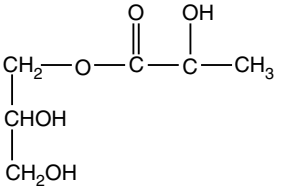
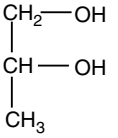
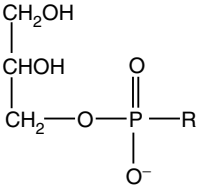
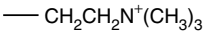
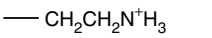
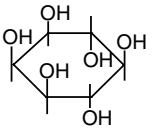
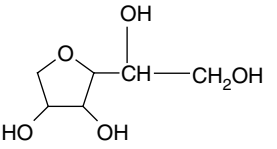
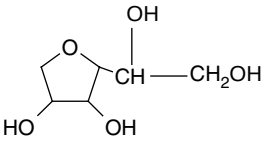
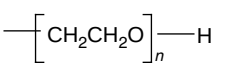
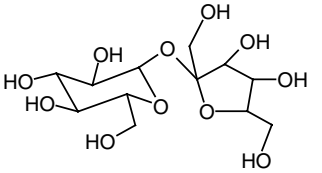
The hydrophilic/lipophilic balance (HLB) value of an emulsifier is a measure of its relative affinity for the hydrophilic and lipophilic phases. The HLB ranges from 1 to 20, with a value of 20 indicating the maximum in affinity for hydrophilic materials. Commercial preparations of monoglycerides are usually mixtures of monoglycerides and diglycerides, with the HLB increasing as the concentration of monoglycerides increases. The HLB values for mono- and diglyceride emulsifiers are generally in the range 2.8–3.5. The HLB can be useful in screening emulsifiers for particular functions, although difficulties are encountered in its application (Graciaa et al., 1989).

As a surface-active agent, an emulsifier will adsorb and concentrate at oil–water, water–air, or oil–air interfaces and will reduce the interfacial tensions at these interfaces. Perhaps the most important role of food emulsifiers is the stabilization of emulsion particles. Thus, milk, ice cream, butter, margarine, cake batter, and many other foods are stable emulsions that resist phase separation because of the presence of emulsifiers.

In addition to the most recognized function of emulsion stabilization, food emulsifiers provide other benefits in food formulation. They can interact with hydrophilic food components such as the amylose in starch to prevent staling through retrogradation, the alignment of amylopectin molecules. Emulsifiers bind to wheat gluten in dough to produce a more elastic substance than that made without emulsifiers. The fatty acid component of emulsifiers can be incorporated into the array of fatty acids in triglyceride crystals and alter the polymorphic behavior of the pure triglyceride. As part of their emulsification functions, food emulsifiers also stabilize foams in aerated emulsions.

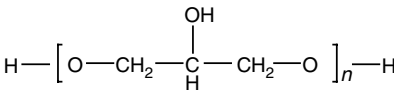
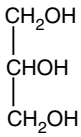
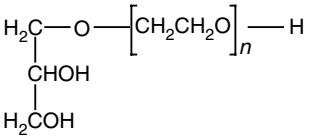
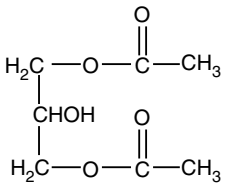
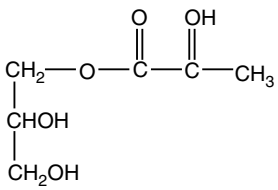
A compilation of fatty-acid-based emulsifiers is given in Table 18.2. Representative molecular structures and the principal uses of commercially important emulsifiers are also presented in that table.

TABLE 18.2
The Structure of the Hydrophilic Groups of Food Emulsifiers (Esterified with One or More Fatty Acids)

	Hydrophilic Group	Emulsifier	Use
Lactic acid		Stearoyl lactate	Dough conditioning
Propylene glycol		Propylene glycol monoesters; PGMs	Cake mixes
Lecithin	 R=  Lecithin  Phosphatidyl ethanloamine  Phosphatidyl inositol	"Lecithin," a mixture of phosphatides, principally lecithin, but containing phosphatidyl ethanolamine, and phosphaditYl inositol	Chocolate confectionery coatings
Sorbitan		Sorbitan esters	Confectionery coatings, fat-crystallization modifier
Ethoxylated sorbitan	 	Ethoxylated sorbitan esters; polysorbates	Dough conditioning, cakes, and icings
Sucrose		Sucrose mono-, di-, and triesters; sucroesters	Dough conditioning; emulsification

Continued

TABLE 18.2 (Continued)

	Hydrophilic Group	Emulsifier	Use
Polyglycerol		Polyglycerol esters	Whipping agents, icings, emulsification
Glycerol		Mono- and diglycerides; GMO	Shortening, icing, bread dough, margarine
Ethoxylated glycerol		Ethoxylated monoglycerides	Dough crumb softening
Acetylated glycerol		Acetylated monoglycerides	Food coatings
Lactylated glycerol		Lactylated monoglycerides	Shortening, cake mixes

Although emulsifiers account for only a small percentage of the total use of fatty acid derivatives in foods, their importance is more than that reflected by this small amount. A large number of processed foods include fatty-acid-based emulsifiers as components that are necessary to the texture and stability of the food. In addition, the emulsifiers that occur naturally in foods, such as the phospholipids in milk products and in eggs, give these foods many of the desirable characteristics that are associated with them.

B. Fatty Acid Derivatives Providing Metabolic Benefits

a. Plant Sterols

The current understanding of the relationship of elevated blood cholesterol levels to the risk of the development of atherosclerosis has resulted in increased emphasis on the development of food products that reduce blood cholesterol concentration. The commercialization of plant sterols in foods is an example of this focus.

It has been known for many years that ingested plant sterols can reduce blood cholesterol concentration. Cytellin®, a suspension of plant sterols, was marketed as a cholesterol-reducing pharmaceutical in the 1950s–1980s. This early use of plant sterols was in a prescription drug, in contrast with currently marketed foods discussed here.

Plant sterols are very similar in chemical structure to cholesterol. They are found in vegetable oils, but not in animal fats. Presumably because of their structural similarity to cholesterol, they compete with cholesterol for micellar solubilization in mixed bile salt micelles in the lumen of the small intestine. If ingested in sufficient quantity, this competition reduces the absorption of cholesterol from the intestine. This reduction in the absorption of dietary and biliary cholesterol can result in reduced levels of circulating LDL cholesterol.

Another very important characteristic of plant sterols is the well-known fact that they are sparingly absorbed from the intestine. In spite of their similarity to cholesterol, their absorption of 0%–5% is markedly less than that of cholesterol, which is generally absorbed in the range of 40%–60% of that ingested. An explanation for this phenomenon has recently emerged with the identification of intestinal transport proteins, ABCG5 and ABCG8, which normally exclude plant sterols from systemic absorption (Hubacek et al., 2001).

A practical problem that has limited the commercialization of plant sterols in drugs and foods is their relative insolubility in edible oils. Cytellin® was a suspension (not a solution) of crystalline plant sterols that was unappealing to many patients. Although plant sterols are consumed in vegetable oils as natural constituents of the oils, their solubility is limited to 1%–2% of the oil. In addition, the presence of water in an oil solution of plant sterols results in precipitation of the crystalline sterols as hydrates that are less soluble than the anhydrous sterols (Jandacek et al., 1977).

A solution to this solubility problem was disclosed by Erickson (1973). He found that plant sterols esterified with oleic acid were markedly more soluble than the free sterols, and that the presence of water did not cause precipitation of the sterol esters. The absence of the free hydroxyl reduces the interaction of at the oil–water interface (Figure 18.4).

Mattson found that plant sterol esters reduced dietary cholesterol absorption in the manner of the free sterols (Mattson et al., 1982). The observation that dietary plant sterols are efficacious in preventing cholesterol absorption is consistent with their hydrolysis to free sterol and fatty acid

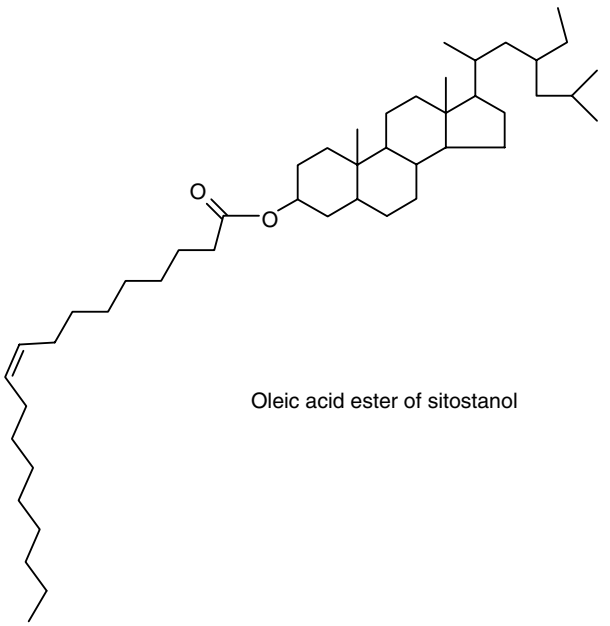


FIGURE 18.4 Sitostanyl oleate, a plant sterol ester, used in margarine and other foods.

in the intestinal lumen, presumably by cholesterol ester hydrolase. The free sterol product is then available for competition with cholesterol in micelles that are necessary for transport and absorption of cholesterol. Consumption of 3.6 g/day of plant sterol esters reduced LDL cholesterol by 9.7% without changing HDL cholesterol relative to a control diet (Judd et al., 2002).

The modification of plant sterols by low-melting fatty acids allows them to be dissolved in edible oils. Vegetable oils with dissolved plant sterol esters are now used in the production of spreads with normal taste and texture. These spreads provide a palatable means for the ingestion of plant sterols by consumers with little or no change in their normal dietary lifestyle.

b. Conjugated Linoleic Acid

Although conjugated linoleic acid (CLA) is a minor component of fatty acids sold and consumed in foods, it has been the subject of numerous studies because of its potentially impactful metabolic properties. CLA is a mixture of octadecadienoic fatty acids, with double bonds at carbons 9 and 11 or at carbons 10 and 12. CLA includes the *cis*,*cis*; *cis*,*trans*; *trans*,*cis*; and *trans*,*trans* isomers of these fatty acids. Biological studies have focused not only on the mixtures of CLA isomers but also on specific isomers. CLA is currently marketed as a free fatty acid provided in gelatin capsules.

Early studies of CLA indicated a protective effect against tumorigenesis in experimental animals (Ha et al., 1987). This observation stimulated numerous investigations into the properties of CLA, and it was found that mice eating CLA gained markedly less weight and fat than control animals that did not eat CLA (Park et al., 1997). On the basis of subsequent studies, it appears that the effect of CLA on weight and fat may be species dependent (Park et al., 2005). Other studies continue to address the differences in the metabolic effects of the CLA isomers (Macarulla et al., 2005; Valeille et al., 2005).

Trials of CLA that address its effects on body weight have generated mixed results (Gaullier et al., 2005; Larsen et al., 2006). Tricon et al. (2005) have reviewed studies of CLA and its specific isomers in humans.

c. Fatty Acids from Single Cell Organisms

Recognition that long-chain polyunsaturated fatty acids provide metabolic benefits has given rise to the production of specific fatty acids by single cell organisms. The principal focus of this production has been on arachidonic acid (ARA, 20:4, ω-6), and DHA (22:6, ω-3). These fatty acids are known to be necessary for the healthy development of infants, and infant formulas now include them. Single cell organisms, such as the dinoflagellates, can produce lipids highly enriched in these fatty acids in a controlled setting that minimizes impurities (Kyle, 1997; Kyle, et al., 1995). These sources also provide long-chain polyunsaturated fatty acids for dietary supplements for purposes other than supplementation of infant formula.

VII. OVERVIEW

This review of the commercial applications of fatty acids in foods illustrates the many roles of fatty acid-based compounds in our diets. Fats provide calories, taste, texture, and both good and bad metabolic effects. Their production is absolutely essential to providing energy and health to a world population that continues to grow. New understanding continues to generate changes in production, processing, and seed compositions. It is clear that these processes will continue to evolve with the sciences of agriculture, health, and nutrition.

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19 Effects of Processing and Storage on Fatty Acids in Edible Oils

Vickie Tatum and Ching Kuang Chow

CONTENTS

I. INTRODUCTION

The goal of commercial oil or fat processing is to obtain the maximum possible yield of oil or fat from the oil-bearing material while maintaining high quality and nutritive value eliminating undesirable impurities, and retaining desirable components (e.g., tocopherols) that may be present (Carr, 1989). The finished product normally consists of 98%–99% triacylglycerols and is odorless, bland, and oxidatively stable (Carr, 1989; Jung et al., 1989).

Crude oils contain naturally occurring compounds such as gums, phospholipids, pigments, color bodies, tocopherols, and free fatty acids (Brekke, 1980a; Pryde, 1980). Crude oils also contain compounds and contaminants formed or introduced during processing such as soaps, hydroperoxides and their breakdown products, hydrogenation catalysts, bleaching clays, moisture, and trace metals (List and Erickson, 1980). Many of these compounds are responsible for the development of undesirable odors, flavors, and colors in the oil; therefore, most steps of processing are carried out to remove these unwanted contaminants.

Some steps of processing are carried out to modify the physical characteristics of the oil or fat for specific applications. Hydrogenation, for example, raises the melting point of fats and oils and affects their plasticity, spreadability, and hardness (Grothues, 1985). This allows oils that are normally liquid to be utilized in solid products such as margarine (Latondress, 1985). However, processing may also induce or promote undesirable changes in the oil. Fats and oils contain essential nutrients such as tocopherols and linoleic acid. Some processing steps reduce the nutritive value of the oil or fat by removing or chemically altering these components. For example, although the naturally occurring *cis*-form of linoleic acid is an essential fatty acid, its *trans*-isomers, which may be formed during processing, have no essential quality and may in fact be harmful (Grothues, 1985; Chow, 1989). In addition, tocopherols are lost at several stages of processing most notably deodorization (Jung et al., 1989).

The most frequently occurring cause of oil quality deterioration during processing and storage is autoxidation (Patterson, 1989). The breakdown products of the autoxidation of unsaturated fatty acids are the major source of off-flavors and odors in the oil (Patterson, 1989). The second most frequent cause of deterioration of fats and oils is hydrolysis, which occurs when a triglyceride reacts with water and the fatty acids are split off from the glyceride backbone (Patterson, 1989). Hydrolysis reduces the final yield of finished product as well as increasing the susceptibility to oxidation.

II. EFFECTS OF PROCESSING ON FATTY ACIDS

Refined oils are usually bland in flavor, more stable to oxidation, and can be stored for extended time period. Fatty acids are affected during refining process in a variety of ways, both direct and indirect. Refining, for example, directly affects the fatty acid content of fats and oils by removing phospholipids, diglycerides and monoglycerides, and free fatty acids that may be present. Hydrogenation, a method of reducing the degree of fatty acid unsaturation, is the primary processing step in which the fatty acid composition of oil and fat is intentionally directly altered. Fatty acid composition may also be affected by processing in an indirect manner. Numerous impurities present in crude oil can act to initiate and/or enhance autoxidation and photooxidation of unsaturated fatty acids or cause hydrolysis of triglycerides. An important goal of oil processing is to remove these impurities, thus preventing undesirable changes in fatty acid content or character.

Table 19.1 lists the major steps involved in the processing of edible oil and the undesirable changes that may be caused by each step. The various stages of processing are briefly described in the following section, with particular emphasis on the ways in which they affect fatty acids.

TABLE 19.1
Steps in Processing of Edible Oils and Fats

Processing Step	Purpose	Side Effects
Seed selection; cleaning	Select nature undamaged seeds; remove dirt, rocks, wood, and scrap iron	—[a]
Drying	Remove moisture	Autoxidation
Dehulling	Remove outer seed coats to facilitate processing	Hydrolysis, autoxidation
Cooking	Inactivate lipoxidase and hydrolytic enzymes, kill molds and bacteria	Autoxidation
Extraction	Separation oil from the oil-bearing material	—[a]
Miscella stripping	Separate oil from solvent and fines	Autoxidation
Degumming	Remove phosphatides and iron	Hydrolysis, isomerization
Caustic refining	Remove free fatty acids, phosphatides, and unsaponifiable material	Autoxidation
Bleaching	Remove pigments, metals, soaps, and oxidation products	Hydrolysis, isomerization, autoxidation
Physical refining	Remove free fatty acids, aldehydes, ketones, and other volatile oxidation products	Hydrolysis, loss of tocopherols
Hydrogenation	Modify physical characteristics and improve oxidative stability	Isomerization, loss of essential fatty acids
Deodorization	Remove free fatty acids, aldehydes, ketones, and other volatile oxidation products	Hydrolysis, loss of tocopherols

[a] Little or no effect.

A. Oilseed Processing

a. Seed Selection and Cleaning

Seeds that are sprouted, immature, or damaged physically or by heat are usually not satisfactory for oil production because oxidative and hydrolytic processes have probably already begun to damage the oil (Becker, 1978; Adhikari et al., 1992). After seeds have been selected for processing, they must be cleaned of dust, chaff, rocks, scrap iron, wood, and so on (Becker, 1978; Carr, 1989).

b. Drying and Storage of Raw Materials

Because moisture promotes deterioration of oils, it is important to reduce oilseed moisture content before storage and keep dry during storage (Becker, 1978; Carr, 1989). Otherwise, both oxidative and hydrolytic damage to the old may occur. Pretreatment of oil seeds affects oxidative stability. For example, crude oil obtained from roasted plus microwaved, or roasted plus steamed sesame seeds has higher oxidative stability than from steamed or roasted seeds (Abou-Gharbia et al., 2000). Moreover, different processing treatments show considerable effects on lipid fractions, and the influence on all components after roasting plus steaming is more pronounced than microwave treatment.

c. Dehulling and Grinding, Rolling, or Flaking

The outer seed coats of some seeds may have to be removed, because they interfere with the next processing step, which is to grind, roll, or flake the seeds or beans in order to rupture oil cells and increase surface area (Carr, 1989). Once this has been done, the oil should be extracted as soon as possible, because the increase in surface area also increases susceptibility to oxidation and hydrolysis of the oil (Becker, 1978).

d. Cooking

Lipoxygenases and lipases are released when the cells are ruptured during grinding, rolling, or flaking. Heating inactivates these enzymes and prevents any enzyme-catalyzed oxidation or hydrolysis (Engeseth et al., 1987; Carr, 1989). Engeseth et al. (1987) found that steaming under pressure was most effective for inactivating lipoxygenases in soybeans. Heating also acts to destroy molds and bacteria (Carr, 1989) that attack fatty acids and yield a wide variety of oxidized products (Patterson, 1989). However, the heating must be carefully controlled because excessive heat can promote autoxidation.

e. Extraction

Expellers or screw presses are used for mechanical extraction of oil from oil-bearing materials. Production of some oils, such as virgin olive oil, consists solely of mechanical extraction followed by centrifuge separation (Di Giovacchino et al., 1994). Oil products in this way are often of high quality, because very little heating is required (Carr, 1989). For some seeds, mechanical extraction is followed by extraction with solvents, most commonly hexane. The use of other solvents, such as methanol or isopropanol, has been explored, but has not been widely adopted (Hoffmann, 1989; Proctor and Bowen, 1996; Thobani and Diosady, 1997).

When carbon dioxide (CO_2) is heated under pressure, it reaches a physical state known as the supercritical fluid state, in which it has properties of both a liquid and a gas. Supercritical CO_2 can be used to dissolve, suspend, and remove undesirable impurities from edible oils and fats. Supercritical CO_2 offers several advantages over traditional solvent (most commonly hexane) and steam processing techniques, including the fact that removal of the extraction "solvent" is easy and that the extraction capacity of supercritical CO_2 can be "fine tuned" by adjusting the pressure and/or temperature of the system or by adding modifying solvents, such as pentane or ethanol, to the supercritical fluid. In addition, CO_2 is nonflammable, nontoxic, low cost, and easily available. Additionally, its critical temperature and pressure are low (31°C, 7.38 MPa), and its solvent capacity for numerous compounds is appreciable at pressures of 10–40 MPa (Fattori et al., 1988).

The usefulness of supercritical CO_2 for the extraction or refining of cereal, seed, or vegetable oils and animal fats has been examined (Ziegler and Liaw, 1993; Merkle and Larick, 1995; Young and Chao, 1995; Garcia et al., 1996; Ooi et al., 1996; Zhiping et al., 1996; Dunford and Temelli, 1997). Maheshwari et al. (1992) measured the solubility of various fatty acids in supercritical CO_2 over a range of temperatures and pressures, and concluded that free fatty acids could effectively be separated from vegetable oil by supercritical CO_2. Similarly, Ziegler and Liaw (1993) reported that supercritical CO_2 extraction removed both odorous volatiles and residual free fatty acids from peanut oil. The total amount of oil recovered from canola seeds by CO_2 extraction was found to be strongly dependent on the pretreatment, and the amount of oil extractable from flaked and cooked seeds was comparable to that recoverable by conventional hexane extraction (Fattori et al., 1988).

The ability to modify the extraction characteristics of supercritical CO_2 through changes in system temperature and pressure has also been explored. Merkle and Larick (1995), for example, extracted beef fat under various processing conditions in an effort to alter the fatty acid composition of various lipid fractions. They reported that beef lipid fractions high in monounsaturated and polyunsaturated fatty acids could be produced and suggested commercial utilization of beef fat in foods might be improved through production of such specific fractions. Similarly, Eller and King (2001) investigated the supercritical CO_2 extraction of fat from ground beef and the effects of several factors on the gravimetric determination of fat. Oven-drying ground beef samples prior to extraction inhibited the subsequent extraction of fat, whereas oven-drying the extract after collection decreased the subsequent gas chromatographic fatty acid methyl ester fat determination. None of the drying agents tested was able to completely prevent the coextraction of water, and silica gel and molecular sieves inhibited the complete extraction of fat. The findings suggest that supercritical CO_2 can be used to accurately determine fat gravimetrically for ground beef.

Palm oil processed by supercritical CO_2 extraction alone retains a similar triglyceride composition with reduced levels of free fatty acids, monoglycerides and diglycerides (Ooi et al., 1996). Addition of ethanol to the CO_2 produces oils with 0.04% free fatty acids, trace amounts of monoglycerides, and much reduced diglycerides. A higher processing temperature results in a very different product, one with lower C_{48} and C_{50} triglycerides, much reduced diglycerides, and higher carotene content. Similarly, Dauksas et al. (2002) have shown that ethanol (1%) increased the yield of liquid CO_2 extraction of *Nigella damascena* seeds by 50%, and increased the yield two times at 150 bars. Linoleic acid was the major fatty acid (43.71%–50.83%), followed by oleic (14.87%–23.65%), stearic (15.07%–23.24%), and palmitic (10.13%–12.07%) acids. Elemenes (21.38%–29.16%) were the most abundant volatile constituents, free fatty acids constituted from 35.04% to 51.18%, the majority being linoleic (32.83–40.58) and oleic acids (4.96–13.32).

As supercritical CO_2 extraction procedure has the capacity to modify extraction selectivity by relatively minor changes system conditions, the extraction procedure offers the edible oil and fat processor unique opportunities to control the final composition of their products. However, this same quality poses additional challenges to those processors, and operating conditions must be optimized according to the main objective of the refining process as well as according to the nature of the starting product (Maheshwari et al., 1992). Dunford and King (2000) evaluated supercritical CO_2 fractionation technique as an alternative process for reducing the free fatty acid content and minimizing the phytosterol loss of rice bran oil during the process, and examined the effects of pressure (20.5–32.0 MPa) and temperature (45°C–80°C) for isothermal operation of the column on the composition of the resultant fractions. They found that low-pressure and high-temperature conditions were favorable for minimizing triglycerides and phytosterol losses during the free fatty acid removal from crude rice bran oil. Rice bran oil fractions with <1% free fatty acid, about 95% triglycerides, and 0.35% free sterol with 1.8% oryzanol content could be obtained utilizing the described supercritical CO_2 fractionation technique.

High-intensity ultrasound may facilitate oil extraction by reducing the time required. Li et al. (2004) evaluated the application of 20 kHz high-intensity ultrasound during extraction of oil from two varieties of soybeans using hexane, isopropanol, and a 3:2 hexane–isopropanol mixture. In a simplified extraction procedure, ground soybeans were added to solvents and ultrasonicated between 0 and 3 h at ultrasonic intensity levels ranging from 16.4 to 47.6 W/cm^2. Using hexane as a solvent, yield generally increased as both application time and intensity of ultrasound increased. Solvent type influenced the efficiency of the extraction, that is, the highest yield was obtained using ultrasound in combination with the mixed solvent. Gas chromatography analysis of ultrasonicated soybean oil did not show significant changes in fatty acid composition.

f. Miscella Stripping

Miscella is the material obtained by solvent extraction. It is a mixture of crude oil, solvent, sediments, and moisture (List and Erickson, 1980). The crude oil must then be separated from the other components of the miscella. Sediments are readily separated from the miscella by filtration. Hexane and moisture are removed by evaporation followed by flashing and low-pressure steam stripping to give 98% crude oil containing less than 0.15 wt.% moisture and volatile content (Becker, 1978; Mustakas, 1980). During miscella stripping it is important to use the lowest temperature possible, because high temperatures can promote oxidation, remove antioxidants, and cause color fixation (List and Erickson, 1980).

g. Degumming

Solvent-extracted oils contain up to 3% gums, which are mostly phosphatides. Phosphatides act as emulsifiers in the oil during refining and can reduce yields of purified oil (Carr, 1989). The presence of phosphatides has also been associated with the development of an off-flavor after deodorization (Ohlson and Svensson, 1976).

TABLE 19.2
Effects of Processing Steps on Some Minor Components of Soybean Oil[a]

Oil Sample	Phosphorus (ppm)	FFA (%)	Chlorophyll (ppm)	PV (mEq/kg)	Tocopherol (ppm)
Crude	510.0	0.74	0.30	2.4	1.67
Degummed	120.0	0.36	na	10.5	1.58
Refined	5.0	0.02	0.23	8.8	1.55
Bleached	1.4	0.03	0.08	16.5	1.47
Deodorized	1.0	0.02	nd	nd	1.14

[a] FFA, free fatty acids; PV, peroxide value; na, not available; nd, not detected.

Source: Adapted from Jung, M.Y., et al. (1989). *J. Am. Oil Chem. Soc. 66*: 118–120.

The principal reason for degumming crude oil is to remove phosphatides (Table 19.2). During degumming, the oil is mixed with water or steam to hydrate the gums, which then precipitate out and are separated from the oil by centrifugation. Because the oil is heated during this process, it is recommended that air be excluded to prevent oxidation (Carr, 1989). In addition, the amount of water used should be the minimum required for effective degumming, because excess water will promote hydrolysis (Carr, 1978).

Evidence available suggests that the removal of iron is closely related to phosphatide removal (Jung et al., 1989), and, in fact, the iron may be present in the form of an iron–phosphatide complex (Dijkstra and Van Opstal, 1989). As even a trace amount of iron is capable of catalyzing fatty acid oxidation, its removal from the oil is important. It has been suggested that the development of off-flavors attributed to phosphatides is actually due to the presence of iron (Dijkstr and Van Opstal, 1989).

In most cases, crude oil is pretreated with phosphoric acid before being degummed (Carr, 1978, 1989; List et al., 1978a; Dijkstra and Van Opstal, 1989). This pretreatment is helpful in reducing the iron content; it apparently acts by converting the iron into a form that is more easily removed during processing (Carr, 1978; List et al., 1978a; Brekke et al., 1980; Dijkstra and Van Opstal, 1989).

The reported effectiveness of phosphatide removal from soybean oil is quite variable, ranging from 75% to 98% (Carr, 1978; List et al., 1978a, 1978b; Brekke, 1980b; Jung et al., 1989). Removal of iron from soybean oil varies with the producer as well as with the method chosen for degumming. Water degumming alone led to the removal of 14%–65% of the iron during processing (List et al., 1978a, 1978b), whereas use of phosphoric acid pretreatment increased the removal to 93% from finished oil (List et al., 1978b).

h. Caustic Refining

Crude or degummed oil is treated with caustic soda to saponify impurities. The mixture is heated to break the emulsion, and then the soapstock is removed through centrifugation (Carr, 1976, 1978, 1989; Cowan, 1976). This refined oil is washed with warm water to remove the last traces of soap, separated again by centrifugation, and then dried under vacuum (Carr, 1989). The primary actions of the caustic are to react with free fatty acids to form soapstock, hydrolyze phosphatides, and remove unsaponifiable matter (Carr, 1976, 1978).

Jung et al. (1989) found that degumming and caustic refining were the most important steps of processing for removing free fatty acids from soybean oil. In their study, the free fatty acid content of 0.74% in crude oil was reduced to 0.36% by degumming and to 0.02% by caustic refining (Table 19.2).

Because refining is carried out at high temperatures, the susceptibility to oxidation is increased. For this reason, hermetic centrifuges are used to exclude air during refining, and refined oil is cooled before being placed into storage tanks (Carr, 1989).

i. Bleaching

Degummed or refined oil is heated and mixed with natural or acid-activated bleaching clay to remove various impurities such as chlorophyll, carotenoids, phospholipids, metals, soaps, and oxidation products (Cowan, 1976; Richardson, 1978; Boki et al., 1989; Taylor et al., 1989). Bleaching is generally carried out under vacuum to minimize possible oxidation (Carr, 1989). The effects of bleaching on several components of soybean oil are illustrated in Table 19.2.

Generally, the goal of bleaching is to reduce the color pigments in order to produce an oil of acceptable color. However, there is another reason to remove some of these pigments. Kirisakis and Dugan (1985) showed that chlorophyll in olive oil acts as a photosensitizer, leading to rapid oxidation of the oil.

Acid-activated clay is usually used because its bleaching power is greater than that of natural clay (Richardson, 1978; Morgan et al., 1985; Boki et al., 1989; Taylor et al., 1989). The mechanism of action is believed to depend on chemical adsorption along with chemical reaction between the acid of the clays and the compounds being affected (Sarler and Guler, 1988; Boki et al., 1989). However, although the acidity of the clay contributes to its activity, it may also lead to two detrimental side reactions: free fatty acid formation and isomerization of the triglyceride alkyl chain (Morgan et al., 1985). These side reactions can be avoided by carefully controlling the temperature of the oil during bleaching (Richardson, 1978; Morgan et al., 1985). The processor must find a compromise temperature high enough to effectively bleach the oil but at the same time minimize the increase in free fatty acids (Richardson, 1978).

The effect of bleaching on peroxide value is variable and depends on the type and amount of clay used (Richardson, 1978). Jung et al. (1989) reported an increase in peroxide value after bleaching with acid-activated clay (see Table 19.2), but others (Brekke, 1980c; Boki et al., 1989) have reported decrease in peroxide value.

j. Physical Refining

Some oils, particularly those with high-free fatty acid and low-phospholipid content, may be refined via physical refining rather than caustic refining (Carr, 1978). In this case, crude oil is degummed and bleached, deaerated, then steam refined for about 1 h at 260°C under a vacuum of 1 mm Hg or less (Carr, 1978, 1989; List et al., 1978c). Exclusion of oxygen is extremely important, because of the high temperatures involved, so both the oil and the steam should be fully deaerated and the vessel used must be completely airtight (Mounts and Khym, 1980). The steam acts to volatilize most of the free fatty acids (Carr, 1978, 1989). This process also removes aldehydes, ketones, and other volatile compounds, which are formed through oxidation, and destroys peroxides and carotenoid pigments (Carr, 1989). Vacuum steam distillation reduces the peroxide value of soybean oil to below measurable levels (Jung et al., 1989; see Table 19.2). However, this process also removes some of the beneficial tocopherols (Cowan, 1976; Jung et al., 1989), so antioxidants may have to be added to the oil after physical refining is completed (Gavin, 1978). In order for physical refining to produce quality oil, the oil must be thoroughly acid-degummed and bleached to remove phosphatides and prooxidant metals such as iron and copper (Carr, 1978; List et al., 1978a, 1978b). Nitrogen has been proposed as an alternative to steam for physical refining. Laboratory studies suggest that nitrogen removes free fatty acids with greater efficiency than steam, while reducing losses of hydrocarbons, sterols, and triglycerides (Ruiz-Mendez et al., 1996).

k. Hydrogenation

The fluidity and susceptibility to autoxidation of oils and fats is dependent on the degree of unsaturation of the fatty acids found in the triglycerides. During hydrogenation, unsaturation is reduced by direct addition of hydrogen to the double bonds of the fatty acid chains of the triglycerides.

TABLE 19.3
Effect of Hydrogenation Catalyst on Fatty Acid Composition of Soybean Oils (wt.%)

Fatty Acid	Unhydrogenated	Nickel-Catalyzed Hydrogenation	Copper Chromate-Catalyzed Hydrogenation
Palmitic	10.2	10.5	10.4
Stearic	3.4	4.2	3.7
Oleic	22.3	47.6	41.3
Linoleic	55.5	34.4	44.2
Linoleic	8.6	3.3	0.4

Source: Adapted from Mounts, T.L., et al. (1978a). *J. Am. Oil Chem. Soc. 55*: 345–349.

The goals of hydrogenation are to increase the melting point, solidity, and oxidative stability of the oil (Allen, 1978; Grothues, 1985; Carr, 1989).

During hydrogenation, the oil is mixed with gaseous hydrogen and a metal catalyst (most commonly nickel) at carefully controlled temperatures, pressures, and agitation rates (Allen, 1978; Grothues, 1985). Generally, mono-, di-, and triunsaturated fatty acids are hydrogenated in stepwise fashion to form the respective saturated, monounsaturated, and diunsaturated fatty acids (Mounts, 1980). As can be expected, hydrogenation has the most profound effect on the fatty acid composition of oil. The effect on the fatty acid content is illustrated in Table 19.3 using soybean oil as an example. In addition, some double bonds may be shifted, and some may be changed from the *cis*- to the *trans*-form (Allen, 1978, 1986; Mounts, 1980). All of these reactions occur during hydrogenation; however, the relative rates can be affected by controlling the reaction conditions and type and concentration of catalyst (Mounts, 1980).

The ideal catalyst selected for hydrogenation is very active and selective, and it hydrogenates rapidly and reduces linoleic acid to oleic acid with little concurrent reduction of oleic to stearic (Grothues, 1985; Allen, 1986). The use of a very selective catalyst produces oils that are oxidatively stable but not overly hard (Allen, 1978, 1986). Nickel catalysts are most commonly used for hydrogenation, and are extremely versatile. By varying the reaction conditions, the selectivity can be controlled and the hydrogenation reaction can be stopped at any chosen end point (Coenen, 1976). However, the use of nickel has several limitations, including the lack of selectivity for linolenic acid over linoleic acid (Coenen, 1976; Allen, 1978). Therefore, in order to eliminate all the linolenic acid when nickel is used, a considerable amount of linoleic acid must be reduced (Coenen, 1976). A second limitation of nickel is that selectivity and isomerization are coupled; high selectivity is always accompanied by high formation of *trans*-isomer (Coenen, 1976).

Copper catalysts are superior to nickel in some respects; they have a much higher preference for linolenic acid (Coenen, 1976; Mounts et al., 1978b) and can produce an oil with a lower melting point than nickel-catalyzed oils that have the same peroxide value (Coenen, 1976). However, copper catalysts are much less active than nickel and can initiate autoxidation. Therefore, the oil requires special treatment to eliminate all traces of copper after hydrogenation is complete because of copper's prooxidant capabilities (Mounts et al., 1978b).

In addition to catalysts type, other processing conditions can affect the selectivity of the hydrogenation reaction as well as the degree of *trans*-fatty acid formulation and reaction rate. Increases in temperature and catalyst concentration, for example, led to corresponding increases in selectivity, *trans* acid formulation, and reaction rate. Increases in pressure and agitation also increase the reaction rate but decrease selectivity and *trans* acid formation (Allen, 1978).

L. Deodorization

The last step in the processing of edible oils, deodorization, is used to remove undesirable substances that occur naturally or are generated during processing (Gavin, 1978; Brekke, 1980d). The process is essentially the same as that used for physical refining (see above). Table 19.2 shows the effects of deodorization on some components of soybean oil.

B. Animal Fat Processing

Meat fats are generally uniform raw materials. This, along with their high degree of saturation, makes the processing of meat fats much easier than that of vegetable oils (Latondress, 1985). The actual processing steps are similar to those used for vegetable oils, but the extent of processing required is usually less.

a. Rendering

Two types of rendering are used: edible and inedible. Only edible rendering produces oils suitable for human consumption. In a typical edible rendering system, fat trimmings are ground and placed in a tank, where they are melted and then pumped into a disintegerator (Prokop, 1985). The disintegrator acts to rupture the fat cells completely and facilitates the separation of protein solids from melted fat. A polished edible fat is separated from solids and moisture via a two-state centrifugation system. Maximum fat quality is achieved by keeping heat exposure to a minimum: generally no more than 30 min at 200°F or less (Prokop, 1985).

b. Caustic Refining

Generally, meat fats are not caustic refined. The only demand for caustic-refined meat fat is for use in French frying potatoes. This product is produced by caustic refining, double water washing, and vacuum drying. It is not bleached or deodorized, contains about 0.5% free fatty acids, and has a fresh tallow flavor (Latondress, 1985).

c. Bleaching

Prior to physical refining, proteinaceous material must be removed from the fat. This can be accomplished by mixing diatomaceous earth or bleaching clay with the fat and then filtering. Bleaching to remove pigments and other impurities is standard procedure for vegetable oils but is rarely done for meat fats (Latondress, 1985).

d. Physical Refining

Once the proteinaceous matter has been removed by mixing with bleaching clay, the primary impurities remaining in the fat are free fatty acids (Latondress, 1985). These can be effectively removed by physical refining, and this is the usual refining method used by processors of meat fats.

e. Hydrogenation

Vegetable oils are hydrogenated to remove their oxidative stability and change their physical characteristics. Meat fats are hydrogenated primarily to change their physical characteristics; since they are already very high in saturated and monounsaturated fatty acids, they do not have the same oxidative stability problems as vegetable oils. In the hydrogenation of meat fats, it is very important to use conditions that lead to the highest selectivity possible. If selectivity is less than perfect, the monounsaturated acids will be converted to saturated acids in large quantities (Latondress, 1985).

Rumenic acid (*cis*-9, *trans*-11 C18:2) accounts for over 80% of total conjugated linoleic acid (CLA) in processed cheeses, and the second main CLA isomers are *trans*-11, *cis*-13 and *trans*-11,

trans-13. Luna et al. (2005) assessed the level and type of CLA isomers in two commercial processed cheeses (portions and slices) and monitor their evolution during the different manufacturing stages. They found that the processing parameters had negligible effects on the CLA content of processed cheese and did not modify the isomer profile in these dairy products, thereby confirming the stability of rumenic acid during manufacturing.

f. Oil Modifications

Fats and oil modification is one of the prime areas in food processing industry and tailored vegetable oils with nutritionally important structured triacylglycerols and altered physicochemical properties have a big potential in the future market. Gupta et al. (2003) have suggested that lipases, especially microbial lipases that are regiospecific and fatty acid specific, can be used for retailoring of vegetable oils and can be carried out at moderate reaction conditions. For example, bulk available cheap oils can be upgraded to synthesize nutritionally important structured triacylglycerols such as cocoa butter substitutes, low-calorie triacylglycerols, polyunsaturated fatty acid-enriched, and oleic acid-enriched oils. It is also possible to change the physical properties of natural oils to convert them into margarines and hard butter with higher melting points or into special low-calorie spreads with short- or medium-chain fatty acids. Once the technologies are established, the demand of lipases in oil industry may increase tremendously in the near future for specific modifications of fats and oils to meet the changing consumers' dietary requirements.

C. Fish Oil Processing

Marine oils were approved for use in the United States as food ingredients only recently (Bimbo, 1987; Dziezak, 1989). Federal approval for the use of partially hydrogenated menhaden oil in food products was granted in 1987. However, the consumption of fish oils as dietary supplements in increasing because of the reported health benefits (Bimbo, 1987; Luley et al., 1988; Kinsella et al., 1990). The processing of marine oils is very similar to the processing of oils originating from oilseeds.

Fish are generally processed using the wet reduction method where they are steam cooked and then pressed to remove the oil and water (Bimbo, 1987). The oil–water mixture is centrifuged to separate the oil from the water, and the oil is refined to remove undesirable substances such as moisture, free fatty acids, trace metals, oxidation products, sulfur, halogen and nitrogen compounds, pigments, sterols, and organic contaminants from the environment (Addison et al., 1978; Bimbo, 1987). Marine oils used for human food products are usually partially hydrogenated, although some oils high in n-3 fatty acids are bottled or put capsules without modification for use as food supplements (Bimbo, 1987).

III. EFFECTS OF STORAGE ON FATTY ACIDS

Degradation of quality during storage is usually a result of moisture, temperature, metal soaps, oxygen, long storage times, and equipment condition or design (Carr, 1978). Moisture can cause hydrolysis of the fat and can also lead to degumming of crude oils within the storage tank (Burkhalter, 1976; Carr, 1978).

The temperature of stored oil should be kept as low as possible, because high temperatures not only increase the oxidation rate but also increase the solubility of water in oil, resulting in an increased rate of hydrolysis (Burkhalter, 1976; Carr, 1978; Woerfel, 1985). If tanks have heating coils, they should also be equipped with a mechanism for agitation, because local overheating near the coil may induce oxidation reactions (Erickson, 1978; Berger, 1985).

The rate and degree of oxidation is also enhanced by contact with air. For this reason storage tanks should be filled from the bottom, and any head space should be filled with inert gas

(Burkhalter, 1976; Carr, 1978). It is important to use proper agitation speeds and refrain from clearing lines by blowing air through them (Erickson, 1978).

Metallic contamination, especially by copper, iron, and brass, is very effective at inducing autoxidation (Burkhalter, 1978; List and Erickson, 1980; Woerfel, 1985; Eboh et al., 2006), so contact with these metals should be avoided. Berger (1985) reported examples of oil deterioration caused by rusty storage tanks, heating coils made from an aluminum-brass alloy, and the use of brass thermometers and sampling instruments. If stored long enough, any fat or oil is subject to deterioration even under ideal conditions (Erickson, 1978). A small quantity of old oil residue in the storage container can speed up the deterioration of fresh oil (Erickson, 1978; Berger, 1985; Woerfel, 1985).

Treatment of almond oil with accelerated electrons (electron beam processing) at the dose of 3 kGy during a storage period of 5 months has no effects on linolenic acid content, whereas, at 7 and 10 kGy, the content in 18:3 disappears from the first moment (Sanchez-Bel et al., 2005). The quality indices of the oil decreased at all doses and remained stable during the time of storage. The peroxide value did not show changes at the doses of 3 and 7 kGy, in nonirradiated samples, but significantly increased when the maximum dose of 10 kGy was applied. These changes were reflected in the sensory analysis, in which the tasters did not find sensory differences between the controls and those irradiated at doses of 3 or 7 kGy, whereas almonds irradiated at 10 kGy exhibited a rancid flavor and a significant decrease in general quality.

The high-stability oils produced from high-oleic oils require less processing and bring additional nutritional benefits such as lower *trans* and saturated fat contents. Accurate and reproducible oxidative stability measurement of these oils is necessary to assess the performance. The accelerated oxidative stability measurement method often used in the fats and oils industry, the oxidative stability index is unreliable for higher stability oils due to poor reproducibility. Kodali (2005) developed a rapid and reproducible pressure differential scanning calorimetry (PDSC) method, to measure the oxidative induction time of oils under high temperature and pressure in the presence of pure oxygen. The oxidative induction times of a number of hydrogenated oils with different unsaturation and oxidative stability are measured. Unlike oxidative stability index data, the PDSC oxidative induction time measurement is highly reproducible and precise and requires only a small sample and a couple of hours. In addition, oil stability can be predicted by high-resolution ^{13}C nuclear magnetic resonance spectroscopy when applied to chromatographically enriched oil fraction (Hidalgo et al., 2002).

IV. UNDESIRABLE CHANGES IN FATTY ACIDS THAT OCCUR DURING PROCESSING AND STORAGE

The main undesirable changes of fats/oils during processes and storage are oxidation and hydrolysis. In oil oxidation, the carbon atoms next to the double bonds in the oils are oxidized, which results in a break of the hydrocarbon chain and the formation of volatile compounds, including aldehydes, ketones, alcohols, short-chain fatty acids, and other compounds that may introduce an off-odor to the oil. In oil hydrolysis, the fatty acids are hydrolyzed off the triacylglycerides at high temperatures in the presence of water, yielding free fatty acids, monoglycerides, diglycerides, and glycerol. These breakdown products will speed up the degradation of the remaining triacylglycerides as they allow greater emulsification of water from the food into the oil.

A. Oxidation

a. Autoxidation

Polyunsaturated fatty acids are more susceptible to autoxidation than monounsaturated or saturated fatty acids, and it is the breakdown products of their oxidation that are the major source of off-flavors in oil (Patterson, 1989). The sequence of events that has been suggested to lead to the development of flavor and odor in oil begins with the oxidation of a fatty acid to a hydroperoxide. Oxygen is

required for this oxidation, and the process is catalyzed by the presence of metals, heat, and light. Hydroperoxides themselves are tasteless and odorless. However, continued oxidation causes these hydroperoxides to be broken down into various short-chain organic compounds such as aldehydes, ketones, alcohols, and acids, which are actually responsible for the strong odors and flavors characteristic of rancidity (Sherwin, 1978; Patterson, 1989).

There are numerous factors, including the presence of free fatty acids, lipoxygenases, metals, light, heat, and oxygen, that are capable of initiating and/or accelerating autoxidation during processing, refining, and storage. Free fatty acids, even at levels as low as 0.5% in the oil, act as prooxidants (Mistry and Min, 1987; Patterson, 1989). Higher levels of volatile compounds and peroxides are found in oils containing free fatty acids than in oils containing no free fatty acids after storage at 55°C for as little as 1 day (Mistry and Min, 1987). Interestingly, saturated fatty acid stearic acid is equally as effective at initiation of oxidation as polyunsaturated fatty acids linoleic and linolenic acids (Mistry and Min, 1987). The findings suggest that the carboxylic group of the free fatty acid is responsible for the prooxidant activity, and that it is essential to the production of good quality oil to remove the free fatty acids through refining.

Lipoxygenases are naturally present in all plant and animal tissues, and act to catalyze the oxidation of polyunsaturated fatty acids (Sherwin, 1978; Engeseth et al., 1987; Patterson, 1989). Metals in trace amounts are very effective prooxidants and are active in the breakdown of hydroperoxides (Sherwin, 1978; Patterson, 1989). Heat greatly increases the rate of oxidation, particularly in the more unsaturated oils, with a doubling in oxidation rate for every 15°C increase in temperature above 60°C (Sherwin, 1978). Light, especially ultraviolet and near-ultraviolet, can provide the energy necessary for both initiation and propagation of oxidation (Sherwin, 1978; Patterson, 1989). Finally, as oxidation cannot proceed without oxygen, any process that increases oil–air contact aeration increases the likelihood of oxidation (Sherwin, 1978; Patterson, 1989).

b. Photooxidation

As mentioned above, ultraviolet light is capable of both initiating and propagating autoxidation. However, light in the visible range is capable of initiating another, mechanically distinct type of oxidation known as photooxidation (Frankel, 1980). Unlike the process of autoxidation, which relies on a free radical mechanism for propagation, photooxidation results from the direct attack of singlet oxygen molecules on fats and oils (Patterson, 1989). Singlet oxygen is produced following the reaction of visible light with a sensitizer such as chlorophyll, riboflavin, hemoglobin, or certain dyes (Frankel, 1980; Patterson, 1989). The excited sensitizer reacts with molecular oxygen to form the excited singlet state oxygen molecule. Highly reactive singlet oxygen then reacts directly with the olefinic groups of lipids to produce lipid hydroperoxides. The breakdown of these lipid hydroperoxides generates, among other things, free radicals, which can in turn initiate autoxidation.

Photooxidation is a much more rapid process than autoxidation, and only trace amounts of sensitizers are sufficient to initiate autoxidation (Frankel, 1980). Most chain-breaking antioxidants that are typically used to prevent autoxidation in oils are ineffective against photooxidation (Patterson, 1989). Singlet oxygen quenchers such as tocopherols and carotenes do, however, inhibit it (Patterson, 1989). Fortunately, the majority of sensitizers such as chlorophyll and dyes are removed during processing. However, most of the singlet oxygen quenchers or free radical scavengers naturally present in the oil are also removed.

Exposure of oil to UV radiation induces changes on the chemical and sensory characteristics of oils. For example, Luna et al. (2006) have shown that even small doses of UV radiation-induced oxidation of the virgin olive oil samples. Total phenols and fatty acid contents decreased during the process as well as the intensity of the bitter and fruity sensory attributes, while the intensity of the rancid sensory attribute notably increased. In addition, acetaldehyde, 2-butenal, 2-pentenal, octane, octanal, hexanal, nonanal, and 2-decenal were increased during the irradiation process, and nonanal, hexanal, and pentanal showed high correlation with the rancid sensory attribute (90%, 86%, and 86%, respectively).

B. Hydrolysis

The next most frequent cause of deterioration of oils and fats during processing and storage is hydrolysis, which occurs when a triglyceride reacts with water and the fatty acids are split off from the glyceride backbone (Patterson, 1989). This has two major implications for the processor. First, the final yield of purified triglycerides is reduced. Second, the free fatty acids that are produced are much more susceptible to autoxidation than those bound to a glyceride backbone (Patterson, 1989). Hydrolysis is catalyzed by the lipolytic enzymes present in plant and animal tissues and can be enhanced by both moisture and heat (Sherwin, 1978).

C. Isomerization

Hydrogenation leads to the creation of both positional and geometrical isomers. The double bonds of unsaturated fatty acids may be shifted to other locations within the fatty acid, and *cis*-double bonds may be isomerized to the *trans*-form (Allen, 1978, 1986). In addition, processes using acids, degumming, for example, may induce *cis–trans* isomerization (Hudson, 1987). *Cis–trans* isomerization affects oil quality in two ways. Increased amounts of *trans*-isomers result in a harder oil than oil with the same degree of hydrogenation but high in *cis*-isomers (Mounts, 1980). In addition, *trans*-fatty acids lack the essential quality of the comparable *cis*-fatty acids (Grothues, 1985). The conventional heating methods of milk did not cause any significant increase in the *trans*-isomer content, with the exception of milk heated at 63°C for 30 min and milk microwaved for 5 min, which were significantly increased by 19% and 31%, respectively. The chemical changes of lipids were generally accelerated with the severity of the heat treatment and duration of storage. The CLA content of cheese heated in a microwave oven for 5 min decreased by 21%, and microwave heating for 10 min caused a decrease of 53% compared with that of freshly boiled cheese (Herzallah et al., 2005).

V. ANTIOXIDANTS AND METAL CHELATORS IN FAT AND OIL PROCESSING

Oxidation during processing and storage is the major cause of quality loss in oils and fats. Autoxidation can be minimized by controlling exposure to conditions that enhance oxidation, such as heat, metal contaminants, and air, and by adding antioxidants and chelating agents to the products. Photooxidation can be minimized by reducing exposure to light and adding appropriate antioxidants.

The oxidative stability of fats and oils is related to their triacylglycerol composition and antioxidants present (Hrncirik and Fritsche, 2005; Mateos et al., 2005) and iron content (Coscione and Artz, 2005). Vegetable oils contain natural antioxidants such as tocopherols (Latondress, 1985; Patterson, 1989), so even without added antioxidants, they exhibit some resistance to oxidation (Kincs, 1985). Del-Carlo et al. (2004) studied the contribution of the phenolic fraction to the antioxidant activity and oxidative stability of extra-virgin olive oil and found no individual compounds was identified as the main cause of the overall antioxidant activity, and the total polyphenol determination was better correlated to antioxidant activity and oxidative stability than each tested polyphenols or polyphenol groups such as o-diphenols.

The effectiveness of commonly used antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ) in vegetable oils has been studied (Erkilla et al., 1978; Kincs, 1985; Hawrysh et al., 1988). Table 19.4 shows the effectiveness of BHA/BHT and TBHQ at preventing a rise in peroxide value during storage of canols oils.

Unlike vegetable oils, for which hydrogenation is the primary method of increasing oxidative stability, animal fats achieve a large degree of their stability from the addition of antioxidants (Latondress, 1985). BHT, BHA, and TBHQ are all very effective in preventing oxidation of meat

TABLE 19.4
Peroxide and Thiobarbituric Acid Values for Antioxidant-Treated Canola Oil during Accelerated Storage at 65°C

	Treatment			
Storage Time (days)	Untreated Control	BHA/BHT (100 ppm ea) + Citric Acid	TBHQ (200 ppm)	TBHQ (200 ppm) + Citric Acid
Peroxide value (mEq/kg)				
0	0.67	0.46	0.61	0.51
4	9.84	5.29	1.21	1.32
8	23.64	20.52	1.99	2.13
16	61.71	40.00	3.34	3.40
TBA value (532 nm)				
0	0.67	0.68	0.68	0.68
4	6.41	3.63	0.91	0.96
8	9.36	8.46	2.57	1.93
16	11.64	10.79	1.70	1.56

Source: Adapted from Hawrysh, Z.J., et al. (1988). *Can. Inst. Food Sci. Technol. J. 21*: 549–554.

fats (Kincs, 1985; Eboh et al., 2006). Heavy metal contaminants adversely affect the composition, storage stability, and fatty acid profiles (Eboh et al., 2006). The use of metal chelators such as citric acid or ethylenediminetetraacetic acid (EDTA) also helps prevent oxidative damage to fats and oils (Sherwin, 1978; Patterson, 1989) by removing trace metals that could act to initiate oxidation.

If antioxidants or chelators are to be used, certain precautions must be taken to achieve the best results (Sherwin, 1978; Patterson, 1989). First, the antioxidant selected must be compatible with the oil or fat and effective in the situation in which it is used. Next, the appropriate concentration must be used; low concentrations may be ineffective, and high concentrations may enhance oxidation. Third, the antioxidant must be thoroughly dispersed throughout the oil to prevent areas of high and low concentration. Finally, the antioxidant must be added at the appropriate stage of processing. If it is not added soon enough, oxidation will begin and once begun cannot be reversed. On the other hand, if added too soon, the antioxidant may be removed during processing, leaving the processor with the false belief that the oil or fat contains sufficient antioxidants (Sherwin, 1978; Patterson, 1989).

VI. SUMMARY AND CONCLUSION

The goal of commercial oil processing is to obtain the maximum yield while at the same time achieving desirable physical characteristics, retaining nutritive value, and improving oxidative stability. Hydrogenation, which reduces the degree of unsaturation, has the most profound effect on the fatty acid composition of oils. Other processing steps, which are mainly designed to remove various undesirable elements, have only minor or indirect effects on the fatty acid composition. The elimination of impurities, such as free fatty acids, phosphatides, pigments, trace metals, and oxidation products, during processing not only improves product quality but also increases oxidative stability.

Various processing steps are generally beneficial to the production of good-quality edible oils. However, processing may also bring about changes in fatty acids that are detrimental to product quality, leading to losses of both esthetic and nutritive value. For example, hydrogenation can cause the formation of isomeric fatty acids and the loss of essential fatty acids. Additionally, during processing and storage, the oil may be exposed to certain conditions that can lead to autoxidation of fatty acids and results in the formation of hydroperoxides, aldehydes, and other undesirable oxidation products.

Factors such as moisture, heat, air, metals, and light can enhance hydrolysis and promote autoxidation of fatty acids. Antioxidants are normally added to edible oils to improve oxidative stability and prolong the shelf life of the products. If potentially damaging conditions are avoided or controlled, each step of processing will contribute to the production of an oil or fat that is bland, free of impurities, and oxidatively stable.

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20 Effect of Heating and Frying on Oil and Food Fatty Acids

Francisco J. Sánchez-Muniz, Sara Bastida, Gloria Márquez-Ruiz, and Carmen Dobarganes

CONTENTS

I. INTRODUCTION

Edible fats and oils usually contain more than 95% triglycerides. The main characteristics of the acyl groups forming triglycerides are chain length (number of C-atoms) and the number and position of unsaturations in the molecules. Before absorption, triglycerides must be hydrolyzed into free fatty acids and monoglycerides. These triglyceride-derived compounds are placed into intestinal micelles and absorbed. Later triglycerides are reesterified within the enterocyte and incorporated to chylomicrons (Sánchez-Muniz and Sánchez-Montero, 1999). Chylomicrons and other lipoproteins participate in the very complex lipoprotein metabolism carrying cholesterol, fatty acids, and other fat-soluble compounds to/between the different body tissues (Elmadfa and Wagner, 1999). The amount and quality of dietary fats and oils may influence the cell, tissue, and body physiology (Elmadfa and Wagner, 1999; Mahungu et al., 1999). Diet supplies some fatty acids that are essential or play very important metabolic regulations. A basic function of the *mother* dietary fatty acids,

oleic (C18:1, n-9), linoleic (18:2, n-6), and linolenic (c18:3, n-3), apart from being metabolic energy substrates, is to be unsaturated and elongated to long-chain fatty acids of 20 and 22 C-atoms. These long-chain fatty acids, once incorporated to membrane phospholipids or cellular membrane regions enriched in esfingomielins (Jump, 2004), can modulate the cell gene expression and generation of several bioactive molecules, through different and complex metabolic ways. Moreover, the balanced quantitative and qualitative intake of fatty acids can reduce the cholesterolemia, the lipid and lipoprotein oxidizability, and the thrombogenesis, and also modify the lipogenesis–lipolysis equilibrium, and so forth (Mensink and Katan, 1992; Massaro et al., 2002, 2006; Mata et al., 2002). Thus, adequate intake of dietary fat is essential for health. At present, it has been accepted by most of the Nutrition Organizations that total fat must contribute up to 30% and saturated fatty acids only less than 10% of the total energy intake (Kris-Etherton, 1999; Ros, 2001).

Mediterranean diet has been considered one of the most healthy standard diets worldwide (Keys et al., 1986; De Lorgeril et al., 1999). Basically, it contains a large proportion of vegetables, cereals, fruits, and olive oil (Trichopoulos et al., 2003). One of the most interesting characteristics of this diet is the frequent inclusion of fried foods (Varela and Ruiz-Roso, 1998; Sánchez-Muniz and Bastida, 2006). According to Henry (1998), fried foods eaten moderately provide important nutrients to our diet although they are often considered to be "bad" foods. Unfortunately, some indiscriminate general recommendations to decrease the consumption of fried foods could negatively affect the dietary habits of certain populations traditionally used to produce and consume fried foods (Bastida and Sánchez-Muniz, 2001a).

Some potential toxic compounds have been suggested to be specifically produced during frying. Thus, the formation of carcinogenic substances in frying oil and in fried foods has been investigated. However, it has to be pointed out that the main compounds associated with mutagenic effects are not specifically related to fatty acids. Otherwise, such compounds are polycyclic heterocyclic compounds formed from proteins or amino acids (Pokorný, 1999), nitrosamine compounds whose precursors are *N*-nitroso derivatives (Buckley et al., 1989); and acrylamide, found to be present in high concentrations in heat-treated food products rich in carbohydrates (Tareke et al., 2002; Boon et al., 2005). Therefore, in this chapter, the postulated formation mechanisms of these potential toxic substances and the methods used for their isolation and quantification will not be described.

Frying is a very old method of cooking food and represents a particular way of cooking food using oil or fat as a fluid heat vector. Frying modifies the physicochemical and organoleptic properties of foods (Varela, 1988), producing, for example, the crispy texture and the rich flavor and aroma that make food a pleasure to eat (Sánchez-Muniz and Bastida, 2006). Normally, less than 10 min are needed to fry any foodstuff (Cuesta and Sánchez-Muniz, 2001; Bastida et al., 2003; Sánchez-Muniz and Bastida, 2006), much less than that required by other culinary techniques.

There are two main frying techniques: *shallow* and *deep-fat frying*. Shallow frying is performed in relatively flat pots or pans containing little oil in which the product is not completely immersed. The food in direct contact with the hot oil is fried and the rest is cooked. In deep-fat frying the entire food is immersed in oil and the frying process takes place throughout the product. This kind of frying is performed in domestic and industrial fryers and in deep pans containing large amounts of oil (Monferrer and Villalta, 1993). According to Bognár (1998), oil uptake in meat and fish fried using the deep-fat frying method is lower than that in shallow frying.

Frying can be carried out either with *frequent*, *slow*, or *null replenishment* of fresh oil. As will be discussed in this chapter, frequent addition of fresh oil minimizes oil alteration (Cuesta and Sánchez-Muniz, 1998; Romero et al., 1998, 2000a,b). Frying can also be performed in a *continuous* or *discontinuous* manner, depending on whether the oil is left to cool (sometimes to room temperature) between frying operations. Jorge et al. (1996a) reported that laboratory continuous potato frying displayed lower degree of oil alteration than that after discontinuous frying.

In this chapter, the general modifications occurring during heating/frying will be summarized. The following sections will be dedicated to fatty acid changes in oils/fats used for cooking and oil transfer between food being fried and fryer oils/fats.

II. GENERAL CHANGES IN OILS AND FATS DUE TO HEATING AND FRYING

As the fat or oil is heated, the quality decreases as evidenced by a decrease in heat capacity, surface and interfacial tension, and increases in specific gravity, viscosity, acid values, anisidine values, and polymer content. Surface tension and interfacial tension are reduced by low- and high-polarity oxidative polymers causing excessive oil uptake by the food (Blumenthal and Stier, 1991).

It is well known that unsaturated fatty acids are prone to *autoxidation*, which is a free radical chain process, consisting mainly of three steps (i.e., initiation, propagation, and termination) (Porter et al., 1995; Lambert, 1999; Frankel, 2005) (Figure 20.1):

1. The formation of a lipid radical ($R^{\bullet}$) from an unsaturated fatty acid (RH) is the key event in the *initiation* step. This can occur by thermal or photochemical homolytic cleavage of the RH double bond or by hydrogen atom abstraction from RH by a free radical initiator.
2. *Propagation* normally begins with the addition of molecular oxygen to $R^{\bullet}$ but the rate-limiting reaction consists of abstraction of a hydrogen atom from RH by peroxyl radical ($ROO^{\bullet}$) to form hydroperoxides (ROOH) and another radical $R^{\bullet}$. Propagation observed in oxidation (Figure 20.1) may be more complicated than the simple transfer and addition (Porter et al., 1995). The primary products formed from the peroxidation of lipids generally include oxygen coupling to radical, hydrogen atom, or group transfer from substrate to the chain carrying the peroxyl radical, fragmentation of the chain containing the peroxyl radical to supply oxygen and a lipid radical, rearrangement of the chain containing the peroxyl radical, and cyclization of the peroxyl radicals, essentially by intramolecular radical addition (Frankel, 1991).
3. The *termination* reactions yield nonvolatile compounds of monomeric or polymeric structure, mostly containing oxygenated groups such as hydroxy, hydroperoxy, keto, epoxy

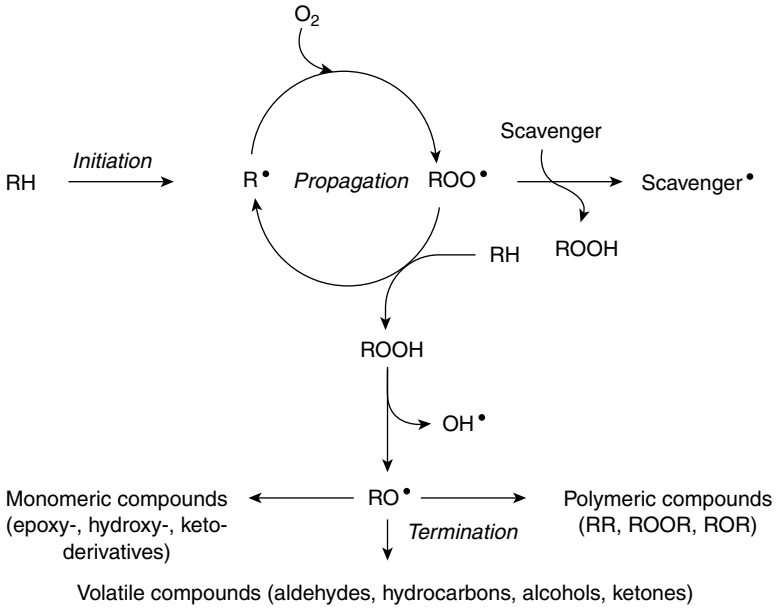

FIGURE 20.1 Major steps in oxidation. RH, a lipid containing an unsaturated fatty acid; $R^{\bullet}$, alkyl radical; $OH^{\bullet}$, hydroxyl radical; $RO^{\bullet}$, alkoxyl radical; $ROO^{\bullet}$, alkylperoxyl radical; and scavenger$^{\bullet}$, radical scavenger.

(Márquez-Ruiz and Dobarganes, 1996a), as well as volatile products (Brewer et al., 1999) including hydrocarbons, aldehydes, and ketones (Frankel, 1991). Alternatively, the propagation reaction also ends when peroxyl radicals combine with a radical scavenger such as vitamin E (Porter et al., 1995) (Figure 20.1).

The heating of oil (in the absence of food) increases the speed of autoxidation (Nawar, 1984). Thus, when the temperature of the reaction medium is relatively low (below 100°C), the formation rate of hydroperoxides is higher than that of their decomposition. Therefore, the compounds formed are mainly monomers of triglycerides with one or more acyl chains at any possible stage of oxidation. All these compounds are classified as oxidized triglyceride monomers.

At higher temperatures, all autoxidation reactions speed up, and the amount of altered compounds formed, mostly secondary oxidation products, depends on the heating time. At temperatures close to 200°C, decomposition and other reactions of hydroperoxides are faster than their formation. In this case, the major compounds generated are dimers and polymers of triglycerides (Nawar, 1984). Thermal alteration is also responsible for the formation of cyclic compounds such as triglyceride monomers showing intermolecular cyclization or triglyceride monomers showing intramolecular cyclization.

Frying clearly differs from heating because the presence of food in the fryer oil makes the situation rather more complex (Figure 20.2). During frying, the food is submerged in fat, which is heated in the presence of air. Therefore, the fat is exposed to the action of moisture from the foodstuff, oxygen from the atmosphere, and high temperature at which the operation takes place. In frying, autoxidation is also accelerated due to the relatively high temperature of the process; oxidation and thermal alterations take place in the unsaturated fatty acids, leading mainly to modified triglycerides with at least one of the three fatty acyl chains altered. Moisture from foods may

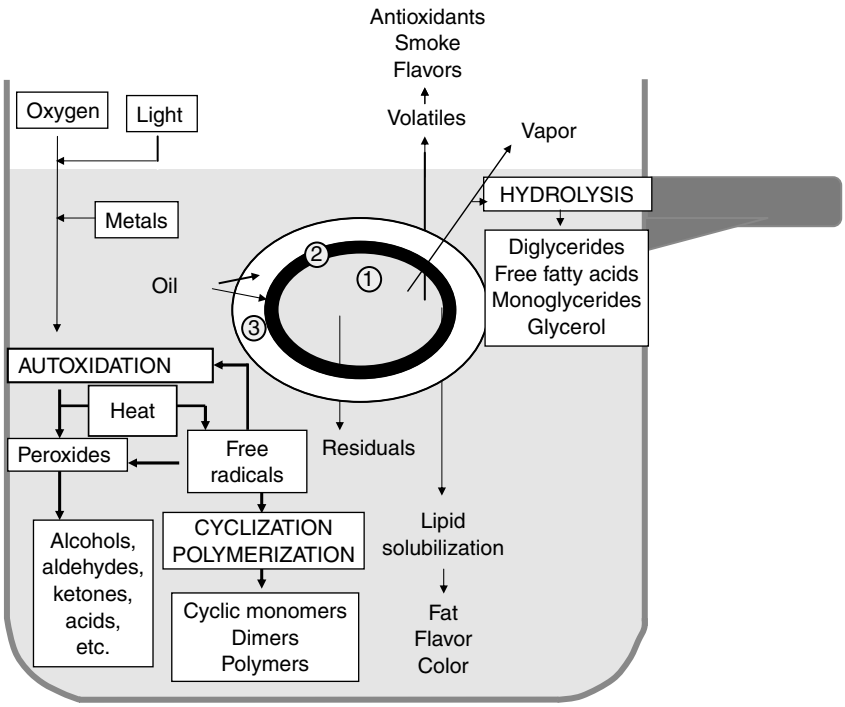

FIGURE 20.2 Major frying changes in frying. (1) Interior of food. Cooking is performed around 100°C due to vapor production. (2) The fat content increases and the water content decreases. (3) Total dehydration occurs. Browning and caramelizing take place.

induce hydrolytic alteration, thus yielding monoglycerides, diglycerides, and free fatty acids that are commonly classified as hydrolytic products (Table 20.1 and Figure 20.2). All these thermally oxidized and hydrolytic compounds are present in different quantities depending on a considerably high number of frying variables (Figure 20.3).

Finally, apart from migration of lipids from the food into the frying oil, it is important to take into account that the presence of some compounds from the foods can substantially affect the thermal oxidation reactions. Thus, (1) amphiphylic compounds such as phospholipids and emulsifiers can contribute to early foaming; (2) lipid-soluble vitamins and trace metals leaching into the frying oil may inhibit or accelerate oil oxidation depending on their antioxidant or prooxidant effects; (3) cholesterol and its oxidation-derived compounds from fatty animal foods can be transferred to vegetable frying oils and then incorporated into nonfatty foods during subsequent frying operations; (4) pigments and Maillard browning products can modify the susceptibility against oxidation of

TABLE 20.1
Main Causes of Alteration Compound Formation in Culinary Fats

Type of Alteration	Causal Agent	Resultant Compounds
Oxidative alteration	Air	Oxidized monomers
		Oxidized dimers and polymers
		Short fatty acids
		Volatiles (hydrocarbons, aldehydes, ketones, alcohols, acids)
Thermal alteration	Temperature	Cyclic monomers
		Dimers and polymers
		Geometric isomers
Hydrolytic alteration	Moisture	Free fatty acids
		Monoglycerides
		Diglycerides
		Glycerol

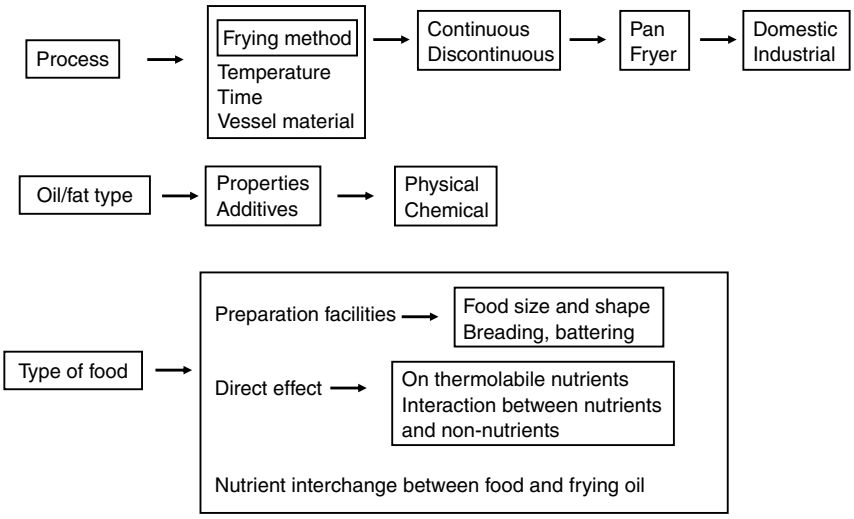

FIGURE 20.3 Factors influencing the alteration of frying fats. (Modified from Sánchez-Muniz, F.J., and Bastida, S. Effect of frying and thermal oxidation on olive oil and food quality. In *Olive oil and Health*, Quiles, J.L., Ramírez-Tortosa, M.C., and Yaqoob, P., eds., CABI. Org., Oxfordshire, U.K., 2006, p. 74. With the publisher permission.)

frying oils and contribute to darkening; (5) phenolic compounds present in the foods or in added spices can increase the frying oil stability; and (6) volatile compounds coming from strongly flavored foods like fish or onions can contribute to specific off-flavors (Figure 20.2)

Although it is not easy to find studies addressed to know the specific effect of minor compounds from foods, some interesting information can be found in general reviews (Pokorný, 1980, 1998, 1999; Stevenson et al., 1984; Fillon and Henry, 1998).

Moreover, during frying the substrate surface is almost totally dehydrated and crust formation occurs (Figure 20.2). According to Varela (1988) the crust acts as a barrier avoiding excessive food fat uptake and dehydration. Furthermore, monounsaturated or nonaltered oils produce a thinner and defined crust than polyunsaturated and thermal oxidized oils. Therefore, the crust contributes to produce leaner, safer, and healthier foods.

III. CHEMICAL CHANGES IN FATTY ACIDS DUE TO HEATING AND FRYING

This section will be dedicated first to the changes at the level of total alteration and second to the major nonvolatile altered fatty acids produced in used frying oils and fats (i.e., modified monomers, dimers, and higher oligomers).

A. Total Changes in the Oils and Fats: Polar Compounds

The overall quality of an oil or fat comprises those attributes that affect the nutritional, acceptability and commercial quality. Consumption of relatively high amounts of altered oil can be harmful to health (Cuesta et al., 1988; López-Varela et al., 1995; Márquez-Ruiz and Dobarganes, 2006; Lambert, 1999; Garrido-Polonio et al., 2004). Thus, adequate fat/oil alteration assessment is of great technological and nutritional importance.

The compounds produced due to changes during heating or frying are known as polar compounds, polar materials, or polar artifacts. In this chapter the term polar compounds will be generally used. Such alteration compounds show higher polarity than the original triglycerides and thus can be measured by techniques based on polarity discrimination.

Adsorption chromatography has been widely used for determining total polar compounds. The standard method uses a classical silica column. Nonpolar and polar fractions are eluted with 150 mL of a mixture hexane/diethyl ether (87:13) and 150 mL diethyl ether, respectively. After solvent evaporation, the polar compounds are determined gravimetrically (Waltking and Wessels, 1981; IUPAC, 1992a) (Figure 20.4a). Nonpolar and polar fractions can be further analyzed by high-performance size-exclusion chromatography (HPSEC), using 100 and 500 Å columns with polysterene divinylbenzene highly cross-linked macroporous packing connected in series; tetrahydrofuran as mobile phase (flow rate: 1 mL/min); and a refractive index detector (Dobarganes et al., 1988). The methodology has been recently standardized by the IUPAC Commission on Oils, Fats and Derivatives with slight modifications, for the analysis of used frying oils, and also for virgin and refined oils (Dobarganes et al., 2000a) (Figure 20.4b).

HPSEC is usually applied for the determination of polymerized triglycerides taking advantage of their molecular weight, much higher than that of the main oil components (IUPAC, 1992b) (Figure 20.4c). Further, the advantages offered by combination of adsorption chromatography–HPSEC (Figure 20.4b) are enormous. First, a substantial increase in the possibilities for quantification of all the groups of alteration compounds is achieved because of the effect of concentration. Second, oxidized monomers, a measurement of oxidative degradation can be determined independently, since the coeluting major peak of nonoxidized triglycerides is separated in the nonpolar fraction. Third, concomitant evaluation of diglycerides as a marker of hydrolytic degradation is possible. Finally, a substantial increase in sensitivity is achieved in quantification of polymerization compounds due to the effect of concentration, overcoming the limitation of the IUPAC Standard Method

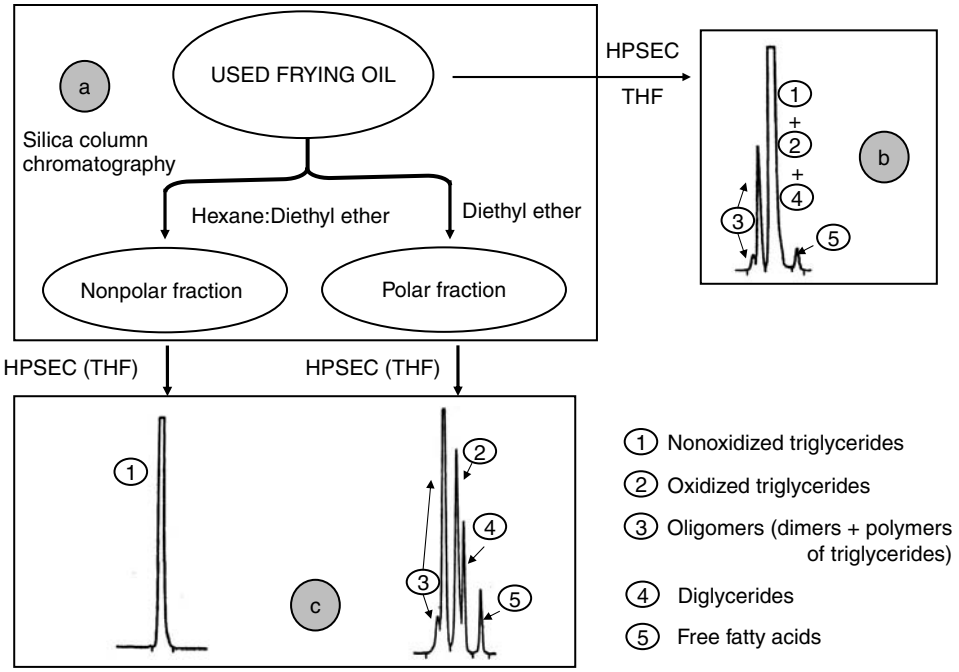

FIGURE 20.4 Analytical procedure for determination of altered oxidized and polymerized compounds. (a) Separation of nonpolar and polar fractions from used frying oils by silica gel chromatography (SGC); (b) direct HPSEC of used frying oils: unaltered and oxidized triglycerides and diglycerides elute together; (c) combination of SGC and HPSEC permits identification of compounds in the nonpolar and polar fractions, and the overlapping of nonaltered triglycerides with oxidized triglycerides and diglycerides. (Modified from Dobarganes, M.C. et al. In *Frying of Food. Oxidation, Nutrient and Non-Nutrient Antioxidants, Biologically Active Compounds and High Temperatures*, Boskou, D. and Elmadfa, I., eds, Technomic Publishing Co. Inc., Lancaster, PA, 1999, p. 143; Sánchez-Muniz, F.J. et al., *J. Nutr.*, 133, 2302, 2003; Sánchez-Muniz, F.J., and Bastida, S. Effect of frying and thermal oxidation on olive oil and food quality. In *Olive oil and Health*, Quiles, J.L., Ramírez-Tortosa, M.C., and Yaqoob, P., eds., CABI. Org., Oxfordshire, U.K., 2006, p. 74. With the publisher permission.)

2.508 to a minimum of 3% content for analyses of total samples by HPSEC. The groups of compounds quantified can be differentiated between thermally oxidized compounds (oxidized monomers, dimers, and oligomers), associated with negative physiological effects, and hydrolytic products (diglycerides and fatty acids), naturally released from lipolysis in the gut before absorption.

An alternative technique intended to reduce the quantity of the sample and solvents, shorten and analysis time is based on the use of silica cartridges for the separation and monostearin as the internal standard. This modified procedure is useful for samples with a wide range of alteration products and especially for those of low levels of degradation. In the latter case, quantification with an internal standard shows significantly lower errors compared to gravimetric determination, based on relative standard deviations (Márquez-Ruiz et al., 1996).

Polar compound determination constitutes the main basis of the used frying oils and fats regulations. The debate on deep-fat frying is primarily focused on the point at which any oil used for frying should be discarded. Many European countries have established legislation that it is compulsory to discard a frying oil when its altered part (polar material) surpasses 25% of total oil mass (Firestone, 1996; Dobarganes and Márquez-Ruiz, 1998; Sánchez-Muniz and Bastida, 2003).

Figure 20.5 shows comparative behaviors of different oils during discontinuous frying of fresh potatoes. Monounsaturated oils were more stable than sunflower oil. This implies that they can be

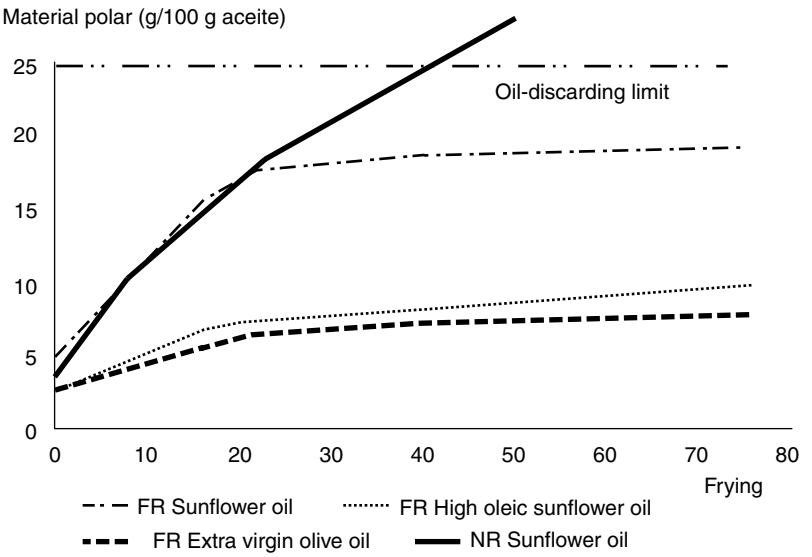

FIGURE 20.5 Polar material content (g/100 g oil) change in extra virgin olive oil, high-oleic acid sunflower oil and sunflower oil used in 75 fryings of fresh potatoes performed with frequent addition of fresh oil, and in 75 fryings of fresh potatoes in sunflower oil performed with null turnover. (Adapted from Arroyo, R. et al., *Fat Sci. Technol.*, 95, 292, 1995; Cuesta, C. et al., *J. Am. Oil Chem. Soc.*, 70, 1069, 1993; Romero, A. et al., *J. Agric. Food Chem.*, 47, 1168, 1999. With the publisher permission.)

used for frying for a higher number of occasions before reaching the 25% polar content cut-off point and, thus, have a longer frying life than other extensively used oils. The comparison between the oil alteration tendencies also suggests that frying life highly depends on the frequency of oil addition. Nonetheless, in a parallel study performed by frying fresh potatoes under null fresh oil addition, it was found that palm olein displayed very high stability (Arroyo et al., 1995). These results are relevant but it has also to be pointed out that frying/cooking with palm olein increases the palmitic acid content of the food giving rise to a dietary energy contribution of saturated fatty acid >10% (Cuesta et al., 1998), which is regrettable taking into account the relationship of these fatty acids and some degenerative diseases, such as cardiovascular diseases (Keys et al., 1986; Mensink and Katan, 1992; Cuesta et al., 1998).

In the past years, a plethora of studies on frying fats and oils based on application of this methodology has been published, and the results obtained have contributed to improve knowledge on important issues related to frying, such as performance of different oils (Dobarganes et al., 1993; Arroyo et al., 1995; Sébédio et al., 1996; Masson et al., 1997, 1999, 2002; Márquez-Ruiz et al., 1999; Abidi and Warner, 2001; Bastida and Sánchez-Muniz, 2001a,b; Abidi and Rennick, 2003; Houhoula et al., 2003; Marmesat et al., 2005), composition of oils absorbed by the fried food and lipid interchange between frying oil and food (Pérez-Camino et al., 1991; Pozo-Díaz et al., 1995; Jorge et al., 1996b; Sébédio et al., 1996), the action of the main variables involved in the continuous and discontinuous frying processes (Jorge et al., 1996a,b), and the effect of oil replenishment during frying on oil quality (Cuesta et al., 1993; Romero et al., 1995, 1998, 1999; Cuesta and Sánchez-Muniz, 1998).

Beside applications on used frying oils, the methodology has also been applied to thermally oxidized fats and oils (Abidi et al., 1999; Barrera-Arellano et al., 1999; González-Muñoz et al., 1999, 2003; Verleyen et al., 2001, 2002; Barrera-Arellano et al., 2002; Kamal-Eldin et al., 2003), and food lipids and oils subjected to microwave heating (Beatriz et al., 1994; García-Ayuso et al., 2000; Caponio et al., 2002; Luque-García et al., 2002).

B. Total Changes in Fatty Acids

The analysis of polar compounds starting directly from the used frying oil is very useful because of their significance in terms of the total new compounds formed. However, from a chemical and even nutritional point of view, the analysis of modified fatty acids provides more interesting information. On one hand, evaluation of polar compounds does not permit to differentiate triglycerides with either one or more polar fatty acyl groups. In this regard, quantification of polar fatty acid methyl esters (FAME) is of great use as it overcomes this limitation and provides additional information. On the other hand, in the past years, nutritional aspects have merged on the potential biological effects of oxidized lipids and there is increasing evidence that they may be detrimental to health, particularly in connection with the development of atherosclerosis, liver damage, and promotion of intestinal tumors (Márquez-Ruiz and Dobarganes, 2006; Dobarganes and Márquez-Ruiz, 2003).

This subsection covers a double aspect concerning formation of new fatty acids during heating and frying: (i) the evaluation of the total modified fatty acids and (ii) the loss of the nonpolar fatty acids present in the oils and fats before heating.

a. Polar Fatty Acids

As already noted, determination of polar fatty acyl groups included in triglyceride molecules is essential to evaluate specifically the oxidative changes undergone in fats and oils after heating or frying since oxidation takes place in the unsaturated fatty acyl groups. In addition, it is of great value in relation to the nutritional consequences of frying oil consumption as it is focused on the fatty acyl groups, which are the products of fat digestion ultimately absorbed. However, even though evaluation of polar compounds in used frying oil is an excellent measurement of oil degradation, it does not permit to differentiate altered triglycerides with either one or more oxidized fatty acyl groups.

Methodologies used to quantify polar fatty acyl groups included in triglyceride molecules are based on the most simple fatty acid derivatives of triglycerides, the FAME. The first approach used enabled direct evaluation of the polar FAME by gravimetric determination following separation in silica column (Dobarganes et al., 1984a; Perrin et al., 1985; Sánchez-Muniz et al., 1990, 1993, 2000a; Cuesta et al., 1991). Briefly, FAME are obtained by transesterification of 1 g of sample with sodium methoxide and hydrochloric acid–methanol. FAME are quantitatively recovered and separated by silica column chromatography in two fractions. When using 150 mL hexane/diethyl ether (95:5), the first fraction includes exclusively the most abundant nonpolar FAME. Then, elution with 150 mL diethyl ether yields a minor fraction of polar FAME. It is interesting to note that two groups of modified FAME elute in the fraction of nonpolar FAME: *trans* FAME and cyclic FAME. These groups have similar polarity than those corresponding to nonmodified FAME and, consequently, they cannot be separated by silica column. However, their quantitative importance as compared to the total new FAME formed as well as to the group of nonmodified FAME is minimal.

Evaluation of polar FAME by silica column in used frying oil samples collected by Food Inspection Services, with polar compound levels close to the limit for frying oil rejection (25%), showed clearly that additional information on the alteration level can be gained (Márquez-Ruiz et al., 1995). Overall, samples at the limit of rejection had alteration levels as high as 10% expressed specifically on polar FAME, which means that significant amounts of altered fatty acids may occur in the diet. In addition, samples with the same percentage of total polar compounds (27.6%) showed different levels of polar FAME (8.7%–11.3%), in part due to the fact that diglycerides form a considerable part of total polar compounds in certain oils and, after transesterification, their resulting FAME will be included in the nonpolar fraction.

A different approach used consists of direct analysis of FAME derivatives by size-exclusion chromatography (Perkins et al., 1973; Perrin et al., 1984; Christopoulou and Perkins, 1989c). In this case, the analysis is very simple as it only requires to inject total FAME, but resolution and detection of polar FAME are very poor due to the presence of nonmodified FAME as major components.

The most complete evaluation of polar FAME can be achieved by combining adsorption chromatography (silica column) to obtain the polar FAME fraction and further analysis by HPSEC to separate groups of compounds possessing different molecular weights (i.e., oxidized monomeric, dimeric, and polymeric FAME) (Márquez-Ruiz et al., 1990). In the case of the samples collected by Food Inspection Services mentioned above, with polar compound levels close to the limit for frying oil rejection, particularly relevant was the high occurrence of oxidized FAME monomers, approximately 30 mg/g, apart from the presence of substantial amounts of oxidized FAME dimers and polymers. Quantification of dimers and polymers directly in the oils and after transesterification of the samples revealed some insight into the complexity of the triglyceride polymer structure. The values found for the fatty acid polymers-to-triglyceride polymers ratio were very low in contrast to those for fatty acid dimers/triglyceride dimers, which ranged from 0.6 to 0.8. These data are evidence to the considerable contribution of dimeric linkages to the structures of trimeric and higher oligomeric triglycerides in this group of samples.

Applications of this latter methodology to animal studies have permitted to evaluate hydrolysis and digestibility of oxidized, dimeric, and polymeric fatty acids (Márquez-Ruiz et al., 1992, 1993; Márquez-Ruiz and Dobarganes, 1995). Following analysis of dietary oils and fecal lipids, high-digestibility coefficients were found for oxidized fatty acid monomers, thus indicating their utmost importance from the nutritional standpoint, supported also by their quantitative relevance in the diet. Polymeric fatty acids showed generally poor digestibility, in great part attributable to low activity of pancreatic lipase on triglyceride polymers.

b. Fatty Acid Composition

Besides the determination of the total content in polar FAME, the complementary analysis of the fatty acids that have not been affected by high temperature and air is also of great interest (Dobarganes and Pérez-Camino, 1985).

Analyses of fatty acid composition after different treatments at high temperature are common in studies on performance of fats and oils. However, the most usual form of expressing the results is the normalized composition (the percentage of each acid on the total of those analyzed). The first part of Table 20.2 shows the changes in major fatty acids for different refined vegetable oils before and after heating at 180°C for 20 h. From the normalized composition, the apparent increase in saturated fatty acids and the decrease in polyunsaturated fatty acids can be observed, while it is not clear why the change undergone by oleic acid that seems to decrease in palm and olive oils and to increase in sunflower and soybean oils.

This form of expression is very useful in the analysis of fats and oils when triglycerides constitute more than 95% of the total sample because it is a good quantitative measurement. However, after formation of new compounds through different reactions quantification of fatty acids becomes necessary to avoid erroneous interpretations.

Determination of total polar fatty acids by column chromatography, as commented above, or quantification of fatty acids with an internal standard (Cuesta et al., 2001; Romero et al., 2000b) is very useful to understand the real changes undergone by the different fatty acids. The second part of Table 20.2 shows the composition of the same oils expressed as wt.% on oil and calculated from the total content of remaining FAME in the samples obtained by silica column (nonpolar methyl esters) and from the normalized composition. The results indicate that the degradation took place in all the unsaturated fatty acids being greater as the fatty acid unsaturation increases. In addition, the loss of oleic acid, the only monounsaturated acid in the samples in significant amounts, was notably influenced by the content of polyunsaturated fatty acids, being higher when the content of polyunsaturated fatty acids was lower. Finally, palmitic and stearic acids remained at their initial levels. The exact loss of each fatty acid can be easily deduced by subtraction from the initial contents (Dobarganes and Pérez-Camino, 1988a,b).

TABLE 20.2
Fatty Acid Composition, Nonpolar Methyl Esters and Quantitative Determination of Major Fatty Acid of Different Vegetable Oils before and after Heating for 20 h at 180°C

		Major Fatty Acids (% Total Fatty Acids)					Nonpolar Methyl Esters	Major Fatty Acids (g/100 g Oil)				
Oil	Heating Period (h)	C16:0	C18:0	C18:1	C18:2	C18:3	(g/100 g Oil)	C16:0	C18:0	C18:1	C18:2	C18:3
Palm	0	38.6	4.2	44.5	11.7	<0.5	98.1	37.9	4.1	43.7	11.5	<0.5
	20	42.8	4.9	43.4	7.6	<0.5	93.0	39.4	4.5	39.9	7.0	<0.5
Olive	0	10.7	2.7	78.0	8.6	<0.5	98.7	10.6	2.7	77.0	8.5	<0.5
	20	11.6	3.2	77.5	7.7	<0.5	91.5	10.6	2.9	70.9	7.0	<0.5
Sunflower	0	7.0	4.5	24.7	63.8	<0.5	97.5	6.8	4.4	24.1	62.2	<0.5
	20	7.7	4.9	26.3	61.1	<0.5	89.5	6.9	4.4	23.5	54.7	<0.5
Soybean	0	11.2	3.6	23.1	55.0	7.0	98.5	11.0	3.5	22.8	54.2	6.9
	20	12.1	3.9	25.2	53.1	5.7	89.2	10.8	3.5	22.5	47.4	5.1

Source: Adapted from Dobarganes, M.C., and Pérez-Camino, M.C., *Grasas y Aceites*, 36, 186, 1985; Dobarganes, M.C., and Pérez-Camino, M.C., *J. Am. Oil Chem. Soc.*, 65, 101, 1988. With the publisher permission.

In addition, determination of polar fatty acids by silica column chromatography in combination with fatty acid analysis has the advantage of making possible the analysis of FAME starting out from the nonpolar fraction, thus avoiding impurification of the column by oxidized and polymeric fatty acids present in the total sample, which would not elute in the gas chromatography analysis.

C. Modified Fatty Acids

The next step in the analysis of the modified compounds formed during frying is the detailed evaluation of the main constituents within the groups of monomers, dimers, and oligomers. In this respect, there is a growing interest in the oxidized monomers because of their possible nutritional implications due to their high absorbability and their presence in used frying fats at nonnegligible levels (Márquez-Ruiz et al., 1995).

In this subsection, the two main groups of modified fatty acids are considered separately:

1. Compounds with molecular weight similar or identical to those of nonmodified fatty acids (i.e., isomeric fatty acids, cyclic fatty acids, and oxidized fatty acids).
2. Polymerization compounds formed through interaction of two or more fatty acids and, thus, with molecular weights much higher than those of nonmodified fatty acids (i.e., nonpolar dimers, polar dimers, and higher oligomers).

a. Modified Fatty Acid Monomers

It is interesting to note that among the groups of modified fatty acids, isomeric fatty acids and cyclic monomers have the same polarity and molecular weight than those of nonmodified FAME and, consequently, when obtaining two fractions of different polarity by silica column they would be included in the nonpolar FAME. However, their quantitative importance as compared to the total new FAME formed as well as to the group of nonmodified FAME is minimal and therefore, quantitative results of nonpolar and polar FAME are representative of nonmodified and modified FAME.

The three groups of modified fatty acid monomers are analyzed by gas chromatography. Improved stationary phases and column design have contributed significantly in recent years to the identification and quantification of specific structures.

i. Positional and Geometric Isomers

Trans polyunsaturated fatty acids and cyclic fatty acid monomers (CFAM) are present in human diets as a consequence of heat treatment of fat and oil (Grandgirard et al., 1984; Sébédio and Grandgirard, 1989), and after catalytic partial hydrogenation. Present nutrition guidelines recommend decreasing the intake of *trans* fatty acids due to the negative effect of such fatty acids on lipoprotein metabolism (Zock and Katan, 1992). These compounds are also formed during refining. In fact, *trans* isomerization of linoleic and linolenic acids can occur during deodorization (Ackman et al., 1974; Devinat et al., 1980). For linoleic acid, isomerization mainly occurs in the Δ9 and Δ15 positions, while minor amounts of isomers with the *trans* double bond in the Δ12 are found (Grandgirard et al., 1984).

According to Sébédio and Chardigny (1996), nonnegligible quantities of linolenic geometric isomers were found in commercial oils while linoleic acid was slightly isomerized. The critical temperature at which some *trans–trans* linolenic isomers are formed is 200°C. Conjugated linoleic acids (*cis*/*trans* and *trans*/*trans* isomers of $C_{18:2}$) were also detected.

Frying different frozen foods with extra virgin olive oil, sunflower oil, and high-oleic acid sunflower oil resulted in relatively negligible amount of *trans* fatty acids (Romero et al., 2000a). The *trans*/*trans* fatty acids were higher in sunflower oil than in the monoenoic oils. Higher amount of these *trans* fatty acids were also found when a null turnover of fresh oil was performed. In that paper, it was probed that frying at 180°C was a poor source of *trans* fatty acids, and a relevant proportion of these fatty acids should come from the prefried frozen foods (Romero et al., 2000a).

In addition, when nonhydrogenated vegetable oils are used in frying of very low-fat content foods, the levels of *trans* oleic and linoleic isomers formed during frying were very low, in the order of mg/kg (Beatriz et al., 1994; Gamel et al., 1999).

ii. Cyclic Monomers

Cyclization is one of the changes occurring through frying. This alteration has been referenced to be more frequent in abused heated oils, and related to temperature, type of oil, and length of heat treatment (Sébédio et al., 1987). CFAM are basically formed in oils from linoleic and linolenic acids after heat treatment of fats and oils. The cyclization between C15 and C10 gives rise to a typical cyclohexane ring, while cyclization between C15 and C11 will produce a cyclopentane ring. Monounsaturated fatty acids are known to cyclize to monocyclic fatty acids (Figure 20.6).

The usual procedure for isolation of the cyclic fatty acids involves conversion to methyl esters followed by chromatography on silicic acid. The nonpolar fraction from such a separation is then fractionated with urea to give the cyclic FAME of up to 99% purity (Hamilton and Perkins, 1997). Since the number of positional isomers can be enormous, it is often necessary to hydrogenate the nonpolar fatty acids. This provides a simpler mixture of compounds for subsequent gas chromatography–mass spectrometry.

Adverse physiological conditions have been described in experimental animals after feeding them with increased amount of CFAM (Cuesta et al., 1988). These adverse effects were more deleterious in animals fed low levels of protein. Nonetheless, the CFAM structure seems relevant because very low toxicity of six-carbon-membered ring cyclic monomers have been suggested due to their rapid urine excretion (Iwaoka and Perking, 1978).

Many studies on the structure and formation of cyclic fatty acids were carried out heating oils under laboratory conditions (Sébédio et al., 1987) but the data from real frying conditions are less available. Available data ranged from 100 to 6600 mg/kg in most altered samples. The CFAM in different oils used to fry fresh potatoes and frozen prefried foods is summarized in Table 20.3. Frying fresh potatoes produces less CFAM content. Moreover, frequent oil addition decreases the CFAM formation with respect to null oil addition in the three oils (Romero et al., 2003, 2006a,b).

The type of CFAM formed depends on the kind of fatty acid involved. Linoleic acid in sunflower oil produces cyclic monoenes (Christie et al., 1993) while linolenic acid in linseed oil produces

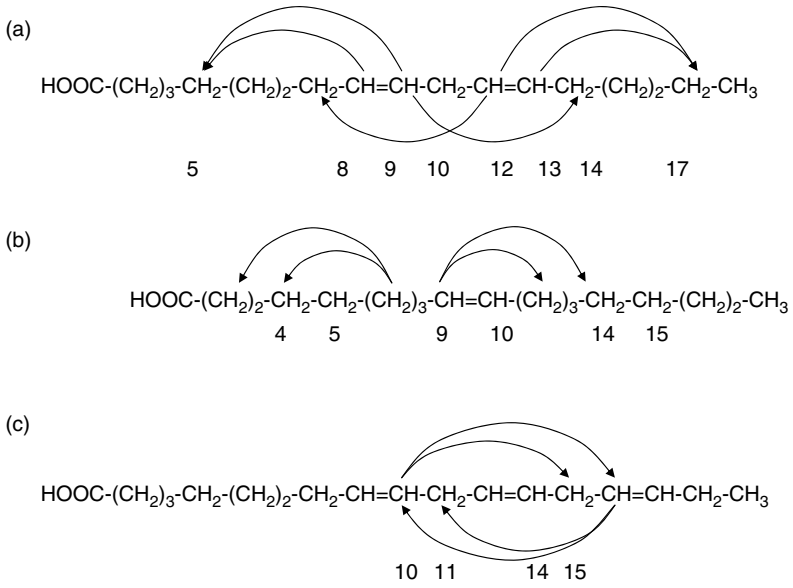

FIGURE 20.6 Possible mechanisms for cyclization: (a) oleic acid, (b) linoleic acid, and (c) linolenic acid. (Modified from Le Quéré, J.L., and Sébédio, J.L., Cyclic monomers of fatty acids. In *Deep Frying. Chemistry, Nutrition, and Practical Applications*, Perkins, E.G. and Erickson, M.D., eds., AOCS Press, Champaign, IL, 1996, p. 49. With the publisher permission.)

cyclic dienes (Mossoba et al., 1994; Dobson et al., 1995) on heating. Most CFAM formed from linoleic acid in heated sunflower oil contain a disubstituted cyclopentenyl ring. The spectra of CFAM esters, before and after hydrogenation, indicated the presence of six major structures. However, for cyclic dienes isolated from heated linseed oil cyclization was always directed internally toward other double bonds and never involved the double bond at position C12. Four major structures were identified (Le Quéré and Sébédio, 1996).

Extra virgin olive oil, high-oleic acid sunflower oil, and sunflower oil used in repeated frying show a trend to increase both cyclopentyl and cyclohexyl ring compounds (Romero et al., 2003). Bicyclic fatty acids were found in significantly higher amounts in SO than in other oils, after 20 fryings of frozen foods, with a null turnover of fresh oil (Table 20.3).

iii. Oxidized Monomers

Oxidized monomeric FAME are characterized by the presence of an extra oxygen in the molecule. They are final stable products formed from hydroperoxides and the expected major compounds are FAME containing different oxygenated groups, mainly epoxy, keto, and hydroxy (Capella, 1989).

Quantification of these compounds in heated and used frying fats has only been reported in recent years (Velasco et al., 2002, 2004a, 2005). Essential requirements of the derivatization technique selected were good repeatability and recovery of the compounds of interest, avoiding artifact formation (Berdeaux et al., 1999a). After selecting the appropriate methodology for FAME derivatization, quantification of monoepoxy compounds in thermal oxidized FAME and triglyceride model systems can be performed (Berdeaux et al., 1999b).

Two distinct mechanisms have been proposed for epoxide formation either at the site of the double bond or nearby the double bond. In the latter case, the original double bond remains (Neff and Byrdwell, 1998). However, only the compounds formed when the oxygen added across an existing double bond (Figure 20.7) were detected by combination of gas chromatography and mass spectrometry in methyl linoleate and oleate heated at 180°C. Thus, two saturated epoxides,

TABLE 20.3
Total and Bicyclic Fatty Acid Monomers in Different Oils before and after Frying Fresh Potatoes or Frozen Prefried Foods with Null or Frequent Fresh Oil Addition to the Fryer Oil

							Effect of Oil Type[a]		
Cyclic Fatty Acids	Fried Food	Total Fryings	Oil Addition	Initial (mg/kg Oil)	Final (mg/kg Oil)	Effect of Oil Addition[a]	EVOO vs. HOSO	EVOO vs. SO	HOSO vs. SO
Total extra virgin olive oil (EVOO)	Fresh potatoes	75	Yes	0	195		**		
Bicyclics extra virgin olive oil (EVOO)	Fresh potatoes	75	Yes	0	2.72		ND		
Total high-oleic acid sunflower oil (HOSO)	Fresh potatoes	75	Yes	64	330				
Bicyclics high-oleic acid sunflower oil (HOSO)	Fresh potatoes	75	Yes	3.02	3.48				
Total extra virgin olive oil (EVOO)	Frozen foods	20	Yes	0	574	***	*	***	
Bicyclics extra virgin olive oil (EVOO)	Frozen foods	20	Yes	0	17.9	NS	NS	***	
Total extra virgin olive oil (EVOO)	Frozen foods	20	No	0	684		***	***	
Bicyclics extra virgin olive oil (EVOO)	Frozen foods	20	No	0	33.4		NS	***	
Total high-oleic acid sunflower oil (HOSO)	Frozen foods	20	Yes	64	608	***			***
Bicyclics high-oleic acid sunflower oil (HOSO)	Frozen foods	20	Yes	3.02	18.5	ND			***
Total high-oleic acid sunflower oil (HOSO)	Frozen foods	20	No	64	706				***
Bicyclics high-oleic acid sunflower oil (HOSO)	Frozen foods	20	No	3.02	25.6				***
Total sunflower oil (SO)	Frozen foods	20	Yes	71	697	***			
Bicyclics sunflower oil (SO)	Frozen foods	20	Yes	5.6	70.0	**			
Total sunflower oil (SO)	Frozen foods	20	No	71	855				
Bicyclics sunflower oil (SO)	Frozen foods	20	No	5.6	104.4				

[a] Comparisons between the linear adjustments (ANCOVA) for concentrations at different number of frying ***$p < .001$; **$p < .01$; *$p < .05$. ND = one of both adjustments to be compared were not significantly lineal; NS = not significantly different.

Source: Adapted from Romero, et al., *J. Am. Oil Chem. Soc.*, 80, 437, 2003; Romero, A., et al., *Food Chem Toxicol.*, 44, 1674, 2006; Romero, A., et al., *Eur. J. Lipid Sci. Technol.,* 109, 165, 2007. With the publisher permission.

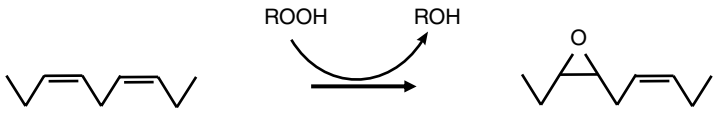

FIGURE 20.7 Suggested mechanism for epoxide formation via external hydroperoxide.

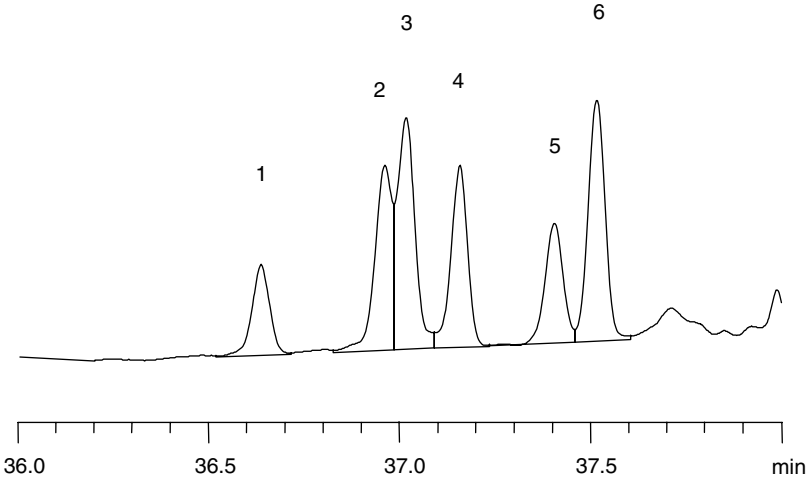

FIGURE 20.8 Partial gas chromatograms showing separation of monoepoxy FAME in used sunflower frying oil. Conditions: HP Innowax capillary column (60 m × 0.25 mm i.d.). Temperature program: 90°C (2 min), 4°C/min, 240°C (25 min). (1) *trans*-9,10-Epoxystearate; (2) *trans*-12,13-epoxyoleate; (3) *cis*-9,10-epoxystearate; (4) *trans*-9,10-epoxyoleate; (5) *cis*-12,13-epoxyoleate; (6) *cis*-9,10-epoxyoleate.

trans-9,10- and *cis*-9,10-epoxystearate, were formed from methyl oleate and four monounsaturated epoxides, *trans*-12,13-, *trans*-9,10-, *cis*-12,13-, and *cis*-9,10-epoxyoleate, from methyl linoleate (Berdeaux et al., 1999b). In the case of heated oils and fats, degradation depends on a large number of triglyceride species. As a consequence, the separation and accurate quantification of the epoxy FAME required more complex analyses and elimination of interference by minor or major components naturally present in fats and oils. After separation of nonpolar FAME by solid-phase extraction, separation, and quantification of six individual epoxy FAME present in used frying oils and fats was possible (Figure 20.8).

Quantification of epoxides in thermal oxidized olive and sunflower oils, and in used frying oils, demonstrated that, for similar levels of polar compounds, monounsaturated oils showed higher levels of monoepoxides than polyunsaturated oils. This was attributable to two concurrent facts for monounsaturated oils: first, their lower tendency to polymerize, and, second, their greater stability. Hence, accumulation of the major monoepoxides formed in monounsaturated oils (i.e., monoepoxystearates), in contrast to susceptibility to further reactions of the most abundant monoepoxides found in polyunsaturated oils (i.e., monoepoxyoleates). It was also found that monoepoxides were major oxidized compounds, accounting for about 25% of the total oxidized monomers in used frying oils at the limit of rejection (Velasco et al., 2004a).

As compared to epoxy FAME, quantification of specific structures of hydroxy FAME and keto FAME is more difficult due to the higher unsaturation of the compounds formed and the existence of conjugated double bonds in polyunsaturated molecules. Thus, from methyl linoleate, two and three conjugated double bonds are expected in hydroxy FAME and keto FAME, respectively. Figure 20.9a shows the significant part of chromatograms of polar FAME from thermal oxidized

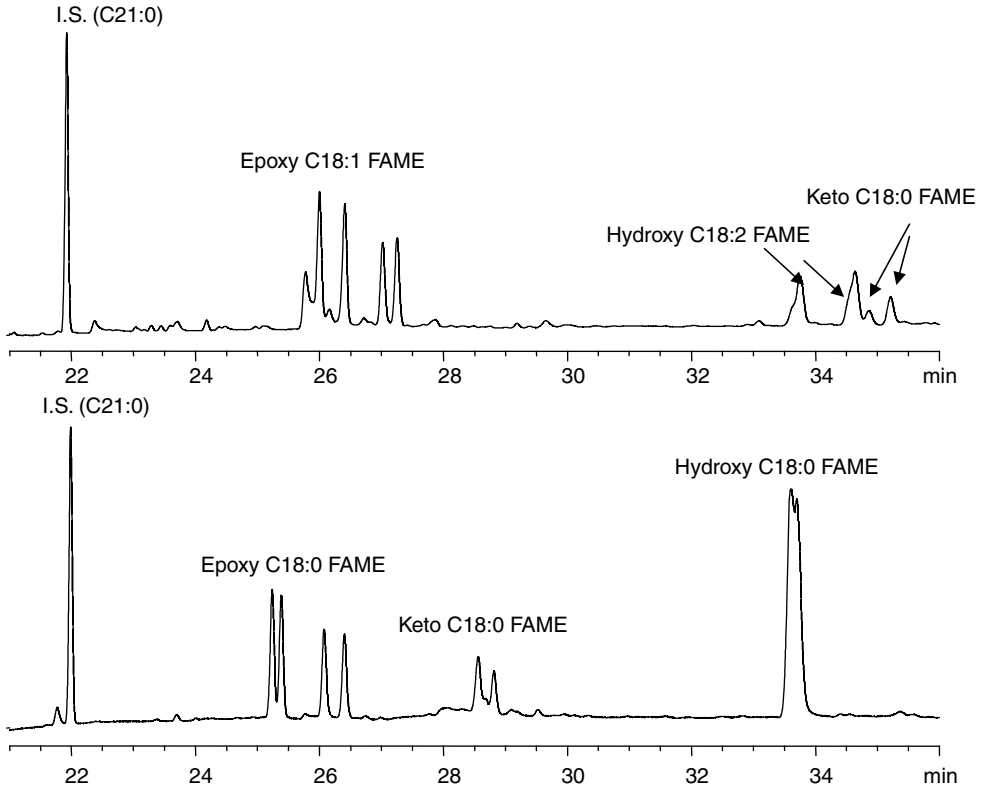

FIGURE 20.9 Partial gas chromatograms showing separation of major compounds in methyl linoleate heated at 180°C for 15 h. (a) Before hydrogenation and (b) after hydrogenation. Conditions: HP Innowax capillary column (30 m × 0.25 mm i.d.). Temperature program: 90°C (2 min), 4°C/min, 240°C (25 min).

methyl linoleate at 180°C, before and after hydrogenation. The differences between the three groups of compounds can be easily observed. The four monounsaturated epoxides are well separated while the major diunsaturated hydroxy and keto FAME elute at similar retention time. Despite the lower polarity of keto FAME as compared to hydroxy FAME, the existence of three conjugated double bonds in ketodienes increases significantly the retention time in gas chromatography. The quantification of hydroxy FAME and keto FAME can be performed after elimination of nonpolar FAME by solid-phase extraction in silica cartridges and further hydrogenation of polar FAME. In these conditions, the separation of three well-defined groups of epoxy FAME, keto FAME, and hydroxy FAME are obtained as shown in Figure 20.9b. From the analysis of used frying fats it is deduced that these three groups of compounds accounted for more than 80% of total oxidized monomers (Dobarganes, Velasco, and Marmesat, unpublished data).

Separation and identification of the main structures in FAME from used frying fats indicate that the fraction of oxidized monomers also contains significant amounts of compounds with lower molecular weight than that of the original fatty acids. Among these compounds are the short-chain n-oxo FAME, originally triglyceride-bound aldehydes, resulting from homolytic β-scission of the alkoxyl radical from allylic hydroperoxide (Kamal-Eldin and Appelqvist, 1996; Kamal-Eldin et al., 1997). The quantification of the nonvolatile aldehyde derivatives in used frying fats and fried products is of great importance from a nutritional point of view since they remain attached to the triglycerides and thus retained in the fried food ingested by the consumer. In this respect, there are some reports on 9-oxononanoic acid, the major esterified aldehyde in oxidized lipids, which indicate that such structures could induce lipid peroxidation and affect hepatic metabolism (Minamoto et al., 1988; Kanazawa and Ashida, 1991).

Methyl octanoate (C8:0), methyl 9-oxo-nonanoate (9-oxo-9:0), and dimethyl nonanodiate (C9:0 diester) were the major compounds formed in thermoxidized methyl linoleate (Berdeaux et al., 2002); besides lower amounts of methyl heptanoate (C7:0), methyl 8-oxo-octanoate (8-oxo-8:0), and dimethyl octanodiate (C8:0 diester) probably coming from 13-hydroperoxide breakdown of linoleic acid and further oxidative reactions (Berdeaux et al., 1999a) and from 8-hydroperoxide of oleic acid (Márquez-Ruiz and Dobarganes, 1996).

The quantification of C8:0 in heated and used frying fats was the first example of an accurate determination of short-chain compounds formed during thermal oxidation (Peers and Swoboda, 1982). Later, C7:0 formed from 8-hydroperoxide of oleic acid in monounsaturated frying oils was also quantified. Interestingly, the ratio between both methyl esters was very useful to deduce the extent of degradation of the main fatty acids—oleic and linoleic acids—present in edible fats and oils. In addition, it was found that the level of C8:0 + C7:0 in used frying fats—at the rejection limit of 25% polar content—ranged from 1.8 to 2.4 mg/g (Márquez-Ruiz and Dobarganes, 1996) whereas the level of total oxidized FAME found in the samples was between 28.3 and 37.7 mg/g (Márquez-Ruiz et al., 1995).

With respect to analysis of aldehydic acids and diacids, quantification of FAME derivatives has been reported (Velasco et al., 2004b, 2005). These authors showed the detailed quantification of the six major compounds (C7:0, C8:0, 8-oxo-8:0, 9-oxo-9:0, C8:0 diester, and C9:0 diester) in thermal-oxidized olive and sunflower oils as well as in used frying fats of different sources supplied by Food Inspection Services. Among them, the most abundant compounds in thermal-oxidized olive and sunflower oils were C8:0 and 9-oxo-9:0, which were derived from the 9-hydroperoxide of unsaturated fatty acids. A more significant participation of oleic acid, as the level of alteration increased, led to higher formation of the compounds derived from the 8-hydroperoxide. Used frying oils with total polar contents ranging from 18.8 to 55.5 g/100 g oil showed levels of total short-chain compounds between 2.13 and 7.56 mg/g oil. In samples with polar content around 25 g/100 g oil, the content of total short-chain compounds was close to 3 mg/g oil. These results demonstrate that these compounds were not major components of oxidized monomers in frying fats and oils as compared with epoxy, keto, and hydroxy FAME (Velasco et al., 2004b).

D. Fatty Acid Polymers

Formation of dimers and polymers during frying is also associated with the autoxidation process. They represent excellent chemical markers of oil degradation at high temperature. After obtaining the FAME derivatives, three major groups of compounds stand out: nonpolar dimers, polar dimers, and higher oligomers. The scarce information available on specific compounds and on the mechanisms of polymerization reactions has been obtained working with FAME heated under well-defined conditions, either in the absence or in the presence of air. Detailed results can be found in excellent general reviews (Figge, 1971a,b).

a. Nonpolar Dimers

Mechanisms and reactions participating in the formation of nonpolar dimers, that is, compounds formed through C–C linkages without any extra oxygen in the molecule, were studied by using FAME subjected to experimental conditions different from those normally used in frying, that is, temperatures between 200°C and 300°C and absence of air to inhibit oxidative reactions. Mass spectra of isolated dimers before and after hydrogenation gave clear evidence of the number of double bonds and rings present in the original structures by determining the parent mass peaks. Additional evidence of isomeric forms was obtained from the pattern of fragments observed (Sen Gupta, 1969; Wheeler et al., 1970; Dobarganes and Pérez-Camino, 1987; Dobarganes, 1998).

The two main routes described for nonpolar dimer formation are radical reactions involving the allyl radical and Diels Alder reactions when a conjugated double bond is present in the molecule.

Noncyclic, bicyclic, and tricyclic dimers as well as Diels Alder dimers in hydrogenated soybean oil used for frying have already been reported (Christopoulou and Perkins, 1989c). The presence of Diels Alder dimers in the low-polarity fraction isolated from heated soybean oil FAME had previously been reported by Ottaviani et al. (1979). However, at frying temperatures (below 200°C) dehydrodimers (two fatty acids linked by formation of a new bond adjacent to the double bonds) are by far the major compounds (Dobarganes et al., 1984b; Dobarganes and Pérez-Camino, 1987).

b. Polar Dimers

The structure of polar dimers is still largely unknown due to the following facts. First, different oxygenated functions are likely to be present in oxidized monomers before dimer formation, or generated by the oxidation of nonpolar dimers. Second, more than one functional group can be present in the same dimeric molecule. Finally, the oxygen may or may not be involved in the dimeric linkage. Therefore, the large number of possible combinations results in a complex mixture that is difficult to separate. Under these circumstances, studies have paid more attention to defining the composition of alteration products than to elucidating the mechanisms involved in dimer formation.

The basic knowledge on polar dimers has been obtained by heating FAME, triglycerides or fats and oils in the presence of air, or by thermal decomposition of FAME hydroperoxides. Perkins and Kummerow (1959) reported the presence of three different polar dimers corresponding to dihydroxy, trihydroxy, and tetrahydroxy dimers with C–C linkages when they subjected corn oil to thermal oxidation (200°C, 48 h) with forced aeration.

Systematic studies have been performed to increase knowledge of dimeric structures (Christopoulou and Perkins, 1989a–c). Thus, dimers from methyl stearate-containing hydroxy and keto groups were synthesized to represent structures that may be formed during the thermal oxidation of fats (Christopoulou and Perkins, 1989a). Dimers were isolated from used frying fats by size-exclusion chromatography and further separated by gas chromatography and high-performance liquid chromatography (HPLC). Identification by gas chromatography/mass spectrometry provided evidence of the presence of monohydroxy, dihydroxy, and keto groups in the C–C linked dimers of methyl linoleate, together with the previously mentioned structures of nonpolar dimers (Christopoulou and Perkins, 1989c).

c. Higher Oligomers

Definite structures for compounds with molecular weight higher than dimers have not been reported, neither in methyl esters from frying fats nor in FAME model systems. This is not strange, considering that much more research remains to be carried out on structure elucidation and quantification of simpler molecules, that is, oxidized monomers and dimers, which are intermediates in trimer and higher oligomer formation. Moreover, the potential number of different structures in trimer formation increases exponentially with respect to those compounds of lower molecular weight, as many different dimeric structures may interact with many different monomeric structures. Furthermore, the limitation of the techniques for isolation, separation, and identification increases. Nevertheless, some information has been reported on the chemical composition of the residual oligomeric fraction obtained after distillation of dimer methyl esters, solvent fractionation of polymeric material, or a combination of chromatographic techniques. In general, the results reported indicate that the polymers formed were essentially FAME dimers and trimers joined through C–C and C–O–C linkages (Williamson, 1953; Chang et al., 1978; Ottaviani et al., 1979). However, when drastic conditions were applied, even FAME pentamers were found in significant amounts (Perkins and Kummerow, 1959).

At present, the total content of triglyceride dimers and higher oligomers is quantified by HPSEC starting from the oil (IUPAC, 1992b) (Figure 20.4b). Results obtained from numerous samples have clearly shown that polymeric triglycerides constitute the major fraction among the different groups

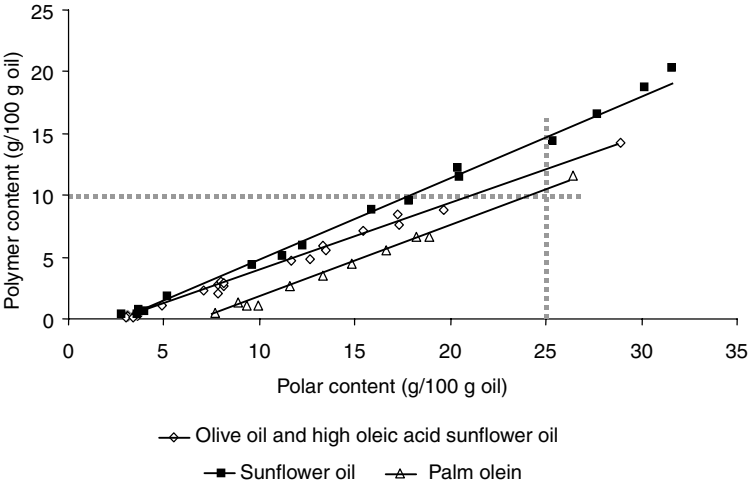

FIGURE 20.10 Relationships between polar compound and polymer contents in vegetable oils of different degree of unsaturation. (Modified from Sánchez-Muniz, F.J., and Bastida, S., *Forum Nutr.*, 56, 345, 2003. With the publisher permission.)

of alteration compounds formed during frying, normally accounting for more than 50%, and their levels have been found to correlate well with those of polar compounds (Perrin et al., 1985; Masson et al., 1997). Sánchez-Muniz and Bastida (2003) found that the 25% polar material content in palm olein correspond to a polymer level of about 10%, while the same polar material level in sunflower oil approximately correspond to a 15% polymer content. In monounsaturated oils, 25% polar content correspond to about 12% polymer content (Figure 20.10).

Some European countries have selected the level of polymers (10% or 16%) as a cut-off point, after which oil must be discarded (Firestone, 1996). Given the simplicity of the HPSEC technique, determination of polymers has been recently proposed as a general good method to control the quality of used frying fats. A maximum content of 12% has been suggested for discarding used frying fats (DGF, 2000).

Identical methodology is applied for quantification of dimers and oligomers starting from FAME derivatives, and finally combination of adsorption chromatography and HPSEC allows independent quantification of three groups of FAME (i.e., nonpolar dimers, polar dimers, and higher oligomers) (Márquez-Ruiz et al., 1990, 1995). However, owing to the complexity of the polymerization compounds, quantification of specific structures of dimers and oligomers has not been possible until now. It is expected that improvements in liquid chromatography systems contribute to the increase in the knowledge on this group in the near future.

As an example, Table 20.4 summarizes the most complete information that can be obtained for modified fatty acids in used frying oils and fats (Márquez-Ruiz et al., 1995; Velasco et al., 2004a,b, 2005). The results correspond to the analysis of samples supplied by Food Inspection Services with levels of polar compounds around the limit of used frying oil rejection (25% polar compounds). As can be observed, the level of modified fatty acids is around 10%. From this total content, around 65% correspond to FAME dimers and higher oligomers and 35% to FAME monomers. Among the latter group, epoxy, keto, and hydroxy FAME are major compounds while the short-chain acids are in much lower amounts.

IV. FAT AND OIL TRANSFER DURING FRYING

Food quality is greatly affected by the quality of used frying fats and oils although the influence of the food being fried on the quality of the frying oil may be of similar importance. Frying implies a

TABLE 20.4
Quantification of Different Groups of Fatty Acids (g/100 g Oil) in Used Frying Fats and Oils around the Limit of Rejection (25% Polar Compounds)

Polar Compounds	Polar Fatty Acids	Fatty Acid Polymers	Fatty Acid Nonpolar Dimers	Fatty Acid Polar Dimers	Fatty Acid Oxidized Monomers	Epoxyacids	Ketoacids	Hydroxyacids	Short-Chain n-Oxoacids	Short-Chain Diacids
23.1	8.1	0.8	3.9	2.0	3.3	0.9	0.4	1.2	0.09	0.13
25.5	10.4	1.1	3.1	2.8	3.4	1.2	0.5	1.5	0.08	0.12
25.7	10.5	1.3	3.0	2.8	3.4	1.1	0.5	1.3	0.08	0.07
26.4	10.8	0.7	3.8	2.9	3.4	1.0	0.6	1.5	0.10	0.09
27.5	10.7	1.1	3.4	2.8	3.4	1.2	0.5	1.3	0.09	0.10
27.6	11.3	1.1	3.8	2.7	3.7	1.4	0.8	1.4	0.13	0.08

Sources: Adapted from Márquez-Ruiz, G., et al., *J. Am. Oil Chem. Soc.*, 72, 1171, 1995; Velasco, J., et al., *J. Agric. Food Chem.*, 52, 4438, 2004a; Velasco, J., et al., *J. Agric. Food Chem.*, 53, 4006, 2005. With the publisher permission.

mass transfer involving a partial exchange between the oil in the pan/fryer and the water/fat in the interior of the food (Ramanna and Sen, 1983; Varela, 1988) (Figure 20.2).

In this section, general modifications on both food fatty acid content and profile and the fatty acid transfer between the food fat and the cooking oil/fat will be described.

A. Food Fatty Acid Changes

The selection of oil seems to be a very important fact because the profile of the fried food may, thus, reflect a more suitable final saturated/monounsaturated/polyunsaturated fatty acid ratio (Sánchez-Muniz et al., 1992a; Sánchez-Muniz and Bastida, 1997; García-Arias et al., 2003).

García-Arias et al. (2003) studied the changes produced by deep-fat frying in the fatty acid composition of chicken meat and chicken-based products, and found that frying with olive oil and sunflower oil increased the percentage of oleic acid and linoleic acid, respectively, in chicken meat. These increases were higher in chicken burgers than in chicken sausages. According to that study, the final fat and fatty acid content of fried chicken and fried chicken-based products depends on (1) the fat content of the raw product, (2) the presence of an edible coating that limits the penetration of fat during frying, and (3) the composition of the oil used.

Sardines fried in olive oil became greatly oleic acid-enriched, while their n-6/n-3 ratio varied moderately from that of their raw counterparts. Sardines fried in sunflower oil, on the other hand, displayed a significant increase in the linoleic acid content and with the n-6/n-3 ratio varying greatly (Sánchez-Muniz et al., 1992a).

Results of these two last studies are of nutritional importance because, according to Varela and Ruiz-Roso (1998), a large percentage (more than 50%–60%) of the fat consumed in the Mediterranean countries comes from culinary oils/fats. Cooking performed with monounsaturated oils enriches the diet in oleic acid, whereas frying with corn, sunflower, safflower, or soybean oils produces linoleic acid-enriched diets. In the same way, culinary use of saturated oils such as palm olein, employed extensively in the sweet roll or prefried food industry (Pantzaris, 1999) implies a palmitic acid enrichment of the diet (Cuesta et al., 1998).

These findings are very important because lipoprotein metabolism, and therefore the cholesterol and low-density lipoprotein (LDL) level, can be modulated by the type of fatty acids available in the liver, which in turn depends on the predominant dietary fatty acids consumed (Dietschy, 1998). According to Dietschy (1998), unsaturated fatty acids (oleic acid more than linoleic acid) increase gene expression of LDL receptors, maintaining the amount and activity of these receptors high and, thus, decreasing the concentration of serum LDL, while palmitic acid in the liver maintains gene expression of LDL receptors low and serum LDL concentration high.

As frying thoroughly changes the fatty acid composition of food, Sánchez-Muniz et al. (1992b, 1996) studied whether the hypolipidemic properties of fatty fish remain intact after frying. Data of both studies indicate that consumption of fried sardines by hypercholesterolemic rats induced both hypotriglyceridemic and hypocholesterolemic effects. Moreover, hypercholesterolemic rats fed fried sardines displayed much lower levels of some cell-damage markers than their respective basal counterparts; probably related to a more balanced n-6/n-3 ratio in cells of rats given the fried sardine diet. It has also been found that growing Wistar rats recover more quickly from hypercholesterolemia when they were fed whole olive oil-fried sardines as the only dietary source of protein and fat, instead of casein plus fat extracted from olive oil-fried sardines or casein plus olive oil (Sánchez-Muniz et al., 2003).

Cooking–freezing–reheating (CFR) has become an alternative system of handling foods in catering, where the prepared food is either frozen or chilled before the reheating procedure is carried out shortly prior to eating (Skjöldebrand, 1984). Thus, in addition to the frying itself, other culinary maneuvers can modify the food fatty acid composition. García-Arias et al. (2003) studied the effect of CFR on fat content and fatty acid composition of sardine fillets using three different ways of cooking (frying, oven-baking, and grilling) and two reheating systems (conventional and

microwave ovens). Both cooking and freezing–reheating significantly affected the fat content. Frying significantly affects the fatty acid composition of sardine, increasing oleic acid and linoleic acid contents. Freezing–reheating significantly affected the fatty acid composition of sardine, increasing oleic and linoleic acids and decreasing eicosapentaenoic and docosahexaenoic acids. Oven-baking and grilling minimally affected the fatty acid content. Freezing–reheating significantly affected the fatty acid composition with the content of oleic acid increasing and those of the n-3 fatty acids decreasing more in microwave oven-reheating than in conventional oven-reheating. Thus, according to the positive effect attributed to n-3 fatty acids, cooked samples with no further treatment would be preferred to their respective CFR counterparts. However, oven-reheating should be used instead of microwave oven-reheating when the CFR system is performed.

B. Fat Interchange during Deep Frying of Fatty Foods

From a quantitative point of view, composition of used frying oils and fats is mainly affected by mixture with fats or lipids from foods that migrate to the frying medium. Two different types of fatty foods can be considered separately; on one hand, fresh fatty foods, that is, fish or meat, normally coated by batter or bread before frying, and hence characterized by a low content of lipids on their surface and, on the other hand, the increasing group of frozen prefried foods (potatoes, fish, vegetables, etc.) where the fat or oil used for prefrying is mainly located on the surface. Lipid exchanges as well as final lipid composition and quality might be very different in both groups.

a. Fresh Fatty Foods

As previously commented, during frying the crust formation occurs. Great differences can be found in lipid composition depending on the method of frying, type of food, and surface composition (i.e., batter, bread, or uncoated food) (Figure 20.3). It has been found that battered fish absorbed less fat than breaded fish. The reason seems to be the rapid formation of a crust that prevents the transfer of oil and water into the battered food while the amount of coating in breaded fish is much lower and less protective (Makinson et al., 1987).

As a consequence of a variable fat absorption, it is difficult to foresee the fatty acid composition of the final product. The higher the amount of oil absorbed, the better the final lipid composition reflects that of the frying fat and the higher the energy density of the food.

During frying, changes in fatty acid composition of used frying oils occur (Dobarganes et al., 2000b) (Figure 20.2). Thus, food can be enriched in some altered and nonaltered fatty acids during frying; therefore, the altered compounds present in it. This fact can change both food digestibility and nutrient utilization (Cuesta et al., 1988; Márquez-Ruiz et al., 1993; Márquez-Ruiz and Dobarganes, 2006; González-Muñoz et al., 1998, 1999, 2003). Moreover, the altered compounds undergo intestinal absorption and thus may present potential toxicity. For general information on this subject see reviews (Márquez-Ruiz and Dobarganes, 2006; Sánchez-Muniz and Sánchez-Montero, 1999).

Some authors (Garrido-Polonio et al., 1994; Pokorný, 1998, 1999) have suggested that slightly higher concentrations of these altered compounds are present in food than in the oil media. Pokorný (1999) indicated that oxidized and other polar lipids enter the fried material relatively more easily than the original frying oil.

The possible diffusion of food lipids into the frying oil during the process is also important from the nutritional point of view. Lipid interchange is obvious in foods such as meat or chicken whose lipid content after frying might even be lower than before frying (Henry, 1998) but exchanges occur as a general fact during frying of fatty foods, as demonstrated by the presence of minor compounds (i.e., cholesterol, phospholipids, vitamins, etc.) of food lipids in the frying oil. Thus, it has been clearly shown that fatty fish release fat during frying (Sánchez-Muniz et al., 1992a). As fish oil contains long-chain polyunsaturated fatty acids, the frying oil can be relatively rapidly deteriorated when frying. Nonetheless, frying with lard or sunflower oil increased the oxidized FAME more

in the fryer oil than when frying with olive oil (Sánchez-Muniz et al., 1990, 1992a). Hamburgers contain high percentage of fat as well, which may be delivered to the frying oil. During frying of animal foods, cholesterol can diffuse to the frying oil/fat and contaminates it with decomposition or oxidation products as well.

Surprisingly, there is no information on lipid transfer during frying of fish in detailed studies (May et al., 1978; Gall et al., 1983; Sánchez-Muniz et al., 1992a) even though lipid exchanges can be easily calculated from the fat content and fatty acid compositions of the foods before and after frying (Pérez-Camino et al., 1991). Reinterpretation of the results obtained from the quoted papers would indicate that a significant percentage of the initial food lipids had passed into the frying medium, mainly from fish with high lipid content. A similar conclusion is deduced from the analysis of fatty acid composition of used frying oils after a high number of frying operations (Sébédio et al., 1993). Consequently, from a nutritional point of view, great care should be taken regarding the composition of the dietary fat ingested as both absorption of the frying oil and migration of lipid into the fryer would contribute to modify the fatty acid composition of the finished fried product, which may be far different from that of the initial food.

Transfer of polar substances and their degradation products from the substrate to the frying oil may either protect the oil against oxidation or increase its alteration level (Sánchez-Muniz and Bastida, 2006). Transport of substances through the surface of fried substrate is inhibited by batter; however, particles separated from the batter in oil soon get deep colored by pyrolitic reactions, and contribute to frying oil degradation.

b. Frozen Prefried Foods

Lipid exchanges in frozen prefried foods are of special interest as the lipid constituents have two specific characteristics:

1. Prefried products contain significant amounts of absorbed used frying fat or oil of unknown composition and quality, depending on the variables of the prefrying process.
2. As a consequence of the previous frying process, the oil is preferably located in the external layers of the food and thereby is in close contact with the frying oil during the second frying operation.

Fat absorption, lipid exchange, and possibilities of preferential adsorption of polar compounds on food surface during frying of frozen prefried foods have been studied in detail (Thomson and Aust, 1983; Sébédio et al., 1990; Pérez-Camino et al., 1991; Pozo-Díaz et al., 1995; Romero et al., 2000a; Cuesta et al., 2001).

Thus, similar fatty acid profiles were found for the oil and the food fried in it, indicating that lipid exchange was very high. When the fatty acid profile of the frozen prefried food and that of the oil used for frying were very different, both the initial lipids remaining in the fried product and those being transferred to the frying oil could be calculated with high accuracy. Irrespective of the oil used and of the prefried food subjected to frying, more than 90% of the fried food lipids came from the frying oil while more than 85% of the prefried food lipids were released into the frying oil (Pérez-Camino et al., 1991). It has also been reported in prefried frozen potatoes that after being fried with high-oleic acid sunflower oil showed that 89%–93% of their fat belonged to the frying oil, while frozen potatoes transferred between 76% and 84% of their initial fat to the bath oil. These figures were 90%–93% and 55%–70%, respectively, when frying with sunflower oil (Romero et al., 2000b; Cuesta et al., 2001).

With respect to the preferential adsorption of polar compounds, no differences was found between the fat food and the oil; although slightly higher values have been reported for the food fat. Thus, Romero et al. (2006b) show that polar material compounds in high-oleic sunflower oil and in the fat extracted from frozen potatoes fried in this oil changed very similarly in repeated fryings

whereas in the case of conventional sunflower oil, the fat extracted from frozen potatoes fried in sunflower oil remains about 1.2–2.5 mg/100 mg higher than in the oil counterpart. Nonetheless, polymers changed very similarly in oils and in the fat extracted from fried potatoes. These results show the benefits of frying potatoes in the monounsaturated oil with respect to the polyunsaturated one because less alteration compounds are formed in the oil and absorbed by the food during frying.

No preferential adsorption of *trans* fatty acids can be deduced from the study of Romero et al. (2000a), although the content of these fatty acids tend to be higher in the fat extracted from frozen potatoes fried in different oils. A percentage of these fatty acids already present in the raw frozen prefried food are retained by the food during the frying. Thus, differences between fried food and oil/fat are not related to *trans* fatty acid formation during frying and/or preferential adsorption. Nonetheless, *trans* fatty acids appear in lower amounts than 5 mg/g in frozen potatoes fried in different oils but data suggest that frequent addition of fresh oil vs. the null addition minimizes the fatty acid alteration contributing to obtain fried foods with less amount of *trans* fatty acids. The consumption of a large ration of those fried potatoes would imply the irrelevant amount of less than 0.13 g of *trans* fatty acids.

Altered fatty acids increased linearly through 20 fryings within frequent and null oil additions in the frying oil and in the fat extracted from fried frozen foods. As expected, changes tend to be higher in the extracted fat of food fried by the null oil addition modality as a consequence of the absence oil renovation and fat exchange between the frying oil and the food. In addition, changes tend to be more evident in the food-extracted fat (Romero et al., 2000b; Cuesta et al., 2001).

Table 20.5 shows changes in significant fatty acids after frying prefried potatoes, prefried hake, and sardines in olive oil to remark the differences between fresh fatty foods and prefried foods. As can be observed, the amounts of selected fatty acids in the fried potatoes and hake were very similar to those present in olive oil while in the case of sardines almost 50% of their characteristic fatty acids ($C_{14:0}$ and $C_{22:6}$) remained in the fried product (Pérez-Camino et al., 1991; Dobarganes et al., 2000b).

C. Food Matrix–Cooking Oil/Fat Interaction

Much attention has been paid to changes occurring in the frying oil but much less to changes occurring in the fried substrate due to interactions between frying oil and food matrix. Thus, apart from oil thermal oxidation and fat exchange, the free peroxy radicals and hydroperoxides, as well as

TABLE 20.5
Quantification of Significant Fatty Acids (g/100 g Lipids) before and after Frying Different Fatty Foods in Olive Oil

Sample	C14:0	C18:1	C18:2	C22:6
Before frying				
Olive oil	—	76.3	8.3	—
Prefried potatoes	—	40.9	2.9	—
Prefried hake	—	28.9	52.8	—
Sardines	6.6	13.2	1.2	11.5
After frying				
Potatoes	—	73.4	7.9	—
Hake	—	72.3	11.2	—
Sardines	3.1	47.9	2.8	6.0

Source: Adapted from Pérez-Camino, M.C., et al., *J. Food Sci.*, 56, 1644, 1991; Dobarganes, M.C., et al., *Eur. J. Lipid Sci. Technol.*, 102, 521, 2000b. With the publisher permission.

other secondary oxidation products may react with the substrate, particularly with thiol, sulfide, disulfide, and primary amine groups of proteins. They are deactivated, and partially remain attached to the protein moiety (Pokorný, 1998, 1999). The protein matrix has great effect on fat transfer. Hamburgers have relatively unstable fat fraction and course protein matrix, thus an easy fat uptake can be expected. In sausage, on the contrary, the fat fraction is relatively stable and meat protein matrix is dense; thus, much lower fat exchange and fat–matrix interaction may occur (Tornberg et al., 1989).

In addition, starch present in vegetable react with oxidized frying oil as well as polypeptides or protein, forming interaction products where triglycerides are bound to starch macromolecules in several ways (i.e., by ether bonds) (Fedelli et al., 1983). According to Pokorný (1998), the frying oil was oxidized more rapidly when frying starch-rich substrates than when frying protein-rich ones. This inhibition could be due to reactions of lipid peroxides with amino acid residues.

V. FINAL REMARKS

Evaluation of all changes occurring in fats and oil fatty acids during frying is a difficult task due to the complexity of the reactions involved and the great variety of compounds formed depending on the frying conditions. Still, efforts made during the past two decades to develop chromatographic techniques have enabled us to gain useful information on the nature and levels of modified fatty acids. Overall, used frying oils and fats close to the limit of rejection, with levels of polar compounds around 25%, contain normally levels of around 10% modified fatty acids. Even though the major part of them is composed of fatty acid dimers and higher oligomers, a relevant proportion are oxidized fatty acid monomers. Among the latter group, epoxy, keto, and hydroxy function compounds are predominant while short-chain acids are present in much lower amounts. In this context, much work remains to be carried out to clarify the specific structures of the new modified fatty acids resulting from frying and to evaluate their nutritional implications at the levels normally found in used frying fats and oils and fried products.

As to the frying methods, while information is abundant on deep-fat frying performed in fryers, less data are available on pan frying, which is the frying method most commonly used in household. Moreover, determination of differences between shallow and deep frying has to be studied in more detail. The accessibility of oxygen is much greater in the former inducing probably earlier hydroperoxide formation.

On the other hand, changes produced in the fryer oil have been more extensively studied than those occurring in food nutrients and minor compounds, or interactions between the frying oil and the food fat and matrix. Since it has been reported that the alteration level could be similar in the fat food than in the corresponding frying oil, special care should be taken when performing frying or cooking with low-stability oils. It is clear that frequent addition of fresh oil is recommended because it decreases the oil alteration, and hence helps to obtain safer foods. Finally, frying with monounsaturated oils rather than with polyunsaturated ones is also recommended to get an adequate balance between nutritional properties (e.g., fatty acid composition) and thermal oxidation stability.

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21 Consumption of Fatty Acids

Sue Joan Chang and Ching Kuang Chow

CONTENTS

I. INTRODUCTION

With improving economy and abundant food supply, excess food intake has been strongly linked to the increasing epidemic proportion of overweight or obesity in the United States in recent decades. Obesity is an important risk factor contributing toward the development of leading causes of death, such as cardiovascular disease, cancer, and diabetes, in the United States (Engelhart et al., 2002; Ernst et al., 1997; Hu et al., 2000; U.S. Department of Health and Human Services, 2001). The medical expenses and lost productivity resulting from these diet-related diseases are already very high, and are continuously increasing at a rapid rate (Heffler et al., 2002; Westerhout, 2006).

Dietary fat serves a number of important functions and is essential for normal growth and maintenance. However, the energy density of fat (9 kcal/g) is much higher than that of protein and carbohydrate (4 kcal/g). Therefore, dietary fat is often blamed as the major source of excess energy, although it is difficult to differentiate the effects of dietary fat and other energy nutrients independent of total energy intake (Arab, 2003). Evaluating trends in energy nutrient intake is important in understanding the role of individual energy nutrient in the development of obesity and obesity-related illness over time. In addition to quantity, the type of dietary fat consumed is also an important factor contributing to the development of cardiovascular disease, cancer, diabetes, and other degenerative

diseases (Das, 2000; Grundy, 1997; Larsson et al., 2004; Slattery et al., 1997; von Schacky et al., 1999). This chapter focuses on the consumption of total fat and major fatty acids in the U.S. populations. For information concerning dietary *trans* fat, fish oil, and other specific types of fats/oils, please refer to the respective chapters in this volume.

II. NATIONWIDE DIETARY INTAKE SURVEYS

For over a century, the U.S. Department of Agriculture (USDA) has maintained a nutrition monitoring system for the U.S. population. A dietary surveillance program conducted by the Food Surveys Research Group, Agricultural Research Service (ARS), USDA, known as the *Continuing Survey of Food Intakes by Individuals* (CSFII), measures foods actually eaten by individuals over a specific period of time (e.g., two nonconsecutive 24-h dietary recalls in the CSFII 1994–1996) (U.S. Department of Agriculture, 1998; U.S. Department of Health and Human Services, 2006a,b). The survey also collects demographic information, such as household size, income, race, age, and sex, and information on where a food was purchased, how it was prepared, and where it was eaten. Therefore, CSFII data are useful in policy formation, regulation, program planning and evaluation, education, and research. The CSFII data provide up-to-date information on food intakes by Americans. In addition, CSFII monitors diet quality and changes over time in the food choices Americans make and the adequacy of their diet. This can be done, for example, by comparing intake data obtained from the CSFII 1994–1995, the Nationwide Food Consumption Survey (NFCS) 1977–1978, and the CSFII 1989–1991. NFCS is another food consumption survey program of USDA. The CSFII 1994–1996 targeted population in all 50 States vs. the 48 conterminous States in the NFCS 1977–1978 and CSFII 1989–1991.

Snice 1960, the National Center for Health Statistics, Division of Health Examination Statistics, Center for Disease Control and Prevention, and U.S. Department of Health and Human Services (USHHS) have conducted a unique series of health and nutrition surveys, known as *National Health and Nutrition Examination Surveys* (NHANES), to obtain health examination data periodically, and in recent years on a continuous basis. The major objectives of the NHANES include (1) estimation of the number and percentage of the population with selected diseases and risk factors; (2) monitoring of trends in the prevalence, awareness, treatment, and control of selected diseases; (3) monitoring of trends in risk behaviors and environmental exposures; (4) analysis of risk factors for selected diseases; (5) study of the relationship between diet, nutrition, and health; (6) exploration of emerging public health issues and new techniques; (7) establishment of a national probability sample of genetic material for future research; and (8) establishment and maintenance of a national probability sample of baseline information on health and nutritional status (U.S. Department of Health and Human Services, 2006a). NHANES provides an ideal setting for collecting high-quality representative data on the health and nutrition data of the U.S. civilian, noninstitutionalized population, and is the most comprehensive and best to represent the changing trend of nutrient intake in the United States.

The first NHANES includes data collected during 1971–1974, the second NHANES during 1976–1980, the third NHANES during 1988–1994, and the fourth one during 1999–2000. New nationwide dietary intake data for the years 2001–2002 have become available for public use. The first NHANES studied persons residing in the contiguous 48 states, and the subsequent NHANES deal with population in all 50 states. All of the surveys consist of a household interview followed by an examination at an examination center, and include a dietary recall interview conducted at the examination center to obtain information on all foods and beverages consumed during the preceding 24 h. The upper age limit is 74 years at the time of the household interview for the first two NHANES (1971–1974 and 1976–1980), and no upper age limit is established for the subsequent NHANES.

In order to examine the changes in dietary intake over time, the analysis of data obtained from the NHANES is restricted to subpopulations consistently sampled in the years available and for which the basic variables of interest are collected. Thus, the analysis included only adults aged

20–74 years to compare estimates across all surveys (Wright et al., 2004). Because of differences in the relative age distribution, estimates for persons aged 20–74 years are adjusted by direct standardization to the 2000 U.S. Census population by using the age groups of 20–39, 40–59, and 60–74 years. The recommended age categories used are based on the survey sample domains (U.S. Department of Health and Human Services, 2005). Persons who reported fasting during the preceding 24 h are excluded from these analyses. Sample weights and design variables are analyzed statistically to generate national estimates (U.S. Department of Health and Human Services, 2006b).

The two nationwide dietary intake surveys, USDA's CSFII and USHHS's NHANES, which collect national dietary intake data, were integrated in 2002 (Dwyer et al., 2001, 2003). Under the integrated framework, USHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. The new integrated survey, also known as *What We Eat in America*, is to collect dietary intake data on a continuous yearly basis. The collection process for foods in the integrated survey consists of an in-person 24-h recall using a computerized five-step method and a second nonconsecutive 24-h recall via telephone. A food frequency questionnaire, which was pilot-tested, is to provide information on the propensity to consume certain foods. Dietary supplement intakes over the past 30 days are assessed for all persons during the household interview (Dwyer et al., 2003). Strengths of the integrated survey include information on food and supplement intakes in a representative sample of the civilian noninstitutionalized population of the United States that can be linked to anthropometric, biochemical, clinical, and disease history information in NHANES (U.S. Department of Health and Human Services, 2006a,b). As discussed in *What We Eat in America*, food intake data are linked to health status data from other NHANES components, the relationship between dietary intake and health state can be explored.

The self-reported data obtained from CSFII and NHANES are subject to recall bias, and changes in the 24-h dietary recall interview method between two different periods may account for some of the differences in average energy intake occurred. In addition, beginning in 1988, dietary recall data are collected for weekend days as well as weekdays because food consumption differs on weekend days. The revised interview format and added questions may allow for collection of more complete dietary intake data than earlier surveys. Since 1980, the U.S. Government has published dietary recommendations, known as *Dietary Guidelines for Americans*, issued jointly by the USDA and USHHS (U.S. Department of Health and Human Services, 2005). On the basis of the information obtained from these dietary intake surveys, the Dietary Guidelines for Americans recommends a reduced consumption of total fat and saturated fat as a way to reduce the risk of chronic diseases.

III. INTAKE OF TOTAL FAT AND FATTY ACIDS IN THE UNITED STATES

The mean total energy intake from individual energy nutrients, carbohydrates, protein, and fats by weight, and percentage of energy contributed by individual energy nutrients, total fats, as well as by saturated, monounsaturated, and polyunsaturated fatty acids among U.S. population, obtained by the NHANES during the period of 1971–2002, and CSFII during 1994–1996, are summarized below.

A. Mean Total Energy Intake

The mean total energy intake in kilocalories (kcal) of the first four NHANES is shown in Figure 21.1. As expected, the mean total energy intake was higher in men than in women during 1971–2000. The average energy intake increased significantly from the period of 1971–1980 to 1988–2000 for both men and women. The average energy intake increased from 2450 kcal to 2618 kcal ($p < .01$) for men, and from 1542 kcal to 1877 kcal ($p < .01$) for women (Wright et al., 2004). The increase in caloric intake is consistent with previously reported trends in dietary intake in the United States

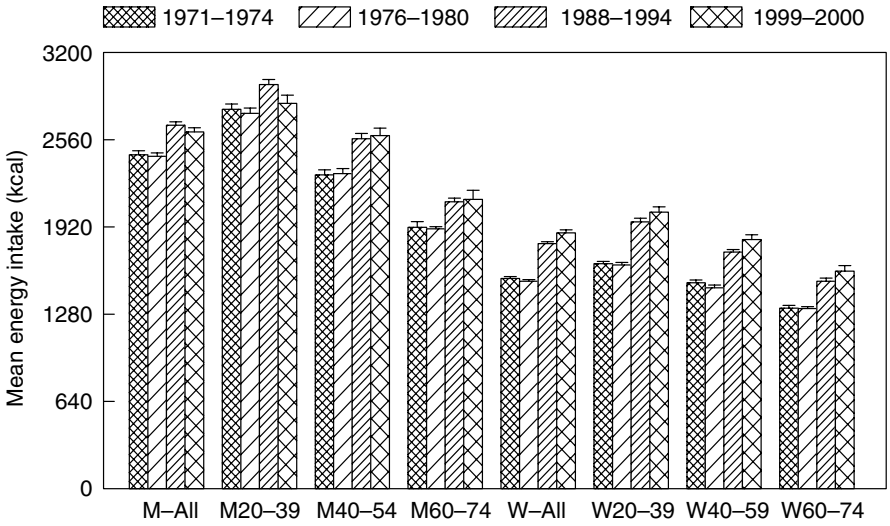

FIGURE 21.1 Estimated mean energy intake among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1976–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). M represents men; W, women; all, all subjects; 20–39, subject age 20–39; 40–59, age 40–59; and 60–74, age 60–74.

(Federation of American Societies for Experimental Biology, 1995). The estimated total energy intake includes kilocalories from alcoholic beverages, which ranged from 2.5% to 4.5% for men and from 0.5% to 2.0% for women (Wright et al., 2004).

B. Mean Energy Intake from Carbohydrates, Proteins, and Fats

The mean energy intake contributed by individual energy nutrients, carbohydrate, protein, and fats, in kcal is shown in Figure 21.2. The energy intake from carbohydrate increased from the period of 1971–1980 to 1988–2000 for both men and women, while energy intake from protein changed little during the same period. The mean energy intake from total fat and saturated fat decreased slightly for men, while those of women increased during the period. Similarly, USDA food consumption survey data from the CSFII 1989–1991 and CSFII 1994–1996 indicate that the increased energy intake was caused primarily by higher carbohydrate intake, with a 62.4-g increase among women ($p < .01$) and a 67.7-g increase among men ($p < .01$) (Chanmugam et al., 2003). The survey data for the period between 1977 and 1996 suggest that factors contributing to the increase in energy intake in the United States include increased consumption of food away from home or increased energy consumption from salty snacks, soft drinks, and pizza (Nielsen et al., 2002), and increased portion sizes (Nielsen and Popkin, 2003).

Similar trend was found for USDA's data on the disappearance of the fats and oils as well as domestic per capita consumption for fats and oils from 1970 to 1995 (Putnam and Allshouse, 1997). Although disappearance data tend to overestimate consumption, by keeping track of disappearance trends over time, relative changes in fats and oils consumed can be determined. In 1995, total fat and oil consumption was composed of approximately 85% vegetable oils and 15% animal fats. Per capita animal fat and oil consumption decreased 28% from 14.1 to 10.2 pounds between 1970 and 1995. During the same period, vegetable oil consumption increased 40% from 38.5 to 53.9 pounds. Thus, despite declines in animal fat consumption, total fat and oil consumption actually rose 22%

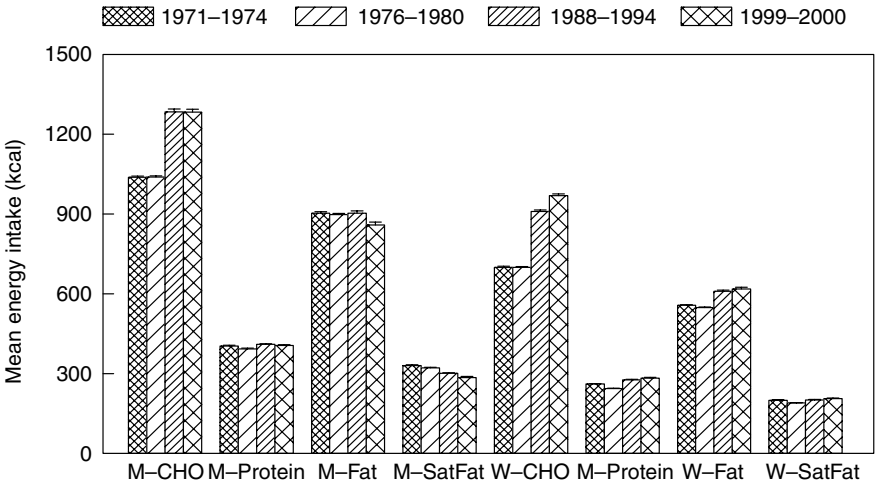

FIGURE 21.2 Estimated mean energy intake from carbohydrate, protein, total fat, and saturated fat among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1876–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). M represents men; W, women; CHO, carbohydrate; and SatFat, saturated fat.

from 52.6 to 64.1 pounds during this period. In addition, since 1970, butter per capita consumption decreased 17% to 4.5 pounds in 1995, and margarine consumption declined 15% from 10.8 to 9.2 pounds. Recent reports of the adversary biological effect of *trans*-fat, however, are likely to shift margarine consumption to butter (Allison et al., 1999).

C. Percentage of Energy Intake from Carbohydrates, Proteins, and Fats

The percentage of energy intake contributed by carbohydrates, proteins, and fats is summarized in Figure 21.3. The percentage of kcal from carbohydrate, between 1971–1974 and 1999–2000, increased from 42.4% to 49.0% ($p < .01$) for men, and from 45.4% to 51.6% ($p < .01$) for women. The percentage of kcal from protein decreased from 16.5% to 15.5% ($p < .01$) for men and from 16.9% to 15.1% ($p < .01$) for women between 1971–1974 and 1999–2000. The percentage of kcal from total fat decreased from 36.9% to 32.8% ($p < .01$) for men and from 36.1% to 32.8% ($p < .01$) for women. The decrease in the percentage of kcal from fat during 1971–1991 is attributable to an increase in total kcal consumed as absolute fat intake in grams was actually increased (Ernst et al., 1997). Total fat intake in grams increased among women by 6.5 g ($p < .01$) and decreased among men by 5.3 g ($p < .01$) during this period. Data from NHANES for 1971–2000 indicate a similar trend (Wright et al., 2004).

D. Percentage of Energy Intake from Total Fats

The percentage of energy intake contributed by total fats is shown in Figure 21.4. The percentage of energy from total fat changed from 36.9% (1971–1974) to 36.8% (1976–1980), 33.9% (1988–1994) and 32.9% (1999–2000) for men, and from 36.1% to 36.0%, 33.4% and 32.8% for women. For both men and women, the rate of decline accelerated between 1985 and 1990. Average daily fat consumption for women is 73.3 g in spring of 1977, and declined significantly by 3.7 g in 1985. Average fat consumption for women decreased significantly by an additional 7.5 g to 62.2 g per day

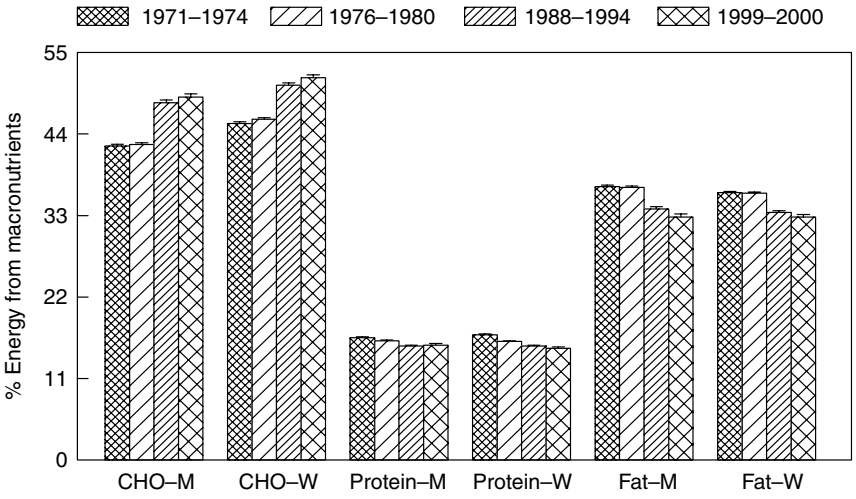

FIGURE 21.3 Estimated percentage energy intake from carbohydrate, protein, and fat among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1876–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). CHO represents carbohydrate; M, men; and W, women.

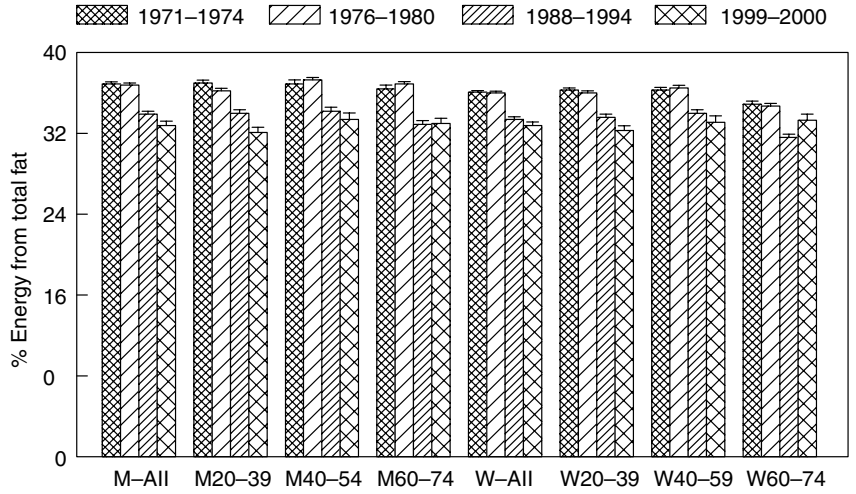

FIGURE 21.4 Estimated percent energy intake from total fat among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1876–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). M represents men; W, women; all, all subjects; 20–39, subject age 20–39; 40–59, age 40–59; and 60–74, age 60–74.

during 1989–1990. For men, fat consumption in summer 1977 was 112.8 g per day, and declined significantly by 5.3 g by 1985, and decreased to 92.6 g by 1989–1990 (Lichtenstein et al., 1998). In each of the age and gender groups reporting consumption of 30% or less of energy from fat, and less than 10% of energy from saturated fatty acids. Fat-modified foods play a more important role in their diets than for people who are consuming higher levels of fat and saturated fat. As a result

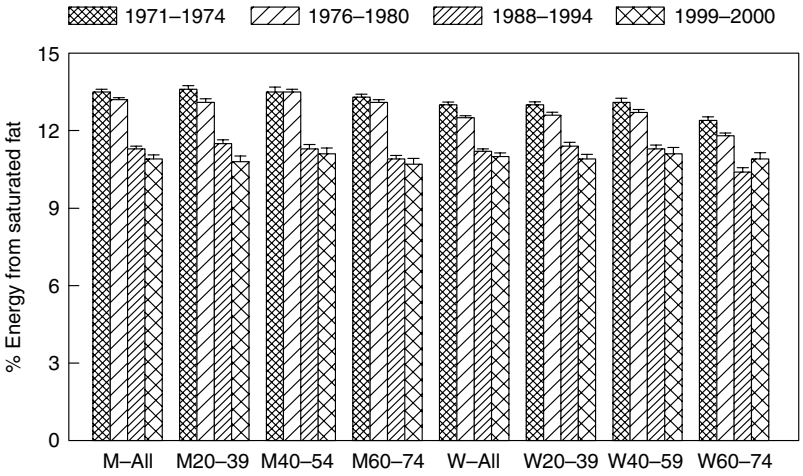

FIGURE 21.5 Estimated percent energy intake from saturated fat among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1876–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). M represents men; W, women; all, all subjects; 20–39, subject age 20–39; 40–59, age 40–59; and 60–74, age 60–74.

of increased consumer awareness of the link between dietary fat and risk of heart disease, annual per capita consumption of added fats and oils declined 7% between 1993 and 1997 (Putnam and Allshouse, 1997).

E. Percentage of Energy Intake from Saturated Fats

The percentage of energy intake derived from saturated fats is summarized in Figure 21.5. As the figure shows, estimated percentage of energy intake from saturated fats decreased from 13.5% in 1971–1974 to 10.9% in 1999–2000, and the decreases are similarly found in every age group for men. The trend is also true for women with the exception of the age group 60–74. Changes in saturated fat consumption are largely parallel changes in fat consumption. As with total fat, the absolute reductions in saturated fat consumption are larger during the 1976–1980 and 1988–1994 periods. For women, the average daily saturated fat consumption was 26.2 g in spring 1977 and 25.2 g in 1985. Consumption was decreased by an additional 3.5 g per day in 1985 to 21.7 g per day in 1989–1990. For men, the average daily saturated fat consumption declined by 1.0 g from 1977 to 1985 and an additional 7.2 g in 1989–1990 to 32.4 g (Lichtenstein et al., 1998).

F. Percentage of Energy Intake from Unsaturated Fats

The percentage of energy intake from unsaturated fats is summarized in Figure 21.6. As the figure shows, the trend of estimated percentage energy intake from unsaturated fat during 1971–2000 is similar to that of total fat or saturated fat, but with a relatively slower rate of decline. As more and more consumers are now more aware of the health consequences of the fats, they tend to include less saturated fat in their diets. Polyunsaturated and monounsaturated oils, rich in safflower, canola, and olive oil, have been suggested to be healthy choices for consumers who wish to reduce the risk of heart disease. Food disappearance data between 1985 and 1994 indicate that the ratio of n-6 to n-3 fatty acids has decreased from 12.4:1 to 10.6:1. This reduction reflects a change in the profile of vegetable oils consumed and, in particular, a big increase in canola oil use. However, the ratio of n-6/n-3 fatty acids remains much higher than the recommended value of 2.3:1 (Kris-Etherton et al., 2000).

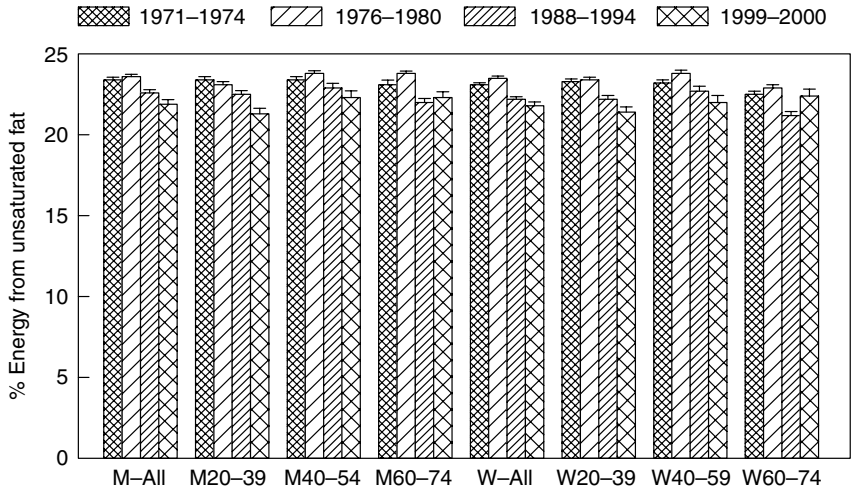

FIGURE 21.6 Estimated percent energy intake from unsaturated fat among U.S. adults aged 20–74. Data (mean ± standard error) are derived from NHANES (Wright et al., 2004). Sample sizes ranged from 4902 men and 7984 women in NHANES I (1971–1974), 5568 men and 6227 women in NHANES II (1876–1980), 6630 men and 7537 women in NHANES III (1988–1994), and 1730 men and 2003 women in NHANES IV (1999–2000). M, represents men; W, women; all, all subjects; 20–39, age 20–39; 40–59, age 40–59; and 60–74, age 60–74.

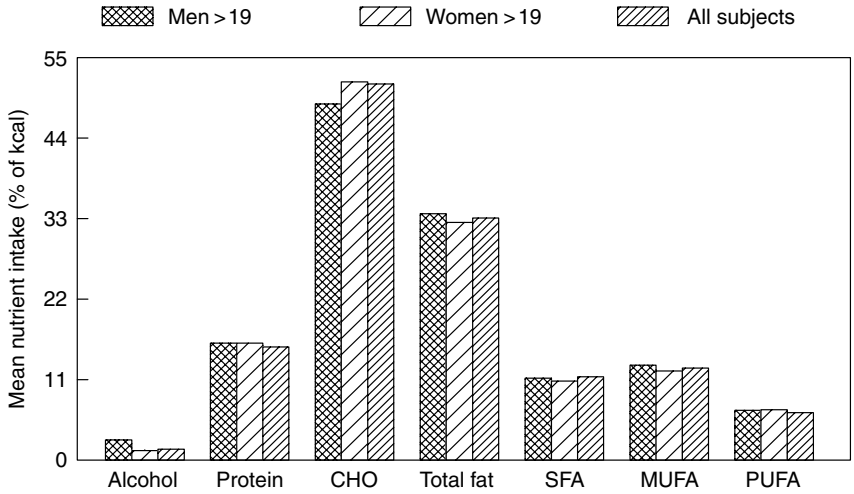

FIGURE 21.7 Mean percentage of calories from alcohol, protein, carbohydrate, and total fat, and from saturated, monounsaturated, and polyunsaturated fatty acids. The data are derived from USDA's 1994 CSFII (U.S. Department of Agriculture, 1998, 2004). Subjects were male aged 20 and over (Men > 19), female aged 20 and over (Women > 19), or all subjects (including those aged 19 and under). The mean food energy intakes were 2460, 1613, and 1985 kcal, respectively, for Men > 19, Women > 19, and All subjects. CHO represents carbohydrate; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

G. Percentage of Energy Intake from Total, Saturated, Monounsaturated, and Polyunsaturated Fats, and Alcohol

The percentage of energy derived from total, saturated, monounsaturated, and polyunsaturated fats, as well as from alcohol, carbohydrate, and protein is shown in Figure 21.7. The one day data is

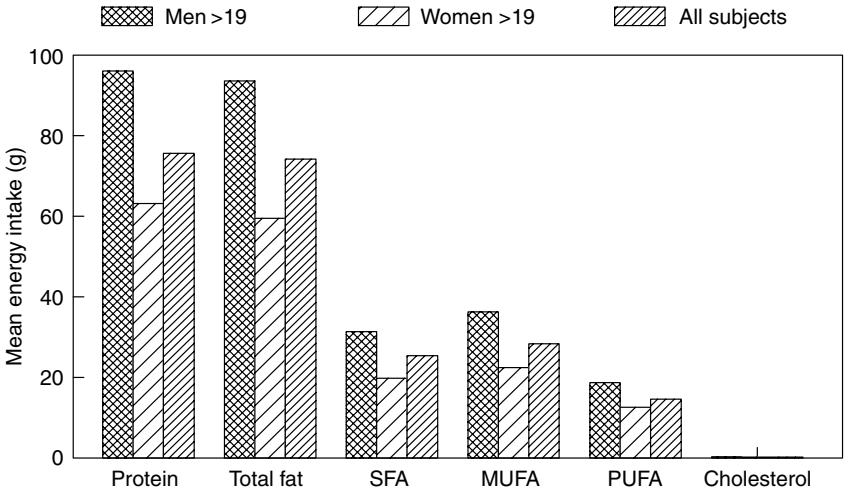

FIGURE 21.8 Mean energy nutrient intake from protein, total fat, and from saturated, monounsaturated, and polyunsaturated fatty acids, and cholesterol. The data are derived from USDA's 1994 CSFII (U.S. Department of Agriculture, 1998, 2004). Subjects were male aged 20 and over (Men > 19), female aged 20 and over (Women > 19), or all subjects (including those aged 19 and under). The mean food energy intakes were 2460, 1613, and 1985 kcal, respectively, for Men > 19, Women > 19, and All subjects. SFA represents saturated fatty acids; MUFA, mono-unsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

derived from USDA's 1994 CSFII. For men and women aged 20 and over, or all subjects (including those under 19), the mean percentage of kcal contributed by proteins, carbohydrates, total fats, and saturated, monounsaturated, and polyunsaturated acids are rather similar. The mean percentage of kcal contributed by carbohydrate is higher for women (51.7%) than men (48.7%) aged 20 and over. In addition, the percentage of kcal from alcohol is higher for men (2.8%) than in women (1.5%) aged 20 and over.

H. Energy Intake from Protein, Total Fat, and Saturated, Monounsaturated, and Polyunsaturated Fats, and Cholesterol

The averaged daily energy intake by weight (g/day) contributed by total, saturated, monounsaturated, and polyunsaturated fats, as well as by cholesterol and protein is shown in Figure 21.8. The one day data are derived from USDA's 1994 CSFII. For men aged 20 and over, the amounts contributed by proteins, cholesterol, total fats, and saturated, monounsaturated, and polyunsaturated acids are all higher than that of women aged 20 and over. Although all subject groups consumed more protein than total fat, the total energy intake contributed by total fat is about twice as much as that of protein. The relative amount of fatty acids in the dietary fat is monounsaturated > saturated > polyunsaturated. In terms of total energy intake, the contribution by cholesterol is insignificant.

I. Percentage of Energy Intake from Snack Foods

The mean percentage of nutrient intake as kcal contributed by foods eaten as snacks (including beverage breaks) is shown in Figure 21.9. The one day data are derived from USDA's 1994 CSFII. Of the mean food energy intakes, the percentage of calories contributed by food eating as snacks averaged 16.1%, 15.2%, and 17.1%, respectively, for men aged 19 and older, for women aged 19 and older, and for all subjects. Individuals eating snacks are 73.3% for men aged 20 and over, 73.5% for women aged 20 and over, and 75.3% for all subjects. The figure also shows that the percentage of calories contributed by snacks is higher in each nutrient intake category for subjects under 19 than

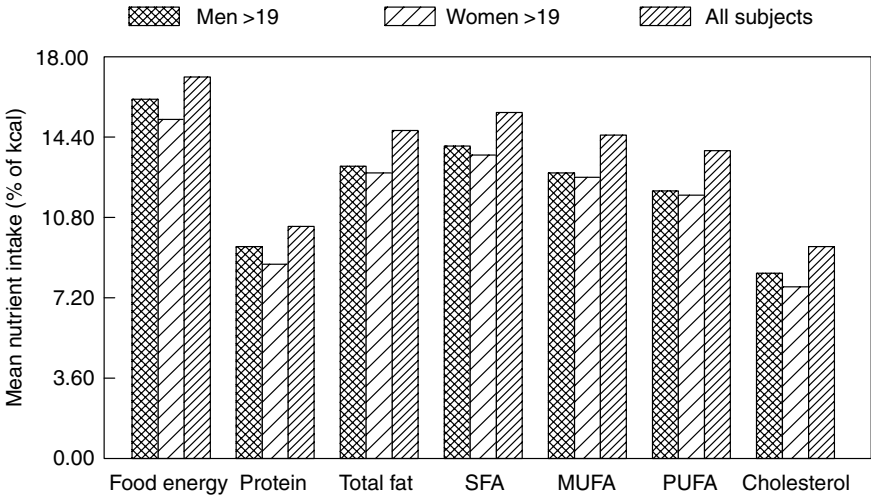

FIGURE 21.9 Mean percentage of nutrient intake as calories contributed by foods eaten as snacks. The data are derived from USDA's 1994 CSFII (U.S. Department of Agriculture, 1998, 2004). Subjects were male aged 20 and over (Men > 19), female aged 20 and over (Women > 19), or all subjects (including those aged 19 and under). The mean food energy intakes were 2460, 1613, and 1985 kcal, and percentage of individuals eating snacks were 73.3, 73.5, and 75.3%, respectively, for Men > 19, Women > 19, and All subjects. SFA represents saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

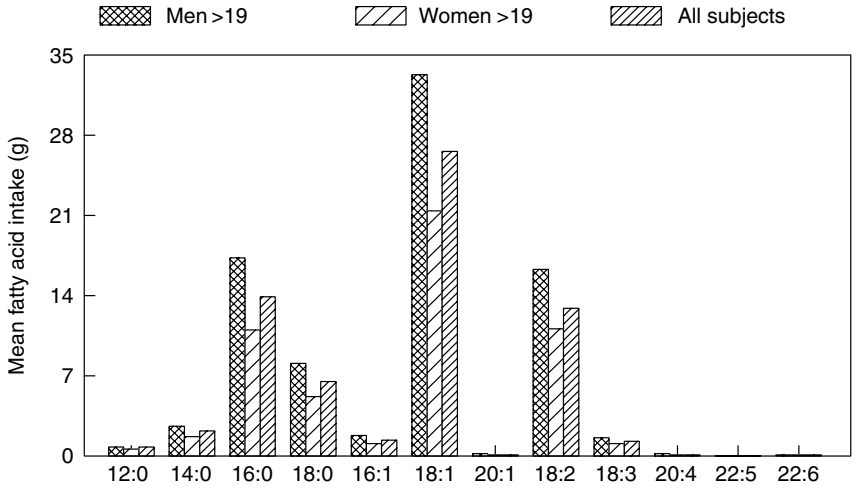

FIGURE 21.10 Mean fatty acid intake. The data are derived from USDA's 1994–1996 CSFII (U.S. Department of Agriculture, 1998, 2004). Subjects were male aged 20 and over (Men > 19), female aged 20 and over (Women > 19), or all subjects (including those aged 19 and under). The mean food energy intakes were 2460, 1613, and 1985 kcal, respectively, for Men > 19, Women > 19, and All subjects.

those aged 20 and over. In addition, for subjects aged 20 and over, men consume relatively more snacks than women.

J. Mean Intake of Major Fatty Acids

The mean one day intake of major fatty acids by grams derived from USDA's 1994–1996 CSFII is shown in Figure 21.10. Among the 16,103 participants, who were asked to recall food intake

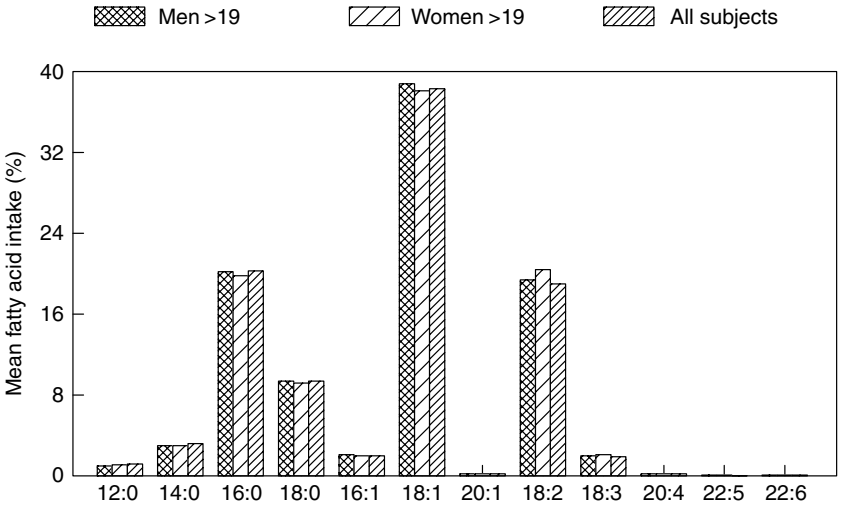

FIGURE 21.11 Mean percentage of fatty acid intake. The data are derived from USDA's 1994–1996 CSFII (U.S. Department of Agriculture, 1998, 2004). Subjects were male aged 20 and over (Men > 19), female aged 20 and over (Women > 19), or all subjects (including those aged 19 and under). The mean food energy intakes were 2460, 1613, and 1985 kcal, respectively, for Men > 19, Women > 19, and All subjects.

information for two separate days, of the CSFII 1994–1996, 5,765 of them were aged 20 and over. As the figure shows, men consumed relatively more fatty acids than that of women in every major fatty acid, and 18:1 is the most abundant fatty acid in the American diet. The next important ones, in decreasing order, are 16:0, 18:2, 18:0, 14:0, 16:1, 18:3, 12:0, and 20:4.

K. Mean Percentage of Fatty Acid Intake

The contribution of important fatty acids as percentage of total fatty acid in the American diet is shown in Figure 21.11. The one day data are derived from USDA's 1994–1996 CSFII. Similar to by weight, the percentage calories contributed by 18:1 is the highest, followed by 16:0, 18:2, 16:0, 14:0, 16:1, 18:3, 12:0, and 20:4. In addition, there is little difference among men and women aged 20 and over, and all subject.

L. Mean Polyunsaturated Fatty Acid Intake

The mean intake of the major polyunsaturated fatty acids, 18:2 and 18:3, by subjects of various age groups is shown in Figure 21.12. The data are derived from *What We Eat in America*, and NHANES 2001–2002. As expected, men consumed more 18:2 and 18:3 than women across all age groups, and all subject groups consumed much more 18:2 than 18:3 during this period.

M. Mean Fatty Acid Intake from Major Food Sources by Male Subjects

The important fatty acids derived from major food sources for male subjects aged 20 and over are shown in Figure 21.13. The one day data are derived from USDA's 1994–1996 CSFII. Meat and fish provide more 16:0, 18:0, and 18:1 than other food sources, and is a rich source for 18:2 for men aged 20 and over. Grain products are the richest source for 18:2 and a good source for 16:0, 18:0, and 18:1. Milk and milk products are good source for 16:0, 18:0, and 18:1, and legumes provide most 18:3.

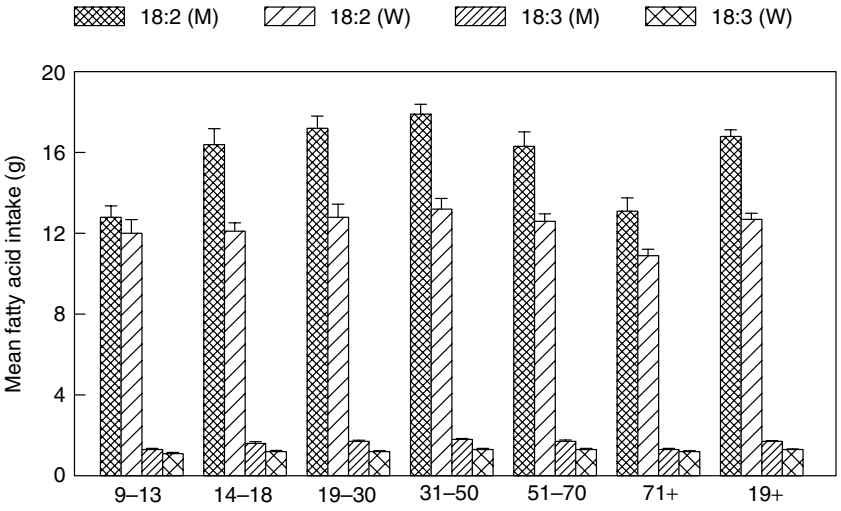

FIGURE 21.12 Mean polyunsaturated fatty acid intake from foods. The data are derived from *What We Eat in America*, NHANES 2001–2002 (U.S. Department of Health and Human Services, 2006a). Subject groups were male aged 9–13 (N = 574), 14–18 (N = 727), 19–30 (N = 552), 31–50 (N = 785), 51–70 (N = 651), 71+ (N = 392), and 19+ (N = 2380), and female 9–13 (N = 597), 14–18 (N = 677), 19–30 (N = 465), 31–50 (N = 754), 51–70 (N = 643), 71+ (N = 405), and 19+ (N = 2267).

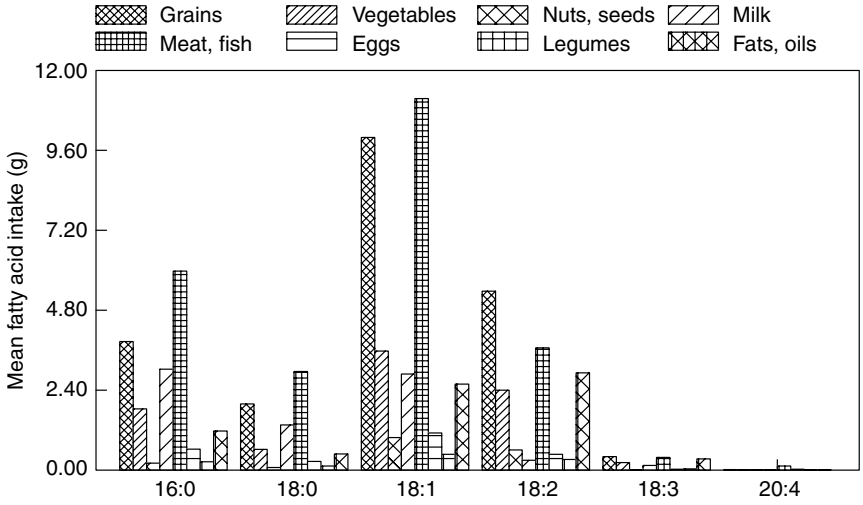

FIGURE 21.13 Mean fatty acid intake from major food sources by males 20 and over (N = 5056). The data are derived from USDA's 1994–1996 CSFII (U.S. Department of Agriculture, 1998, 2004). The category milk includes milk products, and meat includes poultry.

N. Mean Fatty Acid Intake from Major Food Sources by Female Subjects

The important fatty acids derived from major food sources for female subjects aged 20 and over are shown in Figure 21.14. The one day data are derived from USDA's 1994–1996 CSFII. Somewhat different from the male counterpart, grain products provide more 18:1 and 18:2 than other food sources, and are good source for 16:0 and 18:0 for females. In addition, meat and fish are the richest

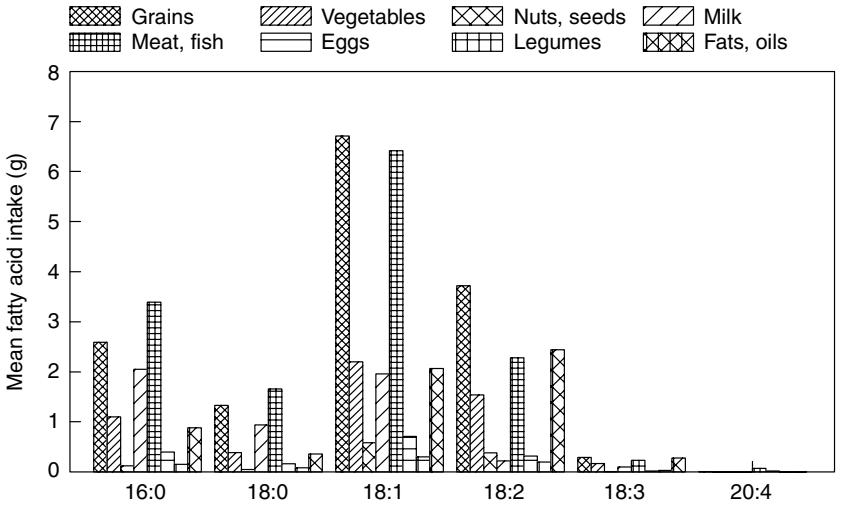

FIGURE 21.14 Mean fatty acid intake from major food sources by females 20 and older (N = 4816). The data are derived from USDA's 1994–1996 CSFII (U.S. Department of Agriculture, 1998, 2004). The category milk includes milk products, and meat includes poultry.

source for 16:0 and 18:0, and good source for 18:1, and fats/oils are rich source for 18:1, 18:2, and 18:3 for women.

A typical American diet consists of high calories contributed by fats, oils, and sweets. In 1995, Americans reported an average of 25% of daily calories from fats added in food or meal preparation and at the table. As total calories from fat averaged 33% of daily calories, most fats consumed are added at the discretion of food processors, consumers, or preparers rather than naturally occurring. Salad and cooking oils are the leading source of added fats, followed by shortening, margarine, and dairy fats. These added fats are consumed in addition to the fats occurring naturally in meats, fish, nuts, eggs, and dairy products. Therefore, in order to achieve the goal of a healthy diet based on the USDA Food Guide Pyramid, consumers need to reduce the amount of added fats by one-third (Putnam and Allshouse, 1997), in addition to reducing total and saturated fats (Ernst et al., 1999).

Although the reported total fat and saturated fatty acid intakes as a percentage of total energy have been declining over the past decades in the United States, most individuals do not consume a diet that meet the levels of fat and saturated fatty acids recommended by the Dietary Guidelines for Americans (Lichtenstein et al., 1998). On a relative basis, saturated fat intake has decreased less than that of total fat intake. In addition, individuals of all ages who consume a diet with 30% or less of energy from fat consistently have lower-energy intakes. The data suggest that fat-modified foods make a more significant contribution to diets of consumers with low-fat intakes, and that the focus on overall diet quality is often lost in the national obsession with lowering fat intake.

From 1971 to 2000, the mean percentage of energy from total and saturated fat has decreased, but remained above recommendations, with overall means of 33.5% of energy from fat and 12.2% of energy from saturated fat. During 1988–1994, only about one in four youths met the recommendations for intakes of fat and saturated fat and three in four met the recommendation for cholesterol intake (Troiano et al., 2000). Beverages contribute 20%–24% of energy across all ages and soft drinks provide 8% of energy in adolescents. The lack of evidence of a general increase in energy intake among youths despite an increase in the prevalence of overweight suggests that physical inactivity is a major public health challenge in this age group. Efforts to increase physical activity and decrease nonnutritive sources of energy are essential to counter the rise in overweight prevalence.

High-fat diets have been associated with increasing obesity, and high intake of total fat and saturated fat is linked to the increased risk of cardiovascular disease and other degenerative diseases.

However, the prevalence of obesity has increased incidence of 14.5%–30.9% in the United States during 1971–2000, while dietary fat intake (both in absolute terms and as a percentage of total dietary energy) has decreased during this period (Flegal et al., 2002). The findings suggest a role of sedentary behaviors in the causation of overweight or obesity (U.S. Department of Health and Human Services, 2001). For example, children who ate fast food, compared with those who did not, consumed more total energy (187 kcal), more energy per gram of food (0.29 kcal/g), more total fat (9 g), more total carbohydrate (24 g), more added sugars (26 g), more sugar-sweetened beverages (228 g), less fiber (–1.1 g), less milk (–65 g), and fewer fruits and nonstarchy vegetables (–45 g) (Bowman et al., 2004). Thus, a concomitant decrease in total dietary energy and modifications of other lifestyle factors, including physical activity, need to be emphasized.

IV. SUMMARY AND CONCLUSION

Data obtained from NHANES show that the mean energy intake from carbohydrate, either by weight or percentage of kcal, has increased, while little changes from protein during 1971–2000 in the U.S. adult population. On the other hand, the mean percentage of total energy intake from total fat, saturated fat, and unsaturated fat has decreased during this period. The total energy intake during 1988–2000 is higher than that of 1971–1980 for both men and women, and the increase is primarily attributable to increased carbohydrate intake. Of the mean food energy intakes, the percentage of calories contributed by food eating as snacks averaged 17.1% for all subjects. Data obtained from CSFII and NHANES 2000–2002 show that men consumed relatively more fatty acids than that of women. The percentage calories contributed by 18:1 is the highest, followed by 16:0, 18:2, 16:0, 14:0, 16:1, 18:3, 12:0, and 20:4. In addition, 18:1 is the most abundant fatty acid in the American diet, and is followed by 16:0, 18:2, 18:0, 14:0, 16:1, 18:3, 12:0, and 20:4. Data obtained from CSFII also show that meat and fish provide more 16:0, 18:0, and 18:1 than other food sources, and are rich sources for 18:2 for men aged 20 and over. On the other hand, grain products provide more 18:1 and 18:2 than other food sources, and are good source for 16:0 and 18:0, and meat and fish are the richest source for 16:0 and 18:0 for females aged 20 and over. Given the increasing rates of obesity in the United States at an earlier age, a reduction in total energy intake is an essential part of an overall strategy to balance energy consumption with energy needs. Focus to achieve total energy balance is fundamental to preventing and reducing obesity in the United States.

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22 Absorption and Transport of Dietary Lipid

Vernon A. Welch and Jürgen T. Borlak

CONTENTS

I. INTRODUCTION

In recent years, there has been considerable interest in determining both the amount and type of fat in the diet that commensurate with good health. This has led both to official reports such as the Committee on Medical Aspects (COMA) of food policy reports (Department of Health and Social Security, 1984; Department of Health, 1991) and to books offering nutritional advice to the general public (e.g., Anderson, 1986; Le Fanu, 1987). This chapter considers the absorption, transport, and desaturation of dietary lipid and some of the possible effects on human health.

II. THE NATURE AND FUNCTION OF DIETARY LIPID

Lipids are important constituents of food that not only provide a concentrated source of energy but also affect the texture and flavor of food and therefore its palatability. The principal lipids found in the diet and in the body include the free fatty acids (FFAs); the esters of glycerol and fatty acids (triacylglycerol, diacylglycerol, and monoacylglycerol); the esters of glycerol that, in addition to fatty acids, also contain a phosphate group (glycerophospholipids); the esters that contain a long-chain hydroxylic base esterified to a long-chain fatty acid and a phosphate group (sphingolipids); the lipids that contain a sugar group (glycolipids); and cholesterol and its esters.

In the body, lipids have several functions—triacylglycerols (TAGs) are stored in adipose tissue, provide insulation, and can be mobilized to meet energy requirements; phospholipids (PLs), glycolipids, and cholesterol play a structural role in cell membranes; and triacylglycerol, diacylglycerol, and monoacylglycerol (MAG), sterols and steryl esters, FFAs, wax esters, and hydrocarbons have a protective function in skin. Lipids also have important metabolic roles to play. They can be oxidized to provide energy (TAGs), and they can serve as precursors for biologically active substances such as prostaglandins (unsaturated PLs) and steroid hormones and bile acids (cholesterol).

Certain lipids such as the fat-soluble vitamins, A, D, E, and K, and the essential fatty acids (EFAs) are not synthesized in the body and have to be supplied in the diet. The fat-soluble vitamins are necessary for vision (vitamin A), regulation of calcium metabolism (vitamin D), prevention of autoxidation of unsaturated lipids (vitamin E), and normal clotting of blood (vitamin K). The role of EFAs will be discussed in more detail later. A comprehensive review of the role of lipids in food is given by Gurr (1984).

III. DIGESTION AND ABSORPTION OF LIPID

Digestion and absorption of lipid has been reviewed by Johnston (1970, 1978), Tso (1985), Zilversmit (1978), Green and Glickman (1981), Norum et al. (1983), Gurr (1984), and Brindley (1985).

A. Digestion in the Intestine

The principal constituent of dietary lipid is TAG, in which mainly long-chain fatty acids and also some medium- and short-chain fatty acids are esterified to glycerol. The other constituents of dietary lipid include PLs, and cholesterol and its esters.

In the stomach, lipid is liberated from lipoproteins by proteolysis, but comparatively little lipolysis takes place in the stomach of the adults. In infants, pancreatic lipase activity is not fully operative, and the activity of a lingual lipase assumes greater importance. This lipase is active at acid pH and preferentially releases the short- and medium-chain fatty acids in milk fat TAG, to leave diacylglycerol (DAG) (Edwards-Webb and Thompson, 1977).

Lipid, emulsified by mechanical action in the stomach, enters the duodenum; the entry of food stimulates secretion of the hormone cholecystokinin–pancreozymin into the proximal small intestine, resulting in the contraction of the gallbladder and the release of bile and pancreatic juice.

The bile acids attach themselves to the emulsion particles, imparting a negative charge and attracting colipase, a protein present in pancreatic juice, that binds to the TAG. In the absence of colipase, pancreatic lipase is inhibited by bile salts, at a concentration exceeding the initial micellar concentration, but colipase overcomes the inhibition by binding pancreatic lipase. The presence of bile salts facilitates lipolysis by increasing the pH optimum of pancreatic lipase from pH 6 to pH 7; the approximate pH of the contents of the upper small intestine (Johnston, 1970).

Pancreatic lipase acts as the oil–water interface of emulsion particles and releases fatty acids from the 1- and 3-positions of the triacyl-*sn*-glycerol, leaving the 2-monoacyl-*sn*-glycerol, which can partially isomerize to 1-monoacyl-*sn*-glycerol. Pancreatic juice also contains a carboxylic esterase that can cause the complete lipolysis of 10%–15% of the TAG and may also hydrolyze the cholesteryl esters (Brindley, 1985). Phospholipases A1 and A2 are also present; they remove fatty acids from the 1- and 2-positions, respectively, of dietary *sn*-PL or *sn*-PL present in the bile; digestion can be complete or can stop at the formation of lysophospholipid. Cholesteryl esters are hydrolyzed by pancreatic cholesterol esterase, whose action is enhanced by bile salts (Dietschy, 1978).

As lipolysis proceeds, the oil phase becomes smaller and the lipolytic products (FFA, MAG, and lysophosphatidylcholine [LPC]), together with the bile salts, form negatively charged polymolecular aggregates, called *micelles*, which are about 1/100 as large in diameter as the emulsion particles (Johnston, 1970). Calcium, present in relatively high concentration in both bile and pancreatic juice, aids the adsorption of lipolytic products from the oil–water interface (Tso, 1985). The presence of a third phase, a lipid crystal phase saturated with bile salts, which may be important in the absorption of MAG and fatty acid in bile salt-deficient patients, has also been reported (Tso, 1985).

B. Absorption of the Products of Digestion

The lipolytic products are taken up by passive diffusion from the micelle by the microvilli of the enterocytes. The bile acid micelles greatly facilitate the uptake of lipid by taking it through the unstirred water layer that is adjacent to the cell membrane and that presents the major barrier to passage of lipid from the lumen to the cell. It is not clear whether micelles can be taken up by the cell, but it seems likely that the aggregated lipid molecules in the micelle are in equilibrium with the monomer forms, which are concentrated at the cell surface and passively absorbed into the cell (Dietschy, 1978; Thomson, 1978).

Bile salts are essential for the uptake of cholesterol (Thomson, 1978), and polar lipids are also necessary for its solubilization. The absorption of polar lipids in the proximal intestine can result in precipitation of cholesterol, and this may provide a partial explanation for why only about half of the cholesterol is absorbed (Norum et al., 1983). The bile salts pass on the distal intestine. There, they are absorbed and returned to the liver, via the portal vein, and incorporated into bile once more, thus completing the enterohepatic cycle (Beynen et al., 1986; Liepa and Sullivan-Gorman, 1986).

C. Metabolism of Absorbed Lipid in the Enterocyte

Fatty acid absorbed into the cell is transported to the endoplasmic reticulum in association with a fatty acid-binding protein. The fatty acids are activated to their coenzyme A (CoA) derivatives by acyl CoA synthetase and reesterified to TAG by either the MAG or the α-glycerophosphate pathway. The enzymes of the MAG pathway, associated mainly with the smooth endoplasmic reticulum (SER),

acyl CoA synthetase, monoacylglycerol transacylase, and diacylglycerol transacylase, are present in one complex called triacylglycerol synthetase, whose preferred substrate is 2-MAG (Tso, 1985).

The other pathway of TAG synthesis, which takes place mainly in the rough endoplasmic reticulum (RER), involves activation of the fatty acids to their acyl CoA derivatives by acyl CoA synthetase, formation of L-α-glycerophosphate from glycerol by glycerol kinase, conversion of L-α-glycerophosphate to phosphatidic acid by glycerophosphate acyl transferase, conversion of phosphatidic acid to DAG by phosphatidate phosphohydrolase, and finally acylation of DAG by diacylglycerol transacylase. Although both pathways synthesize DAG, the DAGs do not equilibrate. Only the DAG synthesized via the α-glycerophosphate pathway can serve as a precursor for *sn*-phosphatidylcholine, which has a different fatty acid distribution in positions 1 and 2 from that of dietary triacyl-*sn*-glycerol. The distribution of fatty acids on the TAG synthesized via the MAG pathway is similar to that of dietary TAG (Johnston, 1970).

About 75%–85% of TAG is synthesized via the MAG pathway, and MAG inhibits the α-glycerophosphate pathway. The α-glycerophosphate pathway assumes more importance when only long-chain fatty acids are absorbed, as in the case of ruminants. Absorbed LPC can be reesterified to phosphatidylcholine by the action of lysolecithin acyltransferase, which is found in both SER and RER (Tso, 1985).

A high proportion of the absorbed cholesterol is esterified. One mechanism for this is by reversal of the action of pancreatic cholesterol esterase, which is absorbed into the cell. Hydrolysis of cholesteryl ester catalyzed by cholesterol esterase is favored by the pH in the intestinal lumen (pH 6.6–8.1), whereas reesterification is favored by the pH in the cell (pH 5.0–6.2) (Thomson, 1978).

D. Assembly of Triacylglycerol-Rich Lipoproteins

In order for lipid to be transported out of the cell and into the lymph, it is bound to protein to form a lipoprotein that is soluble in an aqueous medium. This particle is called a *chylomicron* and consists of a nonpolar core containing TAG and a variable amount of cholesteryl ester that is covered by a surface coat of protein (apolipoprotein), unesterified cholesterol, and PL. The particles vary in size from 75 to 600 nm, depending on the rate of lipid absorption and the nature of the lipid, being larger during the absorption of high-fat loads and when unsaturated fat is being absorbed (Zilversmit, 1978; Norum et al., 1983; Sparks and Sparks, 1985; Tso, 1985).

The enterocyte also produces smaller lipoprotein particles similar in size to the plasma very low density lipoprotein (VLDL), which are termed intestinal VLDL or small chylomicrons, depending on whether they are considered distinct from chylomicrons or not. Some studies suggest that the chylomicrons and the intestinal VLDL are assembled separately, but others indicate one population of TAG-rich lipoproteins that varies in size according to the amount of core lipid and surface material. Intestinal VLDL is the principal form secreted during fasting, but secretion continues when fat is fed (Tso, 1985).

The fatty acid composition of chylomicron TAG depends on the diet, but that of PL and cholesteryl ester does not. The composition of lymph chylomicrons is TAG, 86%–92%; cholesteryl ester, 0.8%–1.4%; unesterified cholesterol, 0.8%–1.6%; PL (PC), 6%–8%; and protein, 1.0%–1.5% (Green and Glickman, 1981). Intracellular chylomicrons or prechylomicrons contain more FFA, cholesterol, and protein and less PL than the lymph chylomicrons, possibly as a result of contamination with intracellular components or because of changes that take place after excretion from the cell (Green and Glickman, 1981). The specialized proteins found in the lipoproteins, termed *apolipoproteins* (apo), are described in more detail below.

It has been demonstrated in the rat that the intestine and the liver are sole sources of the plasma apolipoproteins, and that the intestine is responsible for 19% of the total synthesis (Wu and Windmueller, 1979). The apolipoprotein composition of rat and human lymph chylomicrons is similar and varies according to source and size: apo A-I, 15%–35%; apo C, 45%–50%; apo A-IV, 10%; apo E, 5%; and apo B, 10%. Apo A-II and apo A-V have also been observed (Sparks and Sparks, 1985). The contribution of the intestine to the synthesis of rat apolipoproteins is apo A-IV, 59%; apo A-I, 56%; apo B,

16%; apo C-II, 10%; apo C-II-0, 7%; apo C-III-2 (–1), <10%; apo C-III-3, <1%; and apo E, <1% (Wu and Windmueller, 1979).

During fat feeding, the intestine actively synthesizes apo A-I, apo A-IV, and apo B, and, in the case of humans, also apo A-II. The apo C and apo E are acquired by the chylomicrons after secretion from the cell (see later) (Sparks and Sparks, 1985). Apolipoprotein A-I is synthesized as a preproapolipoprotein with a 24-amino acid extension that is secreted as a proapolipoprotein with a hexapeptide prosegment that is converted extracellularly to apo A-I (Edelstein et al., 1983).

The form of apo B present in chylomicron is apo B_{48}, so-called because its apparent molecular weight is 48% of apo B_{100}, which is found in VLDL of hepatic origin. In the rat, there appears to be two apo B_{48} subunits per chylomicron (Sparks and Sparks, 1985). Apolipoprotein B plays an essential role in the formation and transport of chylomicrons. Small amounts of apo B are synthesized continually during fasting, but synthesis increases during fat feeding. Apo A-I also increases during fat feeding but does not appear to be so important in the secretion of chylomicrons (Tso, 1985).

The lipid of the prechylomicron particle is synthesized mainly in the SER, the protein in the RER, and sugar in the Golgi apparatus, but not exclusively so, and carbohydrate can also be added to the protein in the endoplasmic reticulum. The SER and Golgi apparatus are closely linked to the endomembrane system. Protein synthesized in the RER is incorporated into the TAG-rich particle at the junction of the RER and the SER (Zilversmit, 1978), and the particle is transported to the Golgi apparatus where both protein and lipid can be glycosylated. Prechylomicron particles migrate to the plasma membrane in vesicles that fuse with the plasma membrane and are released into the intercellular space by exocytosis (Sparks and Sparks, 1985; Tso, 1985).

After secretion, changes in the composition of the chylomicron take place in the lymph and, more important, in the plasma. In the rat, very little apo C or apo E is synthesized in the intestine (Krause et al., 1981), although apo C-II may be an exception (Fidge and Nestel, 1986), and the chylomicron acquires these apolipoproteins after secretion, primarily by transfer from high-density lipoprotein (HDL) (Gotto et al., 1986). When hydrolyzed, the chylomicrons lose PL, apo A-I, and apo A-II from the surface to HDL (Tall, 1986a).

E. Absorption of Short-Chain Fatty Acids

The shorter-chain length fatty acids are usually esterified to position 3 of triacyl-*sn*-glycerols and are therefore not found in the 2-monoacyl-*sn*-glycerols. They are easily released from TAG in the stomach and small intestine and can partition into the aqueous phase and be transported, bound to albumin, in the portal vein to the liver (Brindley, 1985). A more prominent role for the portal vein in transporting intestinal lipoproteins has been suggested (Bauchart et al., 1988) and adds weight to much earlier evidence (Barna, 1963).

IV. TRANSPORT OF LIPID ON LIPOPROTEINS

A. Structure of Lipoproteins

Lipoprotein structure and lipid binding have been discussed by Morrisett et al. (1975), Smith et al. (1978), Bradley and Gotto (1978), Nicoll et al. (1980), Tall and Small (1980), Sparks and Sparks (1985), and Gotto et al. (1986). The composition of human plasma lipoproteins is given in Table 22.1.

Because of their hydrophobic nature, lipids are transported in plasma in the form of macromolecular lipid–protein complexes termed *lipoproteins*. These consist essentially of a hydrophobic, neutral lipid core surrounded by a surface film of protein and polar lipid. The lipoproteins may be divided into several classes on the basis of differences in their chemical composition and physical properties. In addition to the chylomicrons synthesized in the intestine, which have already been described, there are the plasma lipoproteins. These can be separated by a variety of techniques, which include selective precipitation with sulfated polysaccharides, electrophoresis, isoelectric focusing, gel filtration, immunoaffinity chromatography, and ultracentrifugation. Many of these are

TABLE 22.1
Human Plasma Lipoprotein Composition

	Protein and Lipid Constituents[a] (g/L)						Apolipoprotein Present[b]					
	Protein	TL	TAG	PL	UC	CE	A-I	A-II	B	C	D	E
VLDL	0.1	0.9	0.6	0.10	0.06	0.05	(+)	(+)	+	+	(+)	+
LDL	0.95	3.1	0.2	0.95	0.35	1.65			+	(+)		
HDL	1.85	1.7	0.15	0.8	0.15	0.55	+	+		(+)	(+)	(+)

Abbreviations: TL, total lipid; TAG, triacylglycerol; PL, phospholipid; UC, unesterified cholesterol; CE, cholesteryl ester; VLDL, very low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; +, major constituent; (+), minor constituent.

Source: Data from [a]Polonovski, J., and Beucler, I. (1983). *Pathol. Biol. 31*: 225; [b]Smith, L.C., et al. (1978). *Ann. Rev. Biochem. 47*: 751.

described in the journal *Methods in Enzymology*, Volumes 128 (Segrest and Albers, 1986) and 129 (Albers and Segrest, 1986).

The traditional way to separate lipoproteins has been to exploit the differences in their hydrated densities and separate them by differential floating in the ultracentrifuge. This has given rise to the classification based on hydrated density: VLDL $d < 1.006$ g/mL; intermediate-density lipoprotein (IDL) $1.006 < d < 1.019 < d < 1.063$ g/mL; and HDL $1.063 < d < 1.21$ g/mL. There are also subclasses of HDL, which will be discussed later. The density intervals quoted are those that have been used to isolate human plasma lipoproteins and may have to be adjusted to separate the lipoproteins of other animal species.

The protein moieties of the lipoproteins are termed *apolipoproteins* or *apoproteins* (apo) and owe their lipid-binding characteristics to the presence of amphipathic helical regions, one face of which consists of hydrophobic amino acids, whereas the other has predominantly hydrophilic polar amino acids. It is thought that lipid binding takes place as a result of hydrophobic interaction between the nonpolar face of the amphipathic helix and the fatty acids of the PLs. The polar face of the helix serves to orient the apolipoprotein at the surface of the particle in contact with the aqueous medium. In addition to PL, the surface coat also contains unesterified cholesterol. The hydrophobic core into which fatty acyl group extends from the surface lipid consists of the nonpolar lipids, TAG, and cholesteryl ester.

The principal apolipoprotein of LDL, apo B, in addition to having regions of amphipathic helix, also has a β-pleated sheet structure. Addition of lipid increases the β-structure of apo B but increases the α-helix of HDL apolipoprotein (Sparks and Sparks, 1985). The apolipoproteins have particular roles to play in the secretion, transport, and metabolism of lipoproteins. These are detailed in Table 22.2 and will be discussed in the appropriate part of the section on lipoprotein metabolism that follows.

It is now recognized that there are discrete lipoprotein families that are characterized by a unique qualitative apolipoprotein composition but that can have varying lipid/protein ratios and hydrated densities and can hence occur in more than one of the traditional lipoprotein classes. These families of lipoproteins are based on apo A or apo B either alone or together with some of the other apolipoproteins. Thus, it has been established that there are three families of lipoproteins based on apo A—LP-A-I, LP-A-I: A-II, and LP-AII—together with subspecies of these that can have apo C, apo E, and so on. There are five major families based on apo B: LP-B, LP-B:C, LP-B:E, LP-B:C:E, and LP-A-II: B:C:D:E (LP-A-II:B complex). The LP-B particle is enriched in cholesteryl ester, whereas LP-B:C, LP-B:C:E, and LP-A-II:B complex are rich in TAG. As the density of apo B-containing lipoproteins increases, the content of apo B also increases, but that of apo C and apo E decreases (Alaupovic, 1988).

The presence of a particular lipoprotein family in a lipoprotein class can confer particular properties. For example, LD-A-I promotes cholesterol efflux from cells, whereas LP-A-I:A-II does not (Barbaras et al., 1987).

TABLE 22.2
Metabolic Role of Apolipoproteins

Role	Apolipoprotein
Lipoprotein secretion	B_{48}, B_{100}
Enzyme activation	
LPL	C-II
LCAT	A-I, C-I, A-IV
HL	A-II
Receptor recognition	
LDL receptor (B/E)	B_{100}, E
Chylomicron remnant receptor (E)	E
HDL receptor	A-I
Inhibition of receptor recognition	C-I, C-II, C-III (A-II)
Lipid transfer	D
Reverse cholesterol transport	A-I, A-IV, E

Abbreviations: LPL, lipoprotein lipase; LCAT, lecithin-cholesterol acyl transferase; HL, hepatic lipase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Source: Data from Gotto, A.M., Jr., et al. (1986). *Methods Enzymol. 128*: 3; Scanu, A.M. (1983). Heterogeneity of plasma lipoproteins: structural considerations and functions, in *Dietary Fats and Health* (AOCS Monograph 10), E.E. Perkins and W.J. Visek, Eds., American Oil Chemists' Society, Champaign, IL, p. 530; Introduction to the plasma lipoproteins. Barbaras, R., et al. (1987). *Biochem. Biophys. Res. Commun. 142*: 63; Stein, Y., and Stein, O. (1988). Reverse cholesterol transport, Abstracts of International Conference, Lipoproteines at Phospholipases Present et Futur, Paris, France; *Bull. Soc. Chim. Biol. 53*: 13.

Dyslipoproteinenmias can be characterized by altered proportions of the lipoprotein families, and these proportions can be selectively changed by drug action (Alaupovic, 1988; Alaupovic et al., 1988).

B. Synthesis of Lipoproteins

The synthesis of the chylomicron and of the intestinal VLDL has already been discussed. The liver is the other major site of lipoprotein synthesis and is the principal source of apo C and apo E (Gotto et al., 1986).

C. Synthesis of VLDL

The large, TAG-rich VLDL is synthesized in the liver. As with the chylomicron, apo B is essential for VLDL formation, but in humans this takes the form of the higher molecular weight apo B_{100}, although in rats, hepatic VLDL contains both apo B_{100} and apo B_{48} (Sparks and Sparks, 1985). Nascent VLDL normally contains only a small amount of cholesteryl ester, but this can be increased when acyl CoA-cholesteryl acyl transferase (ACAT) is stimulated, for example, by cholesterol feeding (Norum et al., 1983; Gotto et al., 1986). Although IDL and LDL are principally derived from VLDL in the plasma, they may also be secreted directly by the liver (Beynen et al., 1987; Shepherd and Packard, 1987b) (also see Section IV.G).

D. Synthesis of HDL

Plasma HDL is a spherical particle that consists of a hydrophobic core of TAG and cholesteryl ester stabilized by a coating of PL and apolipoprotein. The HDL particles in plasma have undergone considerable changes since their formation as precursor or nascent HDL, which are either secreted by the liver and intestine or are products of lipolysis of TAG-rich lipoproteins (Tall and Small, 1980).

Both small, spherical HDL particles containing newly synthesized core and surface components and discoidal forms of HDL have been observed in rat intestinal lymph. There is evidence that the spherical particles have been actively secreted into lymph, but the discoidal particles have not been recovered from intracellular preparations and may not be secreted via the Golgi apparatus (Glickman and Magun, 1986).

The lymph HDLs are relatively enriched in PL and apo A-I and poor in cholesteryl ester and apo E. The discoidal form of nascent HDL has also been observed in liver perfusates and has apo E and apo A-I as its principal apolipoproteins, plus, unlike the intestinal nascent HDL, some apo C. The ratio of apo E to apo A-I is greater than that of plasma or intestinal HDL (Nicoll et al., 1980).

The evidence for the secretion of nascent HDL by human liver or intestine is indirect, and an alternative possibility is that it is formed from surface materials (PL, unesterified cholesterol, and apo C) resulting from the lipolysis of the TAG-rich lipoprotein. An alternative possibility that could account for the presence of discoidal particles in liver perfusates or intestinal lymph is that free apolipoprotein secreted by cells could become associated with PLs derived from cells or lipoproteins during or after secretion. Possibly polar materials could be supplied from both of these sources (Eisenberg, 1984).

Whether or not the nascent HDL particle is formed from surface components during the lipolysis of TAG-rich lipoproteins, there is unequivocal evidence that the conversion of nascent HDL to mature plasma HDL and further HDL metabolism do involve such transfers, and that cells and cell membranes can also contribute PL to this process (Eisenberg, 1986). The conversion of the nascent discoidal form of HDL to the mature spherical form is the result of esterification of cholesterol and the entry of the nonpolar cholesteryl ester into the core of the particle, thereby swelling it and producing the spherical pseudomicellar form (Nicoll et al., 1980; Eisenberg, 1986).

The esterification of cholesterol at the expense of phosphatidylcholine is catalyzed by lecithin-cholesterol acyl transferase (LCAT) that has been activated by apo A-I already present on the nascent particle or transferred to it from the TAG-rich lipoproteins or the free apoprotein pool. The other product of the reaction, LPC, is mainly transferred to albumin and can be subsequently reacylated (Gotto et al., 1986).

E. Lipoprotein Metabolism

A general scheme for lipoprotein metabolism is shown in Figure 22.1 (see also Schaefer et al., 1978; Polonovski and Beucler, 1983; Thompson, 1984; Gotto et al., 1986; Rifai, 1986; Welch, 1986). Some enzymes of importance in lipid and lipoprotein metabolism are listed in Table 22.3.

F. Chylomicron Metabolism

The chylomicrons that are secreted by the intestinal cell and whose surface components have been modified by exchange in plasma, as described previously, then undergo lipolysis by lipoprotein lipase (LPL), which is activated by the newly acquired apo C-II (Kinnunen et al., 1983; McClean et al., 1986). LPL is attached to the endothelial surface of capillaries by heparan sulfate (McClean et al., 1986), and its activity is regulated by those hormones that promote its activity in adipose tissue, such as insulin, and those that depress it, such as catecholamines. Thus, the activity of LPL can be regulated reciprocally between muscle and adipose tissue so as to favor either energy production or storage of TAGs (Brindley, 1985).

As a result of LPL action, most of the TAG and some of the phosphatidylcholine are hydrolyzed rapidly; the liberated fatty acids are taken up by the tissues and either reesterified and stored as TAG (adipose tissue) or oxidized as in muscle in the fasting state. The remaining PL, together with apo A and most of the apo C, is transferred to HDL (Gotto et al., 1986), leaving a smaller remnant particle enriched in cholesteryl ester. The loss of apo C-II renders the remnant less suitable as a substrate for LPL, and so it is released back into the circulation, from where it is removed by the liver, after binding to the chylomicron remnant receptor that recognizes apo E. The presence of apo C on the chylomicron inhibits recognition by the apo E receptor, and its loss facilitates recognition of the remnant.

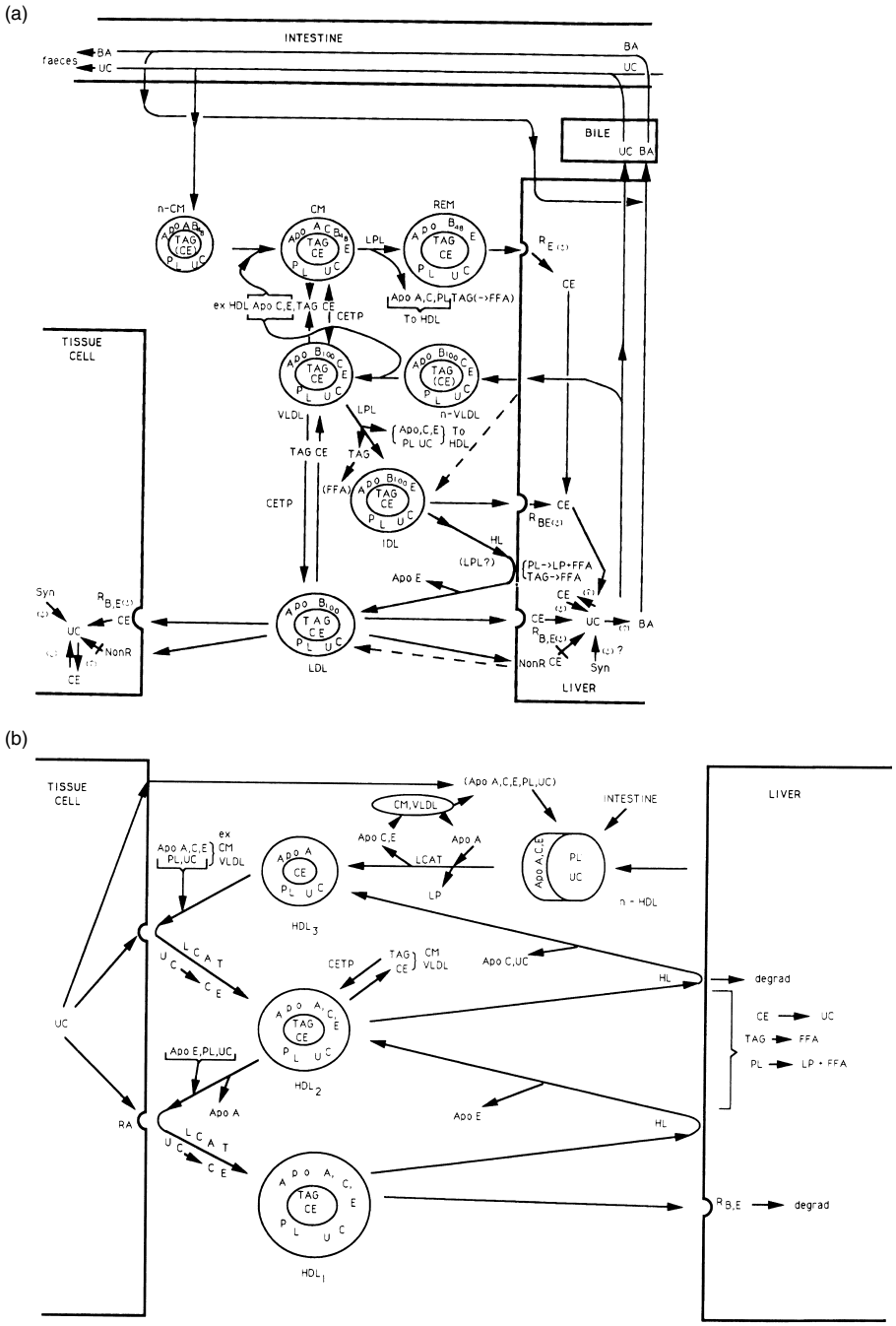

FIGURE 22.1 (a) Triacylglycerol-rich lipoprotein metabolism and (b) high-density lipoprotein metabolism. Outer circles represent surface materials of lipoproteins; inner circles represent core lipids. (↑) Upregulation; (↓) downregulation; and (↕) absence of regulation. Parentheses indicate a component of variable concentration. Solid arrow lines, principal pathways; broken arrow lines, minor pathways. *Abbreviations:* Syn, synthesis; Degrad, degradation; LPL, lipoprotein lipase; HL, hepatic lipase; LCAT, lecithin-cholesterol acyl transferase; CETP, cholesteryl ester transfer protein; apo A, B, C, E, apolipoproteins A, B, C, E; R_E, receptor recognizing apo B and apo E (LDL receptor); R_A, receptor recognizing apo A; non-R, uptake by nonreceptor pathway; CM, chylomicron; VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; REM, remnant; n-, nascent; TAG, triacylglycerol; PL, phospholipid; LP, lysophospholipid; FFA, free fatty acid; UC, unesterified cholesterol; CE, cholesteryl ester; BA, bile acid.

TABLE 22.3
Some Enzymes of Importance in Lipid Transport and Metabolism

Enzyme	Function
Pancreatic lipase	Hydrolysis of fatty acids at position 1 and 3 of emulsified triacyl-*sn*-glycerols in the intestine
Lipoprotein lipase	Hydrolysis of triacylglycerol-rich particles; some phospholipase activity; activated by apo C-II
Hepatic lipase	Hydrolysis of tri-, di-, and monoacylglycerols, acyl-CoA thiol esters, and phospholipids; conversion of HDL_2 to HDL_3; activated by apo A-II
Lecithin-cholesterol acyl transferase (LCAT)	Catalysis of reaction of lecithin with cholesterol to give lysolecithin and cholesteryl ester; activated by apo A-I and apo C-I
Pancreatic cholesterol esterase	Esterification of cholesterol in the intestinal lumen; hydrolysis of cholesteryl ester in the intestinal cell
Acyl CoA-cholesterol acyl transferase (ACAT)	Esterification of cholesterol within cells
HMG CoA reductase	Rate-limiting enzyme of cholesterol biosynthesis

G. VLDL Metabolism

Normal VLDL, similar to the chylomicron, is not taken up by cell receptors directly because of its relatively high apo C content, although small VLDLs can bind to the LDL B_{100}/E receptor (see below), presumably because of greater exposure of apo B (Owen and McIntyre, 1982; Gotto et al., 1986). The VLDLs bind to the endothelial LPL where their TAG is largely hydrolyzed, the rate of lipolysis being a function of size, possibly because more of the LPL cofactor, apo C-II, is associated with the larger particles (Gotto et al., 1986). As with the chylomicrons, surface components including unesterified cholesterol, phopholipid, apo C, and some apo E are transferred to HDL. The resultant VLDL remnant or IDL particle is relatively enriched in unesterified cholesterol, cholesteryl ester, apo B, and apo E. The VLDL remnant, similar to the chylomicron remnant, is a poor substrate for LPL and can either be taken up by the liver directly or be further metabolized to LDL.

Although large IDLs with a high apo E/apo B ratio may be taken up by the hepatic chylomicron remnant apo E receptor, the presence of apo B_{100} normally appears to mask the binding of apo E to the apo E receptor, thereby directing the IDL to the LDL B_{100}/E receptor, where it binds by virtue of the high affinity between apo E and the receptor (Brown and Goldstein, 1983, 1984). Whether IDL is taken up directly by the liver or further metabolized to LDL is dependent on the activity of the hepatic LDL B_{100}/E receptor and the size of the VLDL precursor. The IDL from the largest VLDL is more likely to be removed directly by the liver, possibly because of the greater number of apo E molecules (Gotto et al., 1986; Havel, 1987; Shepherd and Packard, 1987a,b), and since the VLDL of most mammals is larger than that of humans, this could account for the difference in the proportion of VLDL converted to LDL in different species and the generally low level of LDL in species other than humans.

Thus, in the rat, a major part of the VLDL (80%–90%) is converted to IDL and catabolized by the liver, and only 5%–15% is converted to LDL, whereas, in the humans, a greater proportion (although less than originally thought [Havel, 1984a, 1987]) is converted to LDL (Eisenberg, 1983). Another factor is that rat VLDL also contains apo B_{48}, whose presence favors hepatic uptake (Sparks and Sparks, 1985). The large size of the chylomicron and its species of apo B (apo B_{48}) may determine that the chylomicron remnant is taken up by the liver directly with no apparent conversion to a form of LDL (Havel, 1984b; Sparks and Sparks, 1985), although the latter possibility is not completely ruled out (Eisenberg, 1983). The large size of remnant particles also appears to preclude their uptake by extrahepatic tissues (Norum et al., 1983).

The formation of LDL from IDL is more likely to be effected by hepatic lipase than by LPL (Deckelbaum et al., 1982; Havel, 1984a; Eisenberg, 1986). This conversion is accompanied by further loss of TAG, PL, and apo E to give a particle that is enriched in cholesteryl ester and in which the apolipoprotein is almost entirely apo B, which alone of the VLDL apolipoproteins is conserved during the conversion of VLDL to IDL and of IDL to LDL (Eisenberg, 1986). It is unlikely that the conversion of IDL to LDL by hepatic lipase is mediated by the LDL receptor, or that hepatic lipase plays a role in the uptake of VLDL remnants (Havel, 1984a). Subspecies of LDL that differ in size and density have been described (Krauss, 1987; Musliner and Krauss, 1988). Direct hepatic secretion of particles resembling LDL but with an apolipoprotein composition similar to that of nascent VLDL has also been observed (Havel, 1984a). LDLs are removed from circulation by interaction of apo B with the LDL B_{100}/E receptor in hepatic and extrahepatic tissues. Since the affinity of this receptor for apo B is less than that for apo E, the half-life of LDL in circulation is greater than that of IDL, whose binding is mediated by apo E as described previously (Brown and Goldstein, 1984).

H. Receptor-Mediated Uptake of Lipoproteins

a. Chylomicron Remnant Receptors (E)

Most dietary cholesterol is taken up by the liver in the form of chylomicron remnants that bind to the apo E receptor in the liver. The influx of cholesterol depresses the synthesis of cholesterol from acetyl CoA and the activity of the LDL (apo B/E) receptor; however, the number of apo E receptors is not downregulated (Ho et al., 1976; Brown and Goldstein, 1983; Beynen et al., 1986; Gotto et al., 1986). Chylomicron remnants contain apo B_{48}, and high-affinity binding of these remnants, containing apo B_{48} and very little apo E, to hepatic cells has been demonstrated (Sparks and Sparks, 1985). A small proportion of chylomicron remnants containing apo E and apo B_{48} may bind to LDL receptors (Brown and Goldstein, 1983; Smith and Gotto, 1985).

b. LDL Receptors, B_{100}/E

The work of Goldstein and Brown and their coworkers has demonstrated that the uptake of circulating LDL by both hepatic and extrahepatic cells is mediated by a receptor that is specific for apo B_{100} and apo E (Goldstein and Brown, 1976, 1977, 1984; Brown and Goldstein, 1979, 1984; Brown et al., 1981; Schneider, 1989). The dual ligand specificity of the B_{100}/E receptor was established by binding studies with HDL_c, a form of HDL that accumulated in cholesterol-fed dogs and whose sole apolipoprotein is apo E, and LDL whose sole apolipoprotein is apo B.

The affinity of the receptor for apo E was found to be much greater than that for apo B, and this was attributed to the association of apo E with four receptor sites, whereas apo B interacts with only one (Pitas et al., 1979). The ligand–receptor interaction involves the acidic amino acids glutamate and separate the receptors E and B_{100}/E with the basic amino acids lysine and arginine of the ligands (apo E and apo B) (Mahley, 1986). The receptor is a protein of apparent molecular weight 160,000 (Goldstein and Brown, 1984; Schneider, 1989) and is principally located in coated pits on the cell surface. The coated pits, which have a life span of less than 5 min, invaginate to form a coated vesicle. The fusion of several of these vesicles produces an endosome, from which the receptor dissociates and returns to the cell surface. The LDL is digested by lysosomes to amino acids and unesterified cholesterol.

The increase in cellular unesterified cholesterol has the effect of depressing the activity of hydroxymethylglutaryl (HMG) CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, stimulating acyl CoA-cholesterol acyl transferase (ACAT), which converts cholesterol to cholesteryl ester for storage, and suppressing the synthesis of further LDL receptors, thereby reducing further uptake of LDL by the cell. The overall effect is to regulate the level of unesterified cholesterol within the cell.

The nature of the molecules that downregulate cholesterol biosynthesis has not been completely resolved. The fact that release of unesterified cholesterol from the cell to a suitable acceptor (see below) stimulates HMG CoA reductase points to unesterified cholesterol as a regulator per se. However, studies *in vitro* have shown that highly purified cholesterol does not inhibit cholesterol synthesis, whereas oxygenated derivatives such as 25-hydroxycholesterol are potent inhibitors. This has led to the suggestion that it is small amounts of these oxygenated sterols that are responsible for the inhibition. It may be that cholesterol exerts a mass action effect in the regulatory pool and that the effect of the oxygenated sterols is mediated by an oxysterol-binding protein (Smith and Gotto, 1985).

Species and individuals differ in the degree of control of cholesterol metabolism. Although cholesterol synthesis is suppressed in the cholesterol-fed rat, the expression of the receptor is not diminished, and consequently the rat does not become hypercholesterolemic (Goldstein and Brown, 1984). Feedback inhibition of cholesterol synthesis may not operate in human liver (Schneider, 1989), possibly because of a low rate of hepatic synthesis (Grundy, 1983; Dietschy, 1984). The degree of hypercholesterolemia that develops as a result of cholesterol feeding can vary widely among individuals, and the defect in hyperresponders could be inadequate suppression of cholesterol biosynthesis or greater efficiency of cholesterol absorption. In hyporesponders, as in rats, it may be that the number of receptors is not downregulated to the same degree as in hyperresponders (Beynen et al., 1987).

I. Receptor-Independent Uptake of LDL

Even in subjects or animals that lack LDL receptors, there can still be considerable uptake of LDL. The extent of this receptor-independent pathway can be determined by measuring the turnover of LDL in which the lysine or arginine has been suitably blocked, thereby preventing receptor recognition (Shepherd and Packard, 1986). In the liver, endocrine glands, lung, and kidney, over 90% of LDL uptake is receptor mediated, but in the small intestine, and particularly in the spleen, the receptor-independent pathway is more important (Dietschy, 1984).

The receptor-independent pathway has a high capacity for lipoprotein uptake but a low affinity, and is nonspecific insofar as HDL can compete with LDL for binding to the low-affinity binding sites (Durrington, 1982). The LDL that binds to this site may be taken up by both fluid endocytosis and adsorptive endocytosis.

The LDL cholesterol taken up *in vitro* does not downregulate HMG CoA reductase or stimulate ACAT, suggesting that the cholesterol is not in the same pool as that taken up by the receptor-mediated pathway. However, in receptor-deficient states *in vivo*, there does appear to be some regulation, although this may occur at a higher than normal intracellular cholesterol concentration (Shepherd and Packard, 1986).

a. β-VLDL Receptor

Cholesterol feeding results in the appearance of cholesteryl ester rich, apo E-containing VLDLs termed β-VLDLs because of their β mobility on electrophoresis. These are taken up by specific high-affinity receptors on macrophages, and the expression of these receptors can be depressed and the formation of cholesteryl ester stimulated most by β-VLDL. This receptor also takes up chylomicrons and the large VLDL that occurs in hypertriacylglycerolemia, but not normal (VLDL, LDL, or HDL) (Gotto et al., 1986). The β-VLDLs are also taken up by the apo B/E receptor on fibroblasts, smooth muscle cells, and hepatic cells (Daugherty and Schonfeld, 1985; Smith and Gotto, 1985).

b. "Scavenger" or Acetyl LDL Receptor

This has been demonstrated in macrophages, smooth muscle cells, and endothelial cells and is responsible for the uptake of lipoproteins whose negative charge has been increased, as in acetylated lipoprotein. The receptor will also take up lipoprotein that could be modified *in vivo*, by combination

with malondialdehyde or by contact with endothelial cells (Smith and Gotto, 1985). This receptor is not downregulated by the uptake of cholesterol and has been implicated in the pathogenesis of atherosclerosis (Steinberg, 1983).

J. HDL Metabolism

The metabolism of HDL is shown in Figure 22.1b. The conversion of discoidal nascent HDL to the spherical plasma HDL was discussed earlier. The first major mature HDL to be formed in this way is termed HDL_3. It has a molecular weight of 175,000 and a hydrated density in the range 1.125–1.210 g/mL and contains 3 mol/mol HDL of apo A-I and 1 mol/mol HDL of apo A-II. HDL_3 is interconvertible with the large HDL_2, which has a molecular weight of 360,000 and a hydrated density in the range 1.063–1.125 g/mL and has 4 mol/mol HDL of apo A-I, 1 mol/mol HDL of apo A-II, and 1 mol/mol HDL of apo C (Eisenberg, 1983; Gotto et al., 1986). In turn, HDL_2 can be converted into HDL_1, which is larger than HDL_2 but is isolated in the same density range. It contains apo A-I, apo A-II, and variable amounts of apo E up to 60% of total protein. A considerable proportion of rat HDL occurs in this form (Nicoll et al., 1980; Eisenberg, 1984).

When the composition of HDL is considered from the point of view of lipoprotein families, it appears that LP-A-I and LP-A-I:A-II occur in both HDL_2 and HDL_3, but LP-A-I predominates in HDL_2 and LP-A-I:A-II in HDL_3 (Cheung and Albers, 1984). As a consequence of lipolysis of VLDL, HDL_3 can acquire unesterified cholesterol, PL, and apo C and be converted to HDL_2 (Patsch, 1982). Additional apo A-I is also required, and this could come from a free apo A-I pool or from chylomicron surface materials (Eisenberg, 1984; Tall, 1986a). The activity of extrahepatic LPL is strongly correlated with the level of human HDL_2 (Eisenberg, 1984). The HDL_2 level (Cavallero et al., 1988), LCAT activity (Tall, 1986a), and cholesteryl ester transfer rate (Fielding, 1987) (see below) are all increased during lipemia.

The addition of PL and unesterified cholesterol to HDL_3 lowers its density, but unless the cholesterol is esterified by LCAT and transferred to the hydrophobic core, the lipid can be removed again, making the process reversible (Eisenberg, 1984). Polar lipids resulting from the lipolysis of TAG-rich lipoproteins can also be accepted by HDL_2, and the LCAT reaction can produce an even larger particle, HDL_1, particularly in species such as the rat where there is no cholesteryl ester transfer protein (CETP) (see below), which can transfer the cholesteryl ester formed in the LCAT reaction to other lipoproteins. In this conversion, apo E arising from the lipolysis of TAG-rich lipoproteins replaces apo A-I, possibly as a result of the lower affinity of apo A-I for large particles (Eisenberg, 1984, 1986).

By loss of lipid and apolipoprotein, HDL_2 can be reconverted to HDL_3. It is envisaged that the first step is exchange of HDL_2 cholesteryl ester for TAG from chylomicrons and VLDL, which is facilitated by CETP. The TAG-rich HDL_2 is then hydrolyzed, principally by hepatic lipase, although LPL may play a part (Miller et al., 1978; Eisenberg, 1984; Tall, 1986a). Lipolysis removes TAG from the core and PL from the surface, followed by the release of other surface components, unesterified cholesterol, and apolipoproteins; apo C would be transferred to nascent TAG-rich lipoproteins. The regenerated HDL_3 could return to the plasma or be further metabolized (Nikkila et al., 1982).

It has been suggested that HDL_1 could be reconverted to HDL_2 by hepatic lipase action after binding to hepatic cells by virtue of its apo E content, and that the lipid-depleted particle, after dissociation of apo E, could be released back into circulation by retroendocytosis (Kinnunen et al., 1983).

K. Reverse Cholesterol Transport

Since peripheral cells do not catabolize cholesterol, the only way that the cell can lose cholesterol in order to maintain homeostasis is by efflux. Whether there is a net efflux or net influx of cholesterol or an exchange with a suitable acceptor depends on the cholesterol balance between the cell and the medium and the physical properties of the acceptor, particularly the unesterified cholesterol/PL ratio

(Rothblat et al., 1986; Phillips et al., 1987; Steinberg, 1987). In cells in which cholesterol is activity utilized, as in the steroidogenic cells and the liver, unesterified cholesterol can be extracted from lipoproteins (Eisenberg, 1984). The removal of cholesterol from cells is termed reverse cholesterol transport (RCT) and can be effected by PL liposomes and nascent HDL particles, which will accept cholesterol from cells, a process that is enhanced by the presence of apolipoproteins A-I, E, and A-II (Tall and Small, 1980; Daugherty and Schonfeld, 1985; Stein and Stein, 1988).

Similarly, HDL_3 promotes cholesterol efflux from cells (Ho et al., 1980; Oram et al., 1981; Nikkila et al., 1982; Eisenberg, 1984; Phillips et al., 1987; Barbaras et al., 1988). The efflux of cholesterol depends on the presence of LP-I particles that are postulated to bind to specific sites on the cell; apo A-II binds to the same site as apo A-I but does not promote cholesterol efflux and therefore behaves as an antagonist (Barbaras et al., 1988). Downregulation of the HDL receptors suppresses cholesterol efflux (Bierman and Oram, 1987). Both low-affinity nonsaturable (nonspecific) and high-affinity saturable binding sites have been identified on fibroblasts. The binding of HDL is not followed by internalization and degradation and can be upregulated by preloading the cells with cholesterol or downregulated by promoting efflux of cholesterol (Eisenberg, 1984).

If HDL_3 does compare closely to LP-A-I:A-II (Cheung and Albers, 1984), which does not promote cholesterol efflux (Barbaras et al., 1987), then it is curious that it appears to be the principal HDL class responsible for cholesterol efflux. However, it must also be borne in mind that the HDL classes do not have a monopoly of any of the lipoprotein families (Cheung and Albers, 1984). The cholesterol that is removed from the cell by the HDL acceptor is esterified by LCAT and transferred to the hydrophobic lipid core, leaving the surface free to pick up more cholesterol (Paul et al., 1980).

In those species that possess an active CETP, the cholesteryl ester can be exchanged for TAG with the TAG-rich lipoproteins and be ultimately returned to the liver in the form of remnant particle or LDL. In species, such as the rat, that do not possess an active CETP, the cholesteryl ester could be incorporated into HDL_1—an apo E-containing form of HDL—that can be recognized by the LDL B_{100}/E receptor (Nicoll et al., 1980; Scanu, 1983; Havel, 1984b). During lipemia, the activities of LCAT and CETP both rise (Tall, 1986a; Fielding, 1987), and there is an increase in the removal of cholesterol from cells. It has been suggested that postprandial lipemia is a physiological mechanism for the enhanced removal of excess cholesterol from the peripheral cells (Fielding, 1987).

The cholesterol in non-apo E-containing HDL could be removed either by uptake of the whole particle or by preferential removal of cholesteryl ester followed by retroendocytosis and recycling of the particle in the circulation (Phillips et al., 1987). Both of these pathways appear to operate, and the relative proportion of cholesterol metabolized by each route varies with the tissue and cholesterol status. Preloading of fibroblasts with cholesterol effectively inhibits preferential uptake of cholesteryl ester (Rinninger and Pittman, 1988). Whole particle degradation seems highest in liver and intestinal cells and lowest in fibroblasts and endocrine cells (Eisenberg, 1984).

High-affinity binding sites that differ from those of LDL have been demonstrated in the liver, adrenals, ovaries, and kidneys (Eisenberg, 1984) and arterial smooth muscle cells (Bierman and Oram, 1987). It is possible that RCT by means of HDL, LCAT, and CETP may not be wholly responsible for the regulation of tissue cholesterol and that other cholesterol acceptors that do not depend on LCAT on LCAT may be involved (Tall, 1986b). Most of the evidence for RCT has been obtained *in vitro*, but there is also *in vivo* evidence to support the hypothesis (Miller et al., 1985).

L. Lipid Transfer Protein

Many of the interactions between lipoproteins depend on the presence of lipid transfer proteins (LTPs) that are associated with the denser HDL in plasma. Although CETP facilitates exchange and net transfer of cholesteryl ester, TAG, and PL, there is a PL transfer protein (PTP) that does not transfer cholesteryl ester. The PTP promotes the exchange and transfer of PL between VLDL and HDL. The CETP promotes the exchange or transfer of cholesteryl ester and TAG between HDL and

the TAG-rich lipoproteins (Tall, 1986b) and between VLDL and LDL. The latter exchange results in the VLDL having more cholesteryl ester per particle than LDL and a smaller TAG-rich LDL. The excess cholesteryl ester in VLDL could be removed by either LPL or hepatic lipase during conversion to IDL and LDL (Grundy, 1983).

The binding of CETP to both VLDL and HDL and the CEPT-mediated transfer of cholesteryl ester are both stimulated by lipolysis. The increase in CETP activity could result from the increase in TAG-rich lipoproteins, which enhances the exchange of TAG for cholesteryl ester from the HDL (Tall, 1986b). Although PTP activity is high in several species, some mammals, such as dog, pig, rat, and cow, have very low CETP activity, which may be due to the presence of an inhibitor (Calvert and Abbey, 1985; Tollefson and Albers, 1986). In these animals, the LCAT-derived cholesteryl ester remains in the HDL fraction, sometimes in the form of HDL_1.

Since LCAT activity is inhibited by the accumulation of cholesteryl ester, the action of CETP in promoting the transfer of cholesteryl ester could regulate the activity of LCAT and the efflux of cholesterol from cells (Tall, 1986b; Barter et al., 1987). As the species that lack CETP have high levels of HDL and are markedly more resistant to diet-induced atherosclerosis, it has been suggested that high activity of CEPT leads to low-HDL levels and to increases in cholesterol in remnants and LDL, which are believed to be atherogenic (Calvert and Abbey, 1985; Tall, 1986b). However, it has been argued that LDL of normal composition, as distinct from that present in subjects at risk from coronary artery disease, is not atherogenic, and that transfer of cholesteryl ester from cells to VLDL and LDL, whose uptake is well regulated, could be beneficial (Fielding, 1987).

M. Utilization of Cholesterol

The cholesterol taken up by cells or synthesized within the cell can be incorporated into membranes or used for steroidogenesis. Both LDL and HDL contribute cholesterol to the adrenals, with HDL being the major source in the rat. Only HDL is bound with high affinity to membranes in rat testes and is the principal source of cholesterol; in other species, LDL is also utilized (Norum et al., 1983). Loss of cholesterol from the body is affected by sloughing of skin or by excretion in the bile as unesterified cholesterol or after conversion to bile acids. The steroid hormones are also excreted in bile or urine (Dietschy, 1984). The cholesterol taken up by the liver could suppress cholesterol biosynthesis, stimulate esterification, and increase synthesis of bile acids. Cholesterol could also be recirculated in lipoproteins (Grundy, 1983).

N. Desaturation Reactions

Except for a limited number of bacteria (Eubacteriales), the pathway of monounsaturated fatty acid synthesis involves an oxidative mechanism in which a double bond is introduced directly into saturated long-chain fatty acids with oxygen and NADPH as cofactors. The mechanism is not fully understood, although the microsomal electron transport chain apparently undergoes a complex cascade of redox reactions in which NADH-cytochrome b_5 reductase (EC 1.6.2.2) becomes reduced by NADH and in turn reduces an oxidized desaturase that catalyzes the desaturation of the fatty acid-CoA ester. Desaturation involves the removal of two hydrogen atoms that are transferred to molecular oxygen, leading to the formation of a double bond in the fatty acid CoA substrate and water.

The stoichiometry of electron flow was investigated by Rogers and Strittmatter (1974), who showed conclusively the reduction of cytochrome b_5 by NADH. The overall equation can be summarized as follows:

$$\text{NADH} + \text{H}^+ + \text{O}_2 + \text{R–CH2–CH2–R} \rightarrow \text{NAD}^+ + 2\text{H}_2\text{O}\ \text{R–CH=CH=R} \qquad (22.1)$$

The 1:1 molar relationship between NADH utilization and oleyl CoA formation has been confirmed by Strittmatter et al. (1972), who showed that the two electrons required for the introduction

of the double bonds are provided by reduced cytochrome b_5. The reduction of cytochrome b_5 by NADH follows the scheme (from Holloway, 1983)

$$E_{FAD} + NADH \rightleftharpoons E^{NAD}_{FADH_2} \rightarrow E^{NAD}_{FADH_2}$$

$$E^{NAD^-}_{FADH_2} + \text{cyt } b_5 \text{ oxidized} \rightarrow E^{NAD}_{FADH_2} + \text{cyt } b_5 \text{ reduced}$$

$$E^{NAD^-}_{FADH} + \text{cyt } b_5 \text{ oxidized} \rightarrow E^{NAD^-}_{FAD^+} + \text{cyt } b_5 \text{ reduced}$$

$$E^{NAD^-}_{FAD} \quad E_{FAD^+} + NAD^+$$

Holloway (1983) postulated a mechanism by which desaturation and concomitant removal of hydrogen occurs, which is analogous to cytochrome P-450-dependent drug oxidation, as follows:

1. Reduction of the prosthetic group of cytochrome P-450 via the first electron transfer.
2. Binding of molecular oxygen.
3. Oxidation of the prosthetic group and superoxide formation.
4. Second electron transfer.
5. Substrate binding that probably produces an epoxy fatty acid (epoxy acid radical).
6. Cleavage of the oxygen–oxygen bond and transfer of extracted hydrogen atoms to form water and a desaturated product.

Evidence for superoxide involvement in desaturation (Sreekrishna and Joshi, 1980) is shown by superoxide scavengers and copper chelates inhibiting desaturase activity. Enoch and Strittmatter (1979) purified an NADPH-dependent stearoyl CoA desaturase from membranes and reconstituted its activity in lipid vesicles using purified NADPH-cytochrome P-450 reductase. They suggested that NADPH is the physiological reductant rather than NADH.

In animals, the double bond (usually the *cis* configuration) can be introduced at the Δ4, Δ5, Δ6, Δ8, and Δ9 positions but not at the Δ12 or Δ15 positions; these EFAs are of plant origin. Further desaturation and insertion of a methylene group (e.g., elongation) leads to the formation of polyunsaturated fatty acids (PUFAs). Unsaturated fatty acids are important, for example, in regulating the physical properties of membranes and lipoproteins or as precursors for physiologically active metabolites. For example, arachidonic acid is a principal precursor for prostaglandins, prostacyclins, leukotrienes, and thromboxanes, which have important roles in smooth muscle stimulation, control of platelet aggregation, and the release of histamine in anaphylactic shock and other allergic reactions. The mechanism involved in the control of desaturation is poorly understood, although the following observations have been made.

O. Dietary Control

Prasad and Joshi (1979) demonstrated that dietary induction of Δ9 desaturase in chicken liver was due to an increase in the amount of terminal desaturase. Holloway and Holloway (1975) showed that the fourfold decrease in Δ9 desaturase activity of mice fed an essentially fat-free diet compared with mice fed a safflower-supplemented diet could not be related to differences in the lipid composition, NADH-cytochrome b_5 reductase, or cytochrome content of membranes. They concluded that the difference was due to a change in the amount of hepatic Δ9 desaturase. Excess dietary carbohydrate increases Δ9 desaturation, probably to maintain the physical properties of the fatty acyl chains of membrane PLs and hence maintain the optimal "fluidity" of membranes. Brenner (1981) reviewed the regulation of Δ5 and Δ6 desaturases; the latter appears to be less responsive to induction by high-carbohydrate diets, but diets deficient in EFAs cause a fourfold increase in Δ6 desaturation activity and a specific decrease in Δ5 desaturase activity with concomitant accumulation of 20:3n-9, which is characteristic of EFA deficiency.

P. Hormonal Control

The relationship between insulin and Δ9 desaturation activity has been studied in rats (Prasad and Joshi, 1979), embryonic chicken liver explants (Joshi and Aranda, 1979), and cultured 3T3-L1 murine cells (Kasturi and Joshi, 1982). In all cases, changes in desaturase activity were due to changes in the amount of the terminal Δ9 desaturase. In diabetic rats, insulin restored Δ9 desaturase to values in fructose-fed normal rats. With the chicken liver explants, insulin increased the very low amount of Δ9 desaturase, and this stimulation was potentiated by triiodothyroxine and hydrocortisone (Joshi and Aranda, 1979). The authors suggested that induction by insulin may regulate Δ9 desaturase mRNA, as measured by the inhibition of chromatin-bound poly(A) polymerase. 3T3-L1 murine cells also showed a 100-fold induction of Δ9 desaturase in response to insulin.

Lippiello et al. (1979) demonstrated a close relationship between membrane composition, fluidity, and desaturase activity in the tissue of roosters. Desaturase activity was high when membrane fluidity and the proportion of double bonds was low, and the change in activity was shown to be caused by an increase in the amount of enzymatic protein rather than by allosteric effects. Joshi and Aranda (1979) estimated the half-life of Δ9 desaturase to be 4 h. Strittmatter et al. (1972) purified stearoyl CoA desaturase and obtained a single polypeptide with M_r of 53,000 with 458 amino acid residues, of which 62% were nonpolar. Enoch and Strittmatter (1978) suggested that arginyl residues may be part of the binding site for the CoA moiety of the substrate. Prasad and Joshi (1979) isolated and purified Δ9 desaturase from chicken liver, as judged by immunochemical analysis. It is considerably smaller (M_r 33,500) than the rat hepatic enzyme (M_r 53,000). Sreekrishna and Joshi (1980) clearly demonstrated that Δ9 desaturase activity in chicken liver microsomes and in a reconstituted system was inhibited by divalent copper and chelated copper. They concluded that this inhibition is due to the destruction of superoxide (see above), which further substantiated the proposed involvement of cytochrome P-450 in desaturase activity.

Brenner (1981) reviewed the control of Δ5 and Δ6 desaturases. As with Δ9 desaturases, insulin increased their activity, whereas all other hormones tested decreases Δ5 and Δ6 desaturation. For example, Joshi and Aranda (1979) demonstrated loss of Δ6 desaturase activity in chicken liver explants within 3 h of injection of epinephrine (adrenaline). The work of Jones and Gaylor (1979) demonstrated that cytoplasmic protein stimulates microsomal Δ9 desaturase activity despite its inability to bind to acyl CoA esters. Δ9 Desaturases appear less responsive to cytoplasmic stimulation than Δ5 and Δ6 desaturases, which largely depend on the presence of these proteins. Extensive washing of microsomes led to the loss of Δ6 and Δ5 desaturase activities, which were restored by the addition of cytoplasm or a protein fraction isolated from it (Larsson and Brimer, 1979).

V. EFFECT OF DIETARY LIPID ON LIPOPROTEIN CHOLESTEROL

A. Dietary Cholesterol

A comprehensive, retrospective view of the effects of dietary cholesterol is given by McGill (1979a,b). More recent reviews include those of Coates (1983), Goldberg and Schonfeld (1985), Welch (1986), Beynen et al. (1987), McNamara (1987), and Gurr et al. (1989). When interpreting the results of feeding trials, it is important to bear in mind the possibility of a transient response to the diet that could be misleading if the experiment is of short duration. Adaptation and restoration of plasma lipid levels can sometimes occur if the experiment is of sufficient duration (Oliver, 1984; Price et al., 1985; Edgington et al., 1987).

B. Human Studies

According to McNamara (1987), a 70-kg man on a typical North American diet synthesizes 840 mg of cholesterol and absorbs 250 mg/day of a dietary intake of 450 mg/day of cholesterol.

The mechanism for plasma cholesterol homeostasis, in the face of increased cholesterol intake, include decreased absorption, decreased endogenous synthesis, increased biliary excretion, and, to a lesser extent, increased fecal bile acid excretion (McNamara, 1987; Quintao and Sperotto, 1987).

If the intake of cholesterol is relatively moderate (250–750 mg/day), some studies have shown that the compensating mechanisms largely prevent a rise in plasma cholesterol concentration (McNamara, 1987; Gurr et al., 1989). However, other studies have demonstrated an increase in plasma cholesterol concentration with an intake of cholesterol in the range of 0–600 mg/day, with little additional effect above 600 mg/day (McGill, 1979a,b). The response can be greater if the control diet is low in cholesterol and if the initial plasma cholesterol concentration is high. There is some evidence that hypercholesterolemic subjects have a greater response than normolipidemic subjects, but this has not been the experience of all investigators (Gurr et al., 1989).

The variation in the experimental findings could also be explained by the occurrence of hyper- and hyporesponders to dietary cholesterol. The differences between them are possibly due to inadequate suppression of cholesterol biosynthesis, greater efflux of cholesterol in lipoproteins, or more efficient absorption of cholesterol in hyperresponders (Beynen et al., 1987). In some studies, the effect of dietary cholesterol on plasma cholesterol concentration has been potentiated by the cholesterol being given with saturated fat rather than with PUFAs (Goldstein and Brown, 1976; McGill, 1979a,b).

C. Animal Studies

Most animal species are more sensitive than humans to dietary cholesterol and develop high-plasma cholesterol concentrations and aortic lesions that resemble human atheroma (McGill, 1979a,b). It has been claimed, however, that cholesterol-feeding experiments simply lead to lipid storage disease (Stehbens, 1989). Cholesterol supplementation of the diet results in an increase in IDL and LDL, a decrease in HDL_2, the appearance of a cholesteryl ester-rich, apo E-containing form of HDL, termed HDL_c, which is possibly HDL_1 present at a high concentration, and a cholesteryl ester-rich form of VLDL, termed β-VLDL, which contains both apo B and apo E and migrates with β mobility on paper electrophoresis (Mahley, 1978).

The β-VLDL from cholesterol-fed dogs can be fractioned into two classes, one of which resembles chylomicron remnants, and the other, VLDL of hepatic origin. Cholesterol-fed animals develop atherosclerotic lesions in which β-VLDL can be detected (Daugherty and Schonfeld, 1985). The HDL_c appears to represent a form of HDL that is loaded with cholesteryl ester and may play a role in transporting cholesterol from overloaded peripheral cells (Daugherty and Schonfeld, 1985). Lipoproteins similar to β-VLDL and HDL_c have also been identified in humans as a result of diet-induced hypercholesterolemia (Mahley, 1978; Daugherty and Schonfeld, 1985).

D. Dietary Fatty Acid Composition

It is generally believed that, in humans, the fatty acid composition and content of the diet are more important than the cholesterol content in affecting plasma cholesterol concentrations (Beynen et al., 1987). The saturated fatty acids (SFAs) 12:0, 14:0, and 16:0 are those that are principally associated with elevated plasma cholesterol concentration, especially LDL-c. Dietary PUFAs lower the plasma cholesterol concentration, the effect on lipoproteins being dependent on the type of PUFA. The n-6 fatty acids in vegetable oils primarily lower LDL-c, whereas n-3 fatty acids in marine oils primarily lower VLDL-TAG and VLDL-c. When the *P*/*S* ratio of the diet is high, HDL-c can also be lowered (Gurr et al., 1989).

The cholesterol-lowering effects of PUFAs are dependent on the basal conditions, that is, the initial plasma cholesterol concentration and the amount of cholesterol in the diet. It has been demonstrated that when there is no cholesterol in the diet, the increase in the *P*/*S* ratio is ineffective in reducing plasma cholesterol concentration (Connor et al., 1964; Mulvihill and Walker, 1984; Vergroesen, 1986). When the plasma cholesterol concentration is low initially, as in rats or rabbits, any increase in dietary lipid can cause it to increase, whatever the composition of the lipid, although

SFAs are more effective than PUFAs (Gurr et al., 1989). Individual response to dietary saturated lipid varies as with dietary cholesterol, and it appears that human hyperresponsiveness to dietary cholesterol is associated with hyperresponsiveness to SFA (Beynen et al., 1987).

E. Triacylglycerol Structure

The distribution of fatty acids on dietary TAG also has an effect on plasma cholesterol concentration. Thus, randomization of the fatty acids of both peanut oil and butter results in their having a cholesterol-lowering effect as compared to the unmodified materials (Beynen and Kritchevsky, 1986).

F. Level of Dietary Lipid

Nutritional advice intended to produce a lowering of plasma cholesterol has emphasized the need to reduce fat intake, based on the epidemiological evidence, but from nutritional experiments it appears that a high-fat diet with a high *P/S* ratio is as effective in lowering plasma cholesterol as a low-fat, high-carbohydrate diet, and the latter tends to raise VLDL-TAG (Gotenbos, 1985; Berry et al., 1986; Beynen and Kritchevsky, 1986; Gurr et al., 1989). Shepherd and Packard (1984) pointed out that the increase in VLDL production that results from a high-carbohydrate diet would increase VLDL-c and deplete hepatic cholesterol. This would stimulate hepatic cholesterol production and promote hepatic LDL receptors, resulting in a reduction in LDL.

VI. POSSIBLE MECHANISMS OF THE HYPOCHOLESTEROLEMIC EFFECTS OF DIETARY POLYUNSATURATED FATTY ACID

The subject has been reviewed by Paul et al. (1980), Goodnight et al. (1982), Grundy (1983), Welch (1986), McNamara (1987), Beynen et al. (1987), and Gurr et al. (1989).

A. Effects on Cholesterol Metabolism

a. Reduced Absorption of Cholesterol

Evidence in the literature is conflicting (Paul et al., 1980), but it appears that there is little effect of dietary PUFA on dietary cholesterol absorption (McNamara, 1987). Beynen et al. (1987), however, suggested that differences in cholesterol absorption may contribute to the difference in response to dietary cholesterol.

b. Redistribution of Cholesterol to Tissues

There is little evidence for this in humans (Paul et al., 1980; Beynen et al., 1987; Gurr et al., 1989), but there is some evidence in rats (Paul et al., 1980). Edwards-Webb et al. (1986) found that inclusion of PUFA in the diet of guinea pigs led to increases in both PUFA and cholesterol in erythrocyte membranes.

c. Increased Excretion of Cholesterol and Bile Acids

The evidence in animals is conflicting (Paul et al., 1980). In rabbits whose diet was supplemented with either butter- or PUFA-rich margarine plus an equivalent amount of cholesterol, it was found that the proportion of the plasma pool of cholesterol that was excreted daily was significantly greater on the margarine diet (Jadidi, 1989).

Studies in humans suggest that there is a greater fecal excretion of steroids, particularly endogenous cholesterol, as a result of PUFA in the diet (Paul et al., 1980; Liepa and Sullivan-Gorman, 1986). However, it has been suggested that the increased excretion may be a transient response only (McNamara, 1987). An increased intake of PUFA increases both the rate of bile flow and the

degree of unsaturation of biliary PL, which leads to greater solubility of cholesterol in the bile. Thus, increased amounts of cholesterol could be excreted into the intestine via the bile.

Pigs fed on soybean oil had increased concentrations of bile acid compared to pigs fed on tallow, and they also exhibited an increase in coprostanol in the gut, a metabolite of cholesterol that is less readily reabsorbed than cholesterol (J. D. Edwards-Webb and M. I. Gurr, personal communication).

d. Reduced Synthesis of Cholesterol

Most investigators agree that dietary PUFA does not inhibit cholesterol synthesis. However, Strain and Sherry (1985) concluded from balance studies that PUFA did have such an effect.

e. Enhanced Activity of LCAT

The enzyme LCAT catalyzes the reaction between lecithin (phosphatidylcholine) and cholesterol to give lyso lecithin (LPC) and cholesteryl ester. The most favored substrate for the LCAT reaction in humans is linoleate bound to the 2-position of *sn*-phosphatidylcholine (Dobiasova, 1983). An increase in PUFA in the diet increases the linoleate content of HDL PLs (Morrisett et al., 1977; Paul et al., 1980). In an earlier section, it was described how HDL acquires PL from TAG-rich lipoproteins when they are hydrolyzed. The phosphatidylcholine of the chylomicron coat is primarily of biliary origin and is principally 1-palmitoyl-2-linoleoyl-*sn*-phosphatidylcholine (Tso, 1985). It has been observed that patients suffering from acute myocardial infraction have particularly low levels of plasma LPC, which could have been due, at least in part, to a fall in LPC formation *in vivo*. A low level of LPC could exacerbate cardiovascular disease (CVD), because LPC inhibits platelet aggregation (Gillett and Besterman, 1975). There is also a low level of linoleate in the plasma cholesteryl ester of patients suffering from CVD (Grojec et al., 1983).

Wells and coworkers (1986) found that the LCAT molar esterification rate (MER) and LPC concentration were not significantly less than normal in cases of myocardial infraction, but that in patients without myocardial infractions, the severity of coronary atherosclerosis was related to increases in LCAT MER and LPC concentrations and a decrease in unesterified cholesterol. Since LPC was found to mediate the diffusion of LDL through the endothelium of the artery, the authors concluded that increased concentrations of LPC would promote the development of atherosclerosis. However, an increase in LPC would also have the beneficial effects of increasing LDL uptake by the liver (Dobiasova, 1983) and inhibiting platelet aggregation as noted above.

Changes in the fractional esterification rate (FER) are probably of greater significance than changes in MER. Dobiasova (1983) calculated the relative FERs from a number of studies involving comparisons of LCAT activity healthy controls and CVD patients. She concluded that in the disease state, the relationship between LCAT activity and the size of the plasma pool of cholesterol was impaired because either the LCAT activity did not increase proportionally with the plasma cholesterol concentration or the LCAT activity was inhibited at normal cholesterol concentrations. In patients with familial LCAT deficiency, there is a reduced rate of removal of atherogenic remnants of TAG-rich lipoproteins, because they are deficient in apo E, which they normally acquire as a consequence of the LCAT reaction (see Figure 22.1) (Norum et al., 1982).

Hawthorne et al. (1981) suggested that feeding soy lecithin would increase the proportion of linoleic acid in plasma PL and by doing so would lead to an increased production of cholesteryl linoleate via the LCAT reaction and thus facilitate removal of cholesterol from the body. They suggested that the LCAT reaction was more active after lecithin feeding and that sterol excretion increased. An advantage of feeding PUFA in the form of lecithin was that LDL levels were reduced and HDL levels raised. Similar conclusions were reached by Blaton and Hollez (1986). Sinclair (1980, 1984) also drew attention to the greater ease of transport and removal of cholesteryl linoleate from the body compared to cholesteryl oleate, which would be formed in the absence of EFA. Moilanen et al. (1986) reported that the percentage of plasma cholesteryl linoleate was negatively correlated with plasma total cholesterol and LDL concentrations in humans.

It has been suggested that the increased fluidity of the PL resulting from feeding PUFA could increase the rate of LCAT reaction (Morrisett et al., 1977). There are few experimental studies on the effect of dietary lipid on LCAT activity. Gjone et al. (1972) reported a reduction in esterification rate when PUFA replaced saturated fat in the diet. Miller et al. (1975) also reported a lower esterification rate when subjects were fed on PUFA-rich diets. A recalculation of the data of Miller et al. (1975) by Dobiasova (1983) showed that the change from a saturated fat-rich diet to a PUFA-rich one resulted in a decrease in the MER but no change in the FER, so the reduction in the free cholesterol pool was paralleled by a reduction in MER. Janetschek et al. (1983) reported a significant decrease in FER in normal females when a diet high in PUFA and low in cholesterol (*P/S* 1.0) was substituted by a control diet low in PUFA and high in cholesterol (*P/S* 0.3). There was also a nonsignificant decrease in MER.

In an experiment in our laboratory, in which the diet of pigs was supplemented with either butter or PUFA-rich margarine plus an equivalent amount of cholesterol (Jadidi, 1989), there was an increase in plasma cholesterol concentration on the butter diet that was accounted for by an increase in cholesteryl esters, and no significant change in the plasma cholesterol of the margarine-fed pigs, although there was a significant decrease in unesterified cholesterol and a significant increase in cholesteryl ester. The butter diet resulted in a nonsignificant increase in both MER and FER via the LCAT reaction, whereas the margarine diet produced a very significant increase ($p < .001$) in both MER and FER.

It seems likely, therefore, that a diet rich in PUFA leads to an increase in linoleate, the preferred substitute of LCAT, in phospholipids, and increased formation of LPC and cholesteryl linoleate via the LCAT reaction. The increase in linoleate in HDL-PL and HDL-cholesteryl ester could increase the fluidity of the particle and allow easier transfer of cholesteryl ester to the hydrophobic core, thereby leaving the surface free to pick up more cholesterol via the reverse cholesterol pathway (Paul et al., 1980; Blaton and Hollez, 1986).

B. Effects of Lipoprotein Metabolism

a. Reduction in Rate of Synthesis of VLDL and LDL

Substitution of a diet rich in PUFA for a diet rich in saturated fat decreased the synthetic rates of both VLDL apo B and LDL apo B (Cortese et al., 1983). However, other workers reported that a PUFA-rich diet had no significant effect on the synthetic rate of LDL, although they did not rule out a contribution to cholesterol lowering by this means (Shepherd et al., 1980). McNamara (1987) concluded from a study of the literature that large increases in the *P/S* ratio reduced the production of VLDL and LDL apo B to a greater extent than they increased the fractional catabolic rate (FCR) (see below), but the paucity of data prevented a conclusion as to the effect of more modest increases in *P/S* ratio. The lowering of plasma LDL by feeding n-3 fatty acids has been attributed to a reduced synthesis of apo B (Illingworth et al., 1984).

b. Increase in FCR and Changes in Fluidity of Lipoproteins and/or Their Receptors

In the experiment described above, Cortese et al. (1983) found no change in the FCR of LDL on substituting a PUFA-rich diet for a saturated fat-rich one but Shepherd et al. (1980) and others (Goodnight et al., 1982) have observed increases in the FCR. Dietary saturated fat has been found to downregulate LDL receptors (Schonfeld, 1988).

Increases in lipoprotein fluidity as a result of PUFA-rich diets have been reported (Morrisett et al., 1977), and it has been suggested that dietary unsaturated fat may promote increased fluidity of the lipoprotein, which could increase receptor affinity and promote endocytosis (Havel, 1983). Increased fluidity of membranes could also facilitate the incorporation of LDL receptors. It has also

been suggested that because PUFA occupies a greater area than SFA, incorporation of PUFA into LDL could change its configuration and decrease the cholesterol/protein ratio, thereby reducing its capacity to transport cholesterol (Spritz and Mishkel, 1969; Shepherd et al., 1980). LPL will hydrolyze TAGs containing PUFA faster than those containing SFA (Morrisett et al., 1977), and this could lead to enhanced clearance of lipoproteins and increased production of HDL.

c. Change in Size of Lipoprotein Particles

It is generally believed that relative atherogenicity is inversely related to particle size, because small particles such as LDL could permeate the arterial intima more easily than the larger VLDL or IDL (Morris et al., 1984) and because there is a negative correlation between the level of small LDL particles and that of HDL (Shen et al., 1981). It would appear to be paradoxical, therefore, that hyperlipidemia characterized by high levels of VLDL and LDL leads to an enhanced incidence of atherosclerosis. However, it has been demonstrated that a greater range of particle size in the VLDL and IDL occurs in such hyperlipidemias, with the mean particle sizes being lower than normal. The decrease in particle size is accompanied by an increase in TAG content and a decrease in cholesterol content (believed to be mainly cholesteryl ester) and is therefore indicative of a change in core lipid composition. It was concluded that the enhanced atherogenicity was due to the decreased size and altered composition of the particles (Morris et al., 1984).

Similarly, Sniderman and coworkers (Sniderman et al., 1980; Sniderman and Kwiterovich, 1984) attributed enhanced atherogenicity to a class of LDLs found in a subgroup of coronary patients who had a normal LDL cholesterol level but an elevated level of LDL apo B. They suggested that the increased number of LDL particles of smaller size could have resulted from either impaired catabolism or overproduction of VLDL, and that the risk of CVD is more closely related to the number of LDL particles than to the level of LDL-c. Subsequently, it has been demonstrated that in such cases of hyperapobetalipoproteinemia, there is increased production of VLDL and LDL. The situation is exacerbated by a high level of dietary fat, which resulted in higher levels of chylomicrons and VLDLs as a result of delayed clearance (Kwiterovich, 1988). The small LDL is removed from the plasma more slowly than large LDLs (Thompson et al., 1987; Kwiterovich, 1988; Musliner and Krauss, 1988). However, Rudel and coworkers (Rudel et al., 1983, 1986; Johnson et al., 1985; Clarkson et al., 1986) maintain that the size of LDL is positively correlated with coronary atherosclerosis. They found that the American green monkey is a good model for atherogenesis in humans and that the effect of changing from a diet rich in saturated fat to one rich in PUFA was to reduce the plasma cholesterol concentration and both LDL-c and HDL-c. There was a reduction both in the size of LDL and in aortic atherosclerosis. However, similar changes in the diet of cynomologus monkeys produced no change in plasma or LDL cholesterol concentrations, and although there was a decrease in HDL-c, there was an increase in LDL size. The authors suggested that dietary PUFA may be atherogenic in this species of monkey. Jadidi (1989), working with rabbits, also found that dietary PUFA in the form of margarine, as compared to butter, increased the size of LDL.

Large VLDL is removed from the plasma more rapidly than small VLDL and does not give rise to LDL (Shepherd and Packard, 1987a,b). Similarly, large chylomicrons and their remnants are more rapidly cleared from the plasma than smaller ones (Quarfordt and Goodman, 1966; Eisenberg, 1983). Dietary PUFA gives rise to larger chylomicrons than does saturated fat (Caselli et al., 1979; Mattson, 1983), and if it is possible that small chylomicrons (intestinal VLDL) could give rise to a form of LDL (Eisenberg, 1983), then this could be a contributory factor in the hypercholesterolemic effect of saturated fat.

There are differing accounts on the effect of dietary fat on the size of VLDL. Hojackni et al. (1977) found that a dietary supplement of corn oil, as compared to coconut oil, produced large VLDL in cebus monkeys, but Jadidi (1989) found that dietary polyunsaturated margarine, as compared to butter, produced smaller VLDL.

d. Modification of Apolipoproteins

The activation of LCAT by apo A-I and the section of apo A-I in the form of a preapoprotein, which undergoes cleavage to give the active mature form, have been discussed in previous sections. Mengheri et al. (1985) found that when rats were given a high-fat, high-cholesterol diet-containing casein, their LDL-c increased, and this was accompanied by the appearance, in the apolipoprotein electrophoretic pattern of all the lipoproteins, of an anomalous apo A-I band with greater than normal mobility. When fava bean or its ethanol extract was added to the diet, plasma cholesterol concentration was reduced and the apo A-I disappeared from the LDLs, but both the abnormal and the normal form were present in HDL.

The authors suggested that the structure of the cholesterol- and TAG-rich particles leaving the intestine was such that the normal cleavage of preapo A-I to apo A-I was inhibited and that cleavage took place at a different site, to give a smaller form of apo A-I with greater electrophoretic mobility. This form of apo A-I could have a reduced ability to activate LCAT. The addition of fava bean or its ethanol extract could have supplied a polyunsaturated lecithin that could promote the LCAT reaction and facilitate the correct processing of preapo A-I to the mature form of apo A-I.

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23 The Effects of Dietary Fatty Acids on Lipid Metabolism

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CONTENTS

I. INTRODUCTION

Both the amount and type of dietary fatty acids affect the development and progression of several human chronic diseases including cardiovascular diseases (CVD) and cancer. One of the mechanisms by which dietary fatty acids impact human health is by altering lipid metabolism.

In this chapter we review the effects of dietary saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) of chain length C-12 and longer, on the metabolism of fatty acids, cholesterol, and eicosanoids. We will also summarize the sources of fatty acids, their digestion, absorption, and transport, and describe in some detail *de novo* fatty acid synthesis and catabolism. This chapter has been prepared to replace the chapter published by Dr Gary J. Nelson in the second edition of this book (Nelson, 2000).

Results from studies published prior to 2000 will be summarized here and those published since then will be discussed in detail. For details regarding the earlier literature, the readers are referred to the second edition of this book and some other reviews (Sprecher et al., 1995; Sprecher, 1996; Mohammed et al., 1997); for more recent advances in this field the reader is referred to following reviews (Mu and Porsgaard, 2005; Arterburn et al., 2006; Breslow, 2006; Carpentier et al., 2006; Gebauer et al., 2006; Goyens et al., 2006; Hibbeln et al., 2006).

One should recognize that dietary fatty acids, including the essential fatty acids (EFA) are a macronutrient in the human diet. In the Western diet, consumed primarily in Europe and North America, fatty acids can be the major source of calories. (Carbohydrates are usually the largest source of calories in many Asian and third-world countries.) In reality, the influence of dietary fatty acids on

lipid metabolism cannot be separated from the effects of carbohydrate and protein in the diet, but this subject is not within the scope of this chapter.

II. CHARACTERISTICS OF DIETARY FATTY ACIDS

A. Sources and Quantities

Approximately 99% of the fatty acids consumed are as acyl esters, usually triacylglycerols, occasionally mono- or diglycerides (in the Western diet these compounds are often added to processed foods), and, to a lesser extent, phospholipids and other complex lipids found in plant and animal tissues (Norris, 1983). Chemically, triglycerides (TGs) are the triacylglycerols or a glycerol molecule esterified with three fatty acids. Animal fats are a rich source of saturated medium-chain fatty acids and oleic acid (18:1n-9). Plant seed oils are good sources of linoleic acid (18:2n-6, LA) and contain only trace amounts of n-6 fatty acids with chain lengths longer than 18 carbons. Arachidonic acid (20:4n-6, AA) is found in all animal tissues and animal-based food products (Jonnalagadda et al., 1995). Brain and several other organ meats contain hydroxy fatty acids in small amounts (Rouser et al., 1968; Hakamori, 1983), and long-chain saturated fatty acids (SFA) up to 26 carbons in length can be found in small amounts in most animal tissues.

Conjugated linoleic acid (18:2n-6, CLA) and gamma linoleic acid (8:3n-6, GLA), are other important n-6 fatty acids that are found in relatively small amounts in the diets but have significant physiological effects. CLA is a collective term for isomers of LA that have conjugated double bonds. Depending on the position and geometry of the double bonds, more than a dozen isomers of CLA have been reported; two of those isomers, *cis* 9, *trans* 11-CLA (*c*9, *t*11-CLA) and *trans* 10, *cis* 12-CLA (*t*10, *c*12-CLA), have been studied regarding their health effects. The major dietary sources of *c*9, *t*11-CLA are dairy products and ruminant meat, while that of *t*10, *c*12-CLA are partially hydrogenated vegetable oils from margarines and shortenings. Primrose, borage, and black currant seed oils contain considerable amounts of GLA. Dietary fatty acids of the n-3 series include α-linolenic acid (18:3n-3, ALA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). Flax and perilla seed oils are very rich sources of ALA, while deep ocean fish and the fish oils are good sources of EPA and DHA. Another good source of DHA-TG is the oil from genetically engineered algae in which DHA represents approximately 50% of the total fatty acids. In addition, certain animal tissue like brains have significant amount of DHA. Humans cannot insert the first double bond at C3 or C6, but can elongate and desaturate LA and ALA. Therefore, fatty acids with 18, 20, or 22 carbons, with 2–6 double bonds in *cis* configuration, and the first double bond between C3 and C4, or C6 and C7, are considered EFA.

Modern diets based on industrial agriculture are considered to be deficient in n-3 fatty acids (Lands, 1986a; Simopoulos et al., 1994; Broadhurst et al., 1998; Cordain et al., 2005). This is the result of both increased consumption of n-6 fatty acids and decreased consumption of n-3 fatty acids. Other populations, particularly Eskimos, who maintain a traditional life-style, are a prime example, as are the Japanese and other societies who consume a large amount of fish and obtain significant amounts of n-3 fatty acids in their diets and conditions are better health status seen in these populations (Bang et al., 1980; Dyerberg and Jorgensen, 1982). Endogenous synthesis of fatty acids, such as palmitic, stearic, and oleic acids, is triggered by consumption of excessive calories from carbohydrate or protein.

Americans consumed approximately 36%–37% of their calories as fat in 1985 (Ernst, 1991); this was comprised of 13.2% saturated, 13.8% monounsaturated, and 7.0% polyunsaturated fatty acids. According to the data from NHANES 1999–2000 survey (CDC, 2001), the percentage of energy from fat decreased to 32.8% in both men and women. The reduction in fat intake was largely from the reduction in saturated fat intake, which reduced to approximately 11% in both sexes. SFAs consumed are primarily 14:0, 16:0, and some 18:0. The monounsaturated fatty acids are almost exclusively 18:1n-9 unless one is eating much fish or rapeseed oil, containing 20- and 22-carbon

monounsaturated fatty acids. The PUFAs are almost exclusively 18:2n-6 or 18:3n-3, unless fish consumption plays a large role in one's diet, in which case long-chain, polyunsaturated n-3 fatty acids (n-3 PUFAs) such as EPA and DHA can contribute to the composition of dietary PUFAs. However, 18:2n-6 and 18:2n-3 probably account for more than 95% of the PUFAs consumed in the typical Western diet.

Recent data from NHANES III indicate that an average daily intake of n-3 PUFA is about 1.6 g (0.7 en%), out of which EPA and DHA constitute only 0.1–0.2 g/day (Hibbeln et al., 2006). Thus, the current intake of long chain n-3 PUFA is much higher than 50 mg reported in some of the earlier reports (Ernst, 1991; Jonnalagadda et al., 1995). Recommendations for daily intake of n-3 PUFA vary between several scientific and health organizations. American Heart Association recommends eating one serving of oily fish at least twice a week and includes oils rich in ALA if one does not have documented CVD (Kris-Etherton et al., 2002). For patients with documented CVD, the recommendations are 1 g/day of EPA + DHA and for those with hypertriglyceridemia, 2–4 g/day of EPA + DHA is recommended. ISSFAL (2004) recommended for healthy adults 2 en% from LA, 0.7 en% from ALA, and a minimum intake of 500 mg/day of EPA + DHA. Similar but different recommendations have also been made by WHO 2003, NCEP 2002, and USDA dietary guidelines 2005.

III. DIGESTION OF FATTY ACIDS

Although this topic has been discussed extensively, it is necessary to review briefly some salient points as they cannot be easily separated from the topic of this chapter.

Fat digestion and absorption is a multistage metabolic process, the complexity of which is compounded by the insolubility of most lipids in aqueous media (Small, 1983). When fat is consumed, either in natural foods or in a purified form (in experimental dietary protocols), a series of physiological events begin that last for 16–24 h if no other food is consumed after the initial meal (Bisagaier and Glickman, 1983). Initially, the fatty food, if consumed orally and masticated (rather than as capsules or by stomach tube), is mixed with lingual lipase, followed by hydrolysis of the TGs by gastric lipase in the stomach (Muller et al., 1975; Moreau et al., 1988). Both lingual and gastric lipases preferentially hydrolyze short-chain fatty acids at the sn-3 position to produce diacylglycerols (Phan and Tso, 2001; Mu and Porsgaard, 2005). Considerable species differences exist regarding the occurrence and abundance of lingual lipase and gastric lipase. Rats and mice have lingual lipase and essentially no gastric lipase, whereas humans and baboons have high levels of gastric and pancreatic lipase and very little lingual lipase. Gastric lipase has a significant metabolic role in the digestion of fat by human neonates who have a relatively low activity of pancreatic lipase and in patients with pancreatic insufficiencies.

Very short-chain (up to eight carbons) fatty acids are absorbed directly from the stomach into the venous circulation. In addition, small but variable amounts of long-chain free fatty acids (FFAs) can be absorbed directly by the portal circulation (McDonald et al., 1980; Surawicz et al., 1981; Carey et al., 1983; Vallot et al., 1985; Jandacek et al., 1987). In healthy rodents, 30%–70% of the intraduodenally infused long-chain fatty acids bypasses the lymph and directly enters the portal vein (McDonald et al., 1980). Such information in healthy human subjects is not available, however, the portal vein absorption of dietary long-chain unsaturated fatty acids in patients with advanced cirrhosis has been reported (Cabre et al., 2005).

Most of the C_{14}–C_{18} fatty acids, as free or esterified fatty acids, are passed into the intestinal lumen, where pancreatic lipase, colipase, and bile are added to the digestive ferment followed by further lipolysis. The composition of fat entering the upper duodenum is made up of 70% of TGs with the reminder consisting of a mixture of partially digested hydrolysis products formed primarily by gastric lipase. Under normal digestion and absorption, majority of the fatty acid hydrolysis is caused by pancreatic lipase which acts mainly on the sn-1 and sn-3 position of the natural TGs, leaving the 2-monoglyceride intact (Borgstrom, 1974; Tso, 1985; Mu and Porsgaard, 2005). A rearrangement of sn-2 monoglyceride into sn-1(3) monoglyceride may result in complete degradation into

glycerol and FFAs by pancreatic lipase. Even though the pancreatic lipase is active toward fatty acids located in the sn-1(3) positions, the activity toward n-3 fatty acids, particularly EPA and DHA, is lower compared with activity toward other fatty acids at the same position (Mu and Porsgaard, 2005).

Phospholipids are hydrolyzed by pancreatic enzymes to FFAs and their derived lyso compounds (Tidwell et al., 1963; Borgstrom, 1974). Pancreatic phospholipase A_2 acts on the sn-2 position of phospholipids to produce 1-lyso compounds. Pancreatic lipase (1,3-acylglycerol ester hydrolase) also acts on the 1-position of phospholipids (Borgstrom, 1974; Tso, 1985); however, most phospholipids are absorbed by the enterocyte as the 1-lyso compound after being hydrolyzed by phospholipase A_2 (Nilsson, 1969; Borgstrom, 1974). Cholesterol esters, if present, are hydrolyzed completely (Nilsson, 1969) to free cholesterol and FFA by cholesterol esterases.

Once the ingested fats have been hydrolyzed to FFA, monoglycerides, lysophospholipids, and free cholesterol, the hydrolysis products are absorbed by the enterocytes of the intestinal wall. The process of absorption from the intestinal lumen into the enterocyte is by passive diffusion (Carey et al., 1983). Studies by Stremmel have also indicated the possibility of presence of a fatty acid binding protein associated with brush border membrane, and that it might play a role in fatty acid uptake by enterocytes (Stremmel, 1988). However, this carrier-mediated process might play a role only in low fatty acid concentrations. FFA, lysophospholipids, and 2-monoglycerides can be absorbed almost completely in the absence of bile salts (Borgstrom, 1977a), although the presence of bile is obligatory for the absorption of free cholesterol (Borgstrom, 1977b).

IV. FATTY ACID TRANSPORT

In the enterocyte, the lipolysis products are re-esterified in the endoplasmic reticulum to TG, phospholipids, and other complex lipids (Brown and Johnston, 1964). Cholesterol can be synthesized de novo if there are inadequate dietary quantities, but even if no cholesterol is supplied by the diet, the bile supplies appreciable quantities to the enterocyte during fat absorption (Borgstrom, 1977a, b). However, the enterocyte treats dietary cholesterol and endogenous cholesterol differently. For instance, cholesterol derived from the intestinal lumen does not mix evenly with the free cholesterol pool and is preferentially reesterified in the enterocyte for export into TG-rich lipoproteins (Stange and Dietschy, 1985; Pool et al., 1991). The newly synthesized TGs, phospholipids, and cholesterol esters are combined with de novo-synthesized apolipoproteins, mainly apo B-48, with some apo A-I, A-II, A-IV, and E to form chylomicrons (Simmond, 1972). The chylomicrons are then secreted into the lymphatic system and carried by the thoracic lymphatic duct to the superior vena cava, where they enter the blood circulation (Simmond, 1972).

Lipoprotein lipase released by the capillary endothelial cells hydrolyzes the TGs in chylomicrons, releasing FFA and 2-monoglycerides. Lipoprotein lipase is activated in the presence of apo C-II as cofactor (Fielding and Fielding, 1977) and inhibited by apo C-III (Jong et al., 1999). It has positional specificity for the primary ester bonds of acylglycerols (Nilsson-Ehle et al., 1973) and hydrolyzes the TGs to 1,2- and 2,3-diacylglycerols and FFA. It also hydrolyzes 1,2- and 2,3-diacylglycerols to 2-monoacylglycerols and 1- and 3-monoglycerides to free glycerol and fatty acids (Morley et al., 1975). Lipoprotein lipase has no effect on 2-monoglycerides (Scow et al., 1977); 2-monoglycerides are hydrolyzed by lipases in the liver (Havel, 1985).

FFA and monoglycerides released by the lipoprotein lipase are taken up by the peripheral tissue (Fielding and Fielding, 1977). Some of the FFAs released bind to albumin and are cleared by the liver (Bergman et al., 1971). The chylomicron remnants have some apo B-48, apo E, phospholipids, cholesterol, cholesterol ester, and some TGs (Havel, 1982). These remnants are then cleared from circulation by the liver low-density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP) (Redgrave, 2004). Though both contribute to chylomicron remnant clearance, LDL receptor normally predominates.

Apo E is the major apoprotein constituent of the TG-rich lipoproteins. It is a determinant for the receptor-mediated catabolism of very low-density lipoproteins (VLDLs), chylomicrons, and their

remnants. Thus, high Apo E levels are generally expected to increase lipid clearance (Chalas et al., 2002). Apolipoprotein B-48 does not bind to the LDL receptor (Hui et al., 1981) and thus does not aid in the clearance of chylomicron remnants.

Another lipoprotein that plays a crucial role in endogenous lipid transport is high-density lipoprotein (HDL). HDLs are involved in the transport of cholesterol from peripheral tissues to liver, a process referred to as reverse cholesterol transport. HDL particles consist of apo E, apo A-I, apo A-II, and apo C with phospholipids and unesterified cholesterol. It was believed that the apoprotein–phospholipid–cholesterol coat of the chylomicron remnants buds off to form precursor HDL particles (Nicoll et al., 1990). However, more recent findings indicate that nascent HDL particles originate in liver and intestine as lipid free or lipid poor apolipoproteins (Basso et al., 2003; Brunham et al., 2006). The newly secreted HDL particles acquire additional cholesterol and phospholipids via ATP-binding cassette transporter A1 (ABCA1) from the liver to form discoidal HDL particles. ABCA1 is an important cellular protein that facilitates efflux of cellular cholesterol to lipid poor apo A-1 as the preferred acceptor. Targeted disruption of hepatic ABCA1 in mice dramatically reduced circulating HDL (Timmins et al., 2005). These discoidal HDL particles acquire additional cholesterol and phospholipids from cells in extrahepatic tissues via ABCA1-mediated efflux, progressively generating particles that are more cholesterol enriched. The enzyme lecithin cholesterol acyltransferase (LCAT), carried on HDL particles, then esterifies the free cholesterol molecules to form cholesteryl ester, which migrate to the core of the HDL particle to form mature spherical HDL particles (Lewis and Rader, 2005; Krimbou et al., 2006). HDLs are further remodeled by cholesteryl ester transfer protein (CETP), which mediates the transfer of cholesteryl esters from HDL to VLDL and chylomicrons.

The liver catabolizes chylomicron remnants, resynthesizes TGs from fatty acids, and repackages them into VLDLs, consisting predominantly of TGs, small amounts of cholesterol and phospholipids, and release them into circulation (Havel, 1985). Small intestine also synthesizes and secretes VLDL-like particles formed from endogenously synthesized lipids in the fasting and postprandial state (Gangl and Ockner, 1975; Tso and Simmonds, 1984). VLDLs are also substrates for endothelial lipoprotein lipase. They lose TGs by hydrolysis and are transformed to intermediate-density lipoproteins (IDLs) and finally to LDL. LDL is taken up by the LDL receptor (Goldstein et al., 1983) of peripheral tissue and liver by receptor-mediated endocytosis. LDL primarily transports cholesterol esters to the peripheral tissues, where they are hydrolyzed to free cholesterol and then reacylated (Sodhi et al., 1978).

V. FATTY ACID METABOLISM

A. Fatty Acid Synthesis

The liver is the primary site for lipid metabolism (Volpe and Vagelos, 1973; Bloch and Vance, 1977; McGarry and Foster, 1980) and is the organ most susceptible to diet-induced changes in lipid metabolism. Adipose tissue is also a major organ system in which fatty acid synthesis occurs when excess calories are consumed; however, in humans it is less active than in many animal species (Hollands and Cawthrone, 1981; Large et al., 2004; Stich and Berlan, 2004).

In the initial step of fatty acid synthesis, acetate as acetyl CoA is condensed with bicarbonate to form malonyl CoA by acetyl-CoA carboxylase. Acetyl CoA is then combined with a series of malonyl CoA molecules by fatty acid syntheses to form fatty acids of different carbon lengths. For example, four malonyl CoA residues are combined by fatty acid synthase to form lauric (12:0) acid. Two more malonyl CoA residues are added to this to produce palmitic acid (Wakil et al., 1958; Lynen, 1967a; Vagelos and Larrabee, 1967; Arslanian et al., 1976; Stoops et al., 1976). If only one malonyl CoA is added to lauric acid, myristic acid (14:0) would be formed. The synthetic reactions up to this point all take place within the fatty acid syntheses complex (Wakil et al., 1958; Lynen, 1967a; Vagelos and Larrabee, 1967; Tanabe et al., 1975; Arslanian et al., 1976; Stoops et al., 1976).

$$CH_3–CO–S–CoA + 7HOOC–CH–CO–S–CoA + 14NADPH + 14H^+ \longrightarrow CH_3–(CH_2)_{14}–COOH + 8CoA–SH + 7CO_2 + 6H_2O + 14NADP^+$$

FIGURE 23.1 The general chemical equation for the de novo synthesis of palmitic acid from acetyl-CoA in most tissues.

Biotin is a required cofactor for the conversion of acetyl CoA to malonyl CoA by acetyl CoA carboxylase (Lynen, 1967a). Acetyl CoA carboxylase is a ubiquitous enzyme found in most mammalian tissues (Waite and Wakil, 1962; Kleinschmidt et al., 1969) and in other species as well (Bimbaum, 1969). It is the rate-limiting enzyme for fatty acid synthesis in most mammalian tissues (Vagelos and Larrabee, 1967), although in some circumstances fatty acid synthase may be rate-limiting (Chang et al., 1967; Majerus et al., 1968; Numa et al., 1970). Regulation of acetyl CoA carboxylase is usually achieved through the supply of acetyl CoA or citrate produced by the Krebs cycle (Lane et al., 1974, 1979). Palmitoyl CoA (and other medium-chain acyl CoA) is a strong inhibitor of acetyl CoA carboxylase *in vitro* (Taketa and Pogell, 1966; Tsutsumi and Takenaka, 1969; Goodridge, 1973; Nikawa et al., 1979). Regulation by dietary fatty acid may proceed through the formation of medium-chain acyl CoA derivatives (Leveille, 1967a,b; Goodridge, 1969; Numa and Nakanishi, 1970; Kitajima et al., 1975; Jeffcoat and James, 1977; Kamirgo et al., 1979).

The summary of reactions involved in the condensation of malonyl CoA to palmitic acid is shown in Figure 23.1. This reaction is catalyzed by fatty acid synthase and requires one molecule of acetyl CoA, seven malonyl CoA, seven NADPH molecules, and 14 H^+. Fatty acid synthase is a multienzyme complex (Veech and Guynn, 1974; Stoops et al., 1979; Wakil et al., 1983) and the central component of the fatty acid synthase complex is the acyl carrier protein (ACP). Despite having no catalytic properties of its own, ACP is essential to the binding of acyl CoA molecules as they are condensed into longer chain fatty acids within fatty acid synthase complex (Prescott and Vagelos, 1972). Free palmitic acid is the primary product of fatty acid synthase reactions in animal tissues (Volpe and Vagelos, 1973; Brindley, 1978). Some myristic and lauric acids are also formed, and a trace of stearic acid may also be produced (Wakil et al., 1983).

Once free palmitic acid is released from the synthase complex, it can be esterified into complex lipids, elongated to stearic acid, or desaturated to palmitoleic acid (Beare-Rogers, 1977; Brenner, 1989). Different enzymatic pathways are involved in these reactions. The regulation and control of these secondary pathways are poorly understood (Beare-Rogers, 1977). However, little or *no de* novo fatty acid synthesis takes place in humans when adequate or excess calories are consumed in a high-fat diet. Conversely, when carbohydrate is fed in excess of caloric requirements, the conversion of carbohydrate to fatty acids is rapid (Groener and van Golde, 1977) and occurs mostly in the liver. The products are mainly palmitic, stearic, and oleic acids, which are esterified to glycerol to form triacylglycerols (Masoro et al., 1950; Lamb and Fallon, 1974; Brenner, 1989). In liver, these triacylglycerols are incorporated into VLDLs and transported out into circulation, but in adipose tissue they are stored in lipid droplets.

Adipocytes do not absorb VLDLs or intact TGs directly (Kane et al., 1983). The TGs must first be hydrolyzed by lipoprotein lipase localized in the capillary endothelial cells (Kane et al., 1983). The FFA released by this reaction diffuse through the capillary wall and are then taken up by the adipocytes and reesterified to form TGs (Bergman et al., 1971). The fatty acid composition of the adipose tissue reflects that of the diet (Lands et al., 1990), but if a low-fat, high-carbohydrate diet is eaten consistently, the adipose tissue would consist mainly of TGs containing palmitic, stearic, oleic, and LAs. Individuals eating diets containing large amounts of LA will deposit this compound readily in the adipose tissue (Thomas et al., 1987). Rats fed diets high in polyunsaturated 20- and 22-carbon n-3 fatty acids store the excess in the adipose tissue (Nelson et al., 1987).

In individuals eating high-fat diets with excess caloric intake, much of the exogenous fatty acids get stored directly in the adipose tissue without going to the liver through chylomicrons formed in

the enterocytes. Chylomicrons formed in the gut are also hydrolyzed by lipoprotein lipase in the capillary endothelial cells (Scow et al., 1977). Uptake of the liberated FFA can then proceed similarly to that described for FFA liberated from VLDL made in the liver.

Fatty acid synthesis in mammalian systems produces only SFAs and monounsaturated fatty acids of the n-9 series, usually oleic acid (Volpe and Vagelos, 1973; Wakil et al., 1983). Animals lack the ability to desaturate fatty acids in the n-6 or n-3 position of the fatty acid chain. Only plants possess the enzyme systems necessary to affect those reactions (Pollard et al., 1979; Stumpf and Pollard, 1983). Many land plants also lack the n-3 desaturase. Aquatic plants and planktons in colder water produce abundant amounts of the n-3 fatty acids (Lee and Loeblich, 1971). Delta 9-10 dehydrogenases that convert stearic acid to oleic acid are ubiquitous in both the plant and animal kingdoms. Indeed, delta 9-10 dehydrogenase in mammalian tissue is the most active lipid enzyme (Elovson, 1965). It has been postulated that the reason stearic acid is not hypercholesterolemic may be because of its rapid conversion to oleic acid, a presumably non-hypercholesterolemic fatty acid (Bonanome and Grundy, 1988).

In animal tissues the desaturation of de novo synthesized fatty acids stops with the production of the monounsaturated fatty acid with double bond in the 9-10 position of the fatty acid chain. (If palmitate is the substrate for the dehydrogenase, the double bond would appear between n-7 and n-8 position of the chain as the 9,10-dehydrogenase desaturates nine carbons from the carboxyl end of the molecules.) Thus, palmitoleic, oleic, and *cis*-vaccenic acids are the main products of this reaction. Chain elongation by another enzymatic system, the fatty acid elongase, produces eicosaenoic, erucic, and nervonic acids by the elongation of oleic acid by this system (Seubert and Podack, 1973; Bernert and Sprecher, 1977; Ludvig and Sprecher, 1979; Sprecher, 1981).

In the absence of dietary EFA, the same enzymes that desaturate linoleic and α-linolenic acids will desaturate eicosaenoic acid to produce the eicosatrienoic acid, or "Mead acid," characteristic of EFA deficiency (Fulco and Mead, 1959; Mead, 1968). This long-chain PUFA of the n-9 series has double bonds at the n-9, n-12, and n-15 positions of the carbon chain. It is not an EFA and cannot replace AA or alleviate the symptoms of EFA deficiency. However, it would be incorporated into the same tissues and complex lipids as AA. The Mead acid is not a substrate for the cyclooxygenase (COX) reaction, and no eicosanoids are produced from it.

Much of the recent work in the biosynthesis of fatty acids has been concentrated on the conversion of dietary EFA, usually linoleic or α-linolenic acid, to their longer chain, more unsaturated products, such as AA, EPA, docosapentaenoic acid (DPA), or DHA (Emken et al., 1992; Pawlosky and Salem, 1992; Sprecher, 1992; Sprecher et al., 1995; Sprecher, 1996; Goyens et al., 2006). The Δ5 and Δ6 desaturases prefer substrate fatty acids with double bonds in the n-6 and, secondarily, the n-3 position of the carbon chain. The fatty acid 22:5n3 does not accumulate appreciably when adequate n-3 fatty acids are in the diet; 22:6n3 is the dominant product (Sprecher, 1992; Sprecher et al., 1995; Sprecher, 1996).

Dietary EFA are, of course, the precursors of the biologically active eicosanoids (Dustig et al., 1981; Granstrom et al., 1982; Oats et al., 1988). This is an area that in the past 20 years has come to dominate much of the research on PUFA metabolism. Most people except strict vegetarians consume some AAs, but most of the body stores come from the conversion of dietary LA to AA by its desaturation and elongation as shown in Figure 23.2. This is a pathway found in all omnivores (Lands, 1986a).

As Sprecher and colleagues (Sprecher and Lee, 1975; Bernert and Sprecher, 1977; Sprecher, 1991) have shown, dietary PUFAs are not elongated and then desaturated, but are desaturated and then elongated. Figure 23.3 displays the main sequence of reactions that yield PUFAs from their dietary precursors. The Δ5 and Δ6 desaturases, which convert linoleic to AA and α-linolenic acids to EPA, have been extensively studied (Sprecher and Lee, 1975; Brenner, 1977; Sprecher and James, 1979).

In the past several years, Sprecher and colleagues (Sprecher, 1992; Moore et al., 1995; Sprecher et al., 1995; Luthria et al., 1996; Rotstein et al., 1996; Sprecher, 1996) have shown conclusively that the purported Δ4 desaturase that had been assumed to convert 20:3n3 to 20:5n3 and 22:6n3

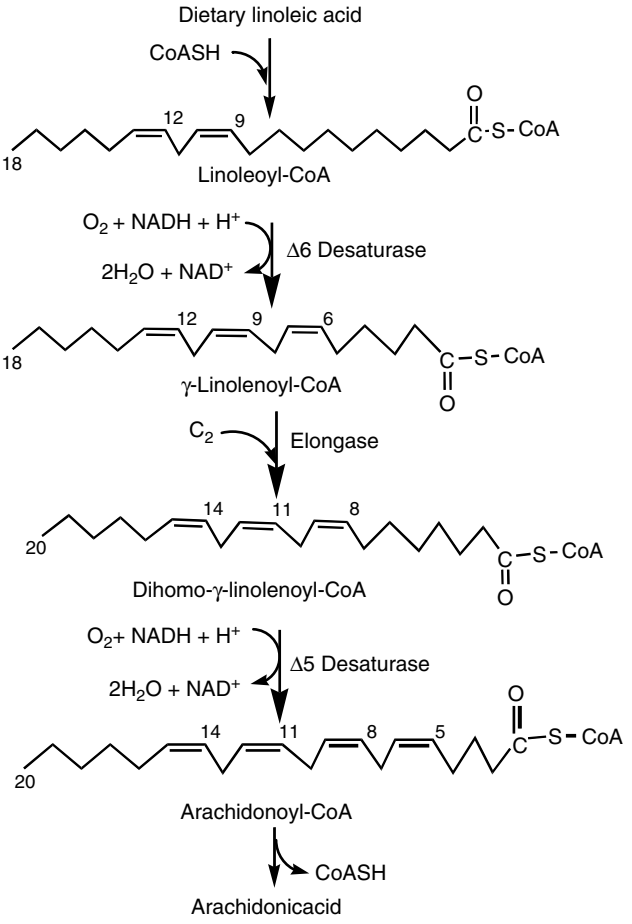

FIGURE 23.2 Metabolic pathway for the conversion of dietary linoleic acid to arachidonic acid *in vivo* by the Δ5 and Δ6 desaturases, and long-chain fatty acid elongases.

does not exist in microsomes. Thus, the metabolic pathway for the biosynthesis of EPA and DHA cannot simply be one of desaturation followed by elongation. In a series of elegant experiments, Sprecher and colleagues (Moore et al., 1995; Luthria et al., 1996; Rotstein et al., 1996) have shown that 20:3n3 is elongated and desaturated to 24:6n3 and that this fatty acid is then retroconverted to 22:6n3 and 20:5n3 by β-oxidation in the peroxisomes. This peroxisomal pathway is also active in converting 22:5n-6 to 20:4n-6. These pathways need only the Δ5 and Δ6 desaturases of the microsomal desaturase system but require a chain-shortening step involving the β-oxidation mechanism in the peroxisomes (Moore et al., 1995). Thus, the metabolism of PUFAs is much more complex than previously thought. Figure 23.4 (modified from the work of Sprecher) is a schematic presentation of the biosynthesis of long-chain PUFAs as the process is now understood.

B. Catabolism

The enzymatic pathways for the catabolism of fatty acids are essentially the same for dietary and endogenously synthesized fatty acids. Still, there may be significant functional differences between the utilization of dietary fatty acids and those synthesized endogenously. Dietary α-linolenic acid is a good example of a fatty acid not readily incorporated into tissue lipids. It is the most rapidly oxidized fatty acid of all unsaturated fatty acids (Nettleton, 1991). Of course, some is converted to

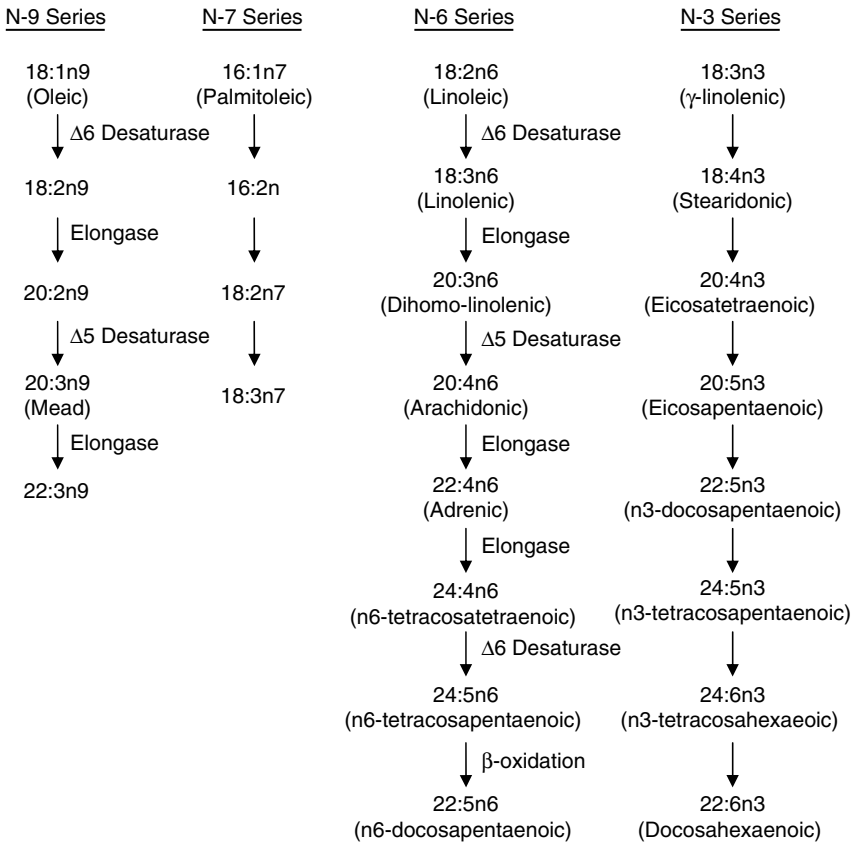

FIGURE 23.3 The metabolic pathways for the conversion of dietary unsaturated fatty acids to their long-chain, polyunsaturated metabolites.

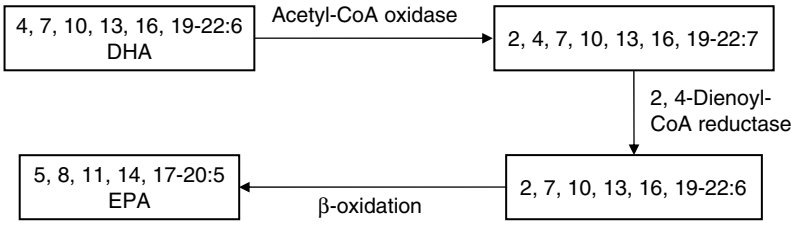

FIGURE 23.4 The probable metabolic pathway for the retroconversion of docosahexaenoic acid to eicosapentaenoic acid *in vivo*. Based on the information from this chapter in the previous edition by Nelson and from papers by Sprecher et al. (2000) and Reddy et al. (2001).

EPA and DHA, but only a small proportion of the α-linolenic acid enters this pathway (de Gomez-Dumm and Brenner, 1975; Aeberhard et al., 1978; Nelson and Chamberlain, 1995; Arterburn et al., 2006). If all other fatty acids are removed from the diet and α-linolenic acid is fed as the only source of dietary fat, then a higher percentage would be incorporated into tissue lipids (Mohrhauer and Holman, 1963). Thus, it is a competitive discrimination that prevents α-linolenic acid from being stored in tissues, not an intrinsic property of this compound.

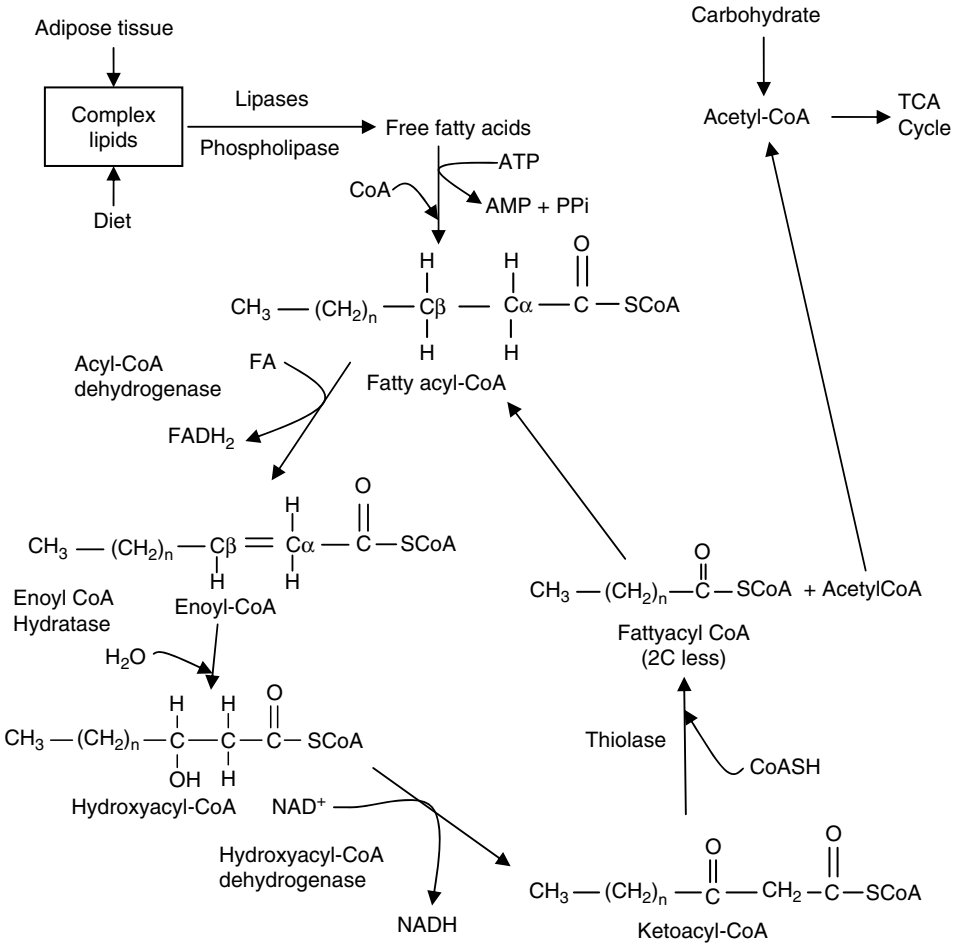

FIGURE 23.5 The classic β-oxidation cycle for the metabolic degradation of fatty acids in mitochondria. This cycle degrades fatty acids of 18 or less carbon atoms. See text for further information on degradation of 20 carbon and longer fatty acids.

Fatty acids are oxidized by β-oxidation to CO_2 and water by enzyme systems in both mitochondria and peroxisomes (Bronfman et al., 1979; Pande and Parvin, 1979; Pande, 1983). The β-oxidation system depends on carnitine as a cofactor (Bieber et al., 1982; Zammit, 1984). Mitochondria, which produce metabolic energy via ATP and the Krebs cycle, use the classical β-oxidation (see Figure 23.5), whereas peroxisomes use a modified β-oxidation system with a fatty acyl CoA oxidase in the first step of the reaction. This step produces hydrogen peroxide and is the rate-limiting step of the oxidation (Foerster et al., 1981). Both pathways use fatty acyl CoA as substrates, but the peroxisomes have a very different preference for fatty acid chains (Osumi et al., 1980; Lazarow, 1981). Fatty acids shorter than 16 carbons are oxidized at the same rate by both systems (Christiansen et al., 1977). Peroxisomes oxidize fatty acids with chain lengths longer than 18 carbons that mitochondria will not normally degrade. Peroxisomes will oxidize the monounsaturated fatty acids such as 20:1n-9, 22:1n-11, 22:ln-9, 24:1n-9, and 22:ln-9*t* that are not substrates for the mitochondrial β-oxidation system (Christiansen et al., 1977). It has been speculated that the peroxisomal oxidation pathway is primarily a chain-shortening mechanism for these long-chain fatty acids so that they can enter the mitochondrial β-oxidation system (Osmunden, 1987). In this manner, peroxisomes can be involved in the regulation of the fatty acid composition of the tissues.

Published results of He et al. (1995) demonstrated that peroxisomes contain all the necessary enzymes to affect the β-oxidation of unsaturated fatty acids through a novel reductase-dependent pathway. These authors reported that peroxisomes from rat liver contain a $\Delta^{3,5}$, $\Delta^{2,4}$-dienoyl-CoA isomerase that is immunologically similar to that found in mitochondria. However, the peroxisome enzyme may be a dimer as its molecular weight is 64 kDa and the enzyme found in liver mitochondria has a molecular weight of 32 kDa. Luthria et al. (1997) have shown that peroxisomal β-oxidation can produce metabolites of arachidonate that can then be reconverted to LA in the microsomes. Thus, there is a potential for recycling of PUFAs within various organelle. Depending on the type and amount of dietary fatty acid, the organism can burn them for energy, convert them to important metabolites such as eicosanoids, isoprostanes, and hydroxyeicosatetraenoic acid (HETEs), or store them in adipose tissue. It has been generally assumed that the regulation of PUFA metabolism is largely dependent on dietary intake coupled with energy expenditure. The work of Sprecher and colleagues (Sprecher, 1996; Luthria et al., 1997; Mohammed et al., 1997) and others (Emken et al., 1992; Pawlosky and Salem, 1992; Pawlosky et al., 2001; Brenna, 2002; Burdge et al., 2002; Wilkinson et al., 2005) have elucidated the fatty acid metabolic pathways and the mechanisms involved.

In addition to the mitochondrial and peroxisomal β-oxidation of the fatty acids, peroxisomal alpha and microsomal omega oxidations need to be mentioned. Fatty acids, such as phytanic acid, with a methyl group at the β-position cannot undergo direct β-oxidation. Phytanic acid first undergoes alpha oxidation whereby the terminal carboxyl group is removed as CO_2. The other product of this alpha oxidation is pristanic acid, a 2-methyl branched-chain fatty acid, which can undergo normal β-oxidation similar to any 2-methyl fatty acid (Reddy and Hashimoto, 2001; Wanders et al., 2001; Wanders, 2004). Microsomal cytochrome P450 and flavin monooxygenase 3 (FMO3) are involved in the ω hydroxylation of the fatty acids and the production of dicarboxylic fatty acids (Sanders et al., 2005; Weng et al., 2005). Once formed, dicarboxylic fatty acids can be shortened from either end of the molecule by β-oxidation. This pathway plays a significant role in overall fatty acid oxidation during starvation and diabetes.

As mentioned above, DHA can be retroconverted to EPA (Kunua, 1968; Stoffel et al., 1970; Sprecher and James, 1979; Schulz and Kunau, 1987). EPA is normally present only in trace amounts in the tissue lipids of animals eating a diet derived largely from terrestrial plants and animals. Interestingly, EPA is not a major fatty acid in the tissue of aquatic mammals (Nelson, 1971). Conversely, DHA is the major n-3 fatty acid in most tissues in mammals; a few tissues contain 22:5n-3 in specific organs (Rouser et al., 1968; Pooviah et al., 1976). As EPA appears to have several unique pharmacological and biochemical actions when consumed in large amounts (Leaf and Weber, 1988; Kremer, 1991; Reddy, 1991; Robinson et al., 1991; Nettleton, 1995), it is possible that DHA, through retroconversion, supplies EPA on demand if there is an inadequate dietary supply of α-linolenic acid.

Consequently, the variable activity of each fatty acid's metabolic pathway acts to produce the fatty acid composition of the tissues. The fatty acid composition of any particular tissue is determined by a complex interplay among synthesis, degradation, and diet. Yet there are limits within which some variation is possible and beyond which it is impossible to alter the composition without interfering with the metabolic function of the tissue. Mammalian species have evolved over a long period, presumably eating a constant diet. Tissues and organs evolved to have specific functional roles. Presumably, the fatty acid composition of any particular tissue has been optimized by evolutionary forces for efficient functioning. One would naturally expect that deviations from normal fatty acid composition in an organ would impair its function. Thus, although it is not often mentioned, the fatty acid composition of a tissue is important to its function and not simply the reflection of yesterday's diet. Even human adipose tissue is not simply a repository of dietary fatty acids but an active endocrine tissue releasing several adipokines that effect metabolism. The fatty acid composition of adipose tissue is determined only partially by diet, a significant portion of its composition is affected by metabolic determinants under genetic control.

Similar to TGs in adipose tissue, FFAs are also released from phospholipids in cellular membranes of other tissues by the action of phospholipase A_2 (Lands and Crawford, 1976). The FFAs

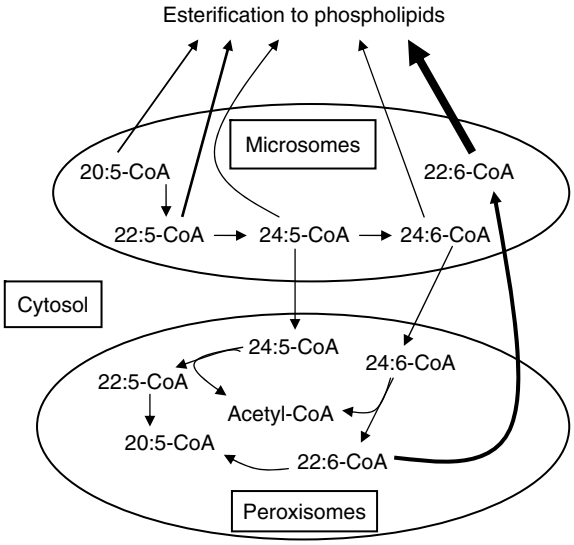

FIGURE 23.6 Metabolism of n-3 long chain PUFAs in peroxisomes and microsomes. Long-chain PUFAs may be oxidized in the peroxisomes or esterified into phospholipids in microsomes. Conversely, fatty acids liberated from phospholipids by phospholipases can migrate to the peroxisomes to undergo chain-shortening by oxidation. Adapted from Luthria et al. (1996). *J. Biol. Chem. 271*: 16020–16025 and Reddy et al. (2001).

are metabolized or reesterified as mentioned earlier. Luthria et al. (1996) have speculated that one mechanism for regulating the fatty acid composition of phospholipids in tissues is the selective esterification of fatty acid metabolites as they move back and forth between peroxisomes and microsomes. Figure 23.6 shows this process schematically.

In both the mitochondria and the peroxisomes, the initial substrate for fatty acid oxidation is an acyl CoA derivative of the fatty acid. Conversely, the COX and lipoxygenase (LOX) systems that produce eicosanoids will accept only the FFA as substrate (Struijk et al., 1966). As FFA does not normally exist in tissues, this is one method by which the production of eicosanoids is regulated. Trauma and disease produce FFA in tissues by liberating acid hydrolases (lipases and phospholipases) from lysosomes in damaged cells (Pitt, 1975). It is also now well recognized that AA can be oxidized to isoprostanes by pathways that do not require either COX I or II (Awad et al., 1996; Morrow and Roberts, 1996). Whether isoprostanes exert their physiological actions through prostanoid receptors is not yet known, but there is some evidence to suggest that they may have their own receptors (Elmhurst et al., 1997; Fukunaga et al., 1997). Obviously, much remains to be learned about the physiological regulation of dietary PUFA metabolism.

VI. PHYSIOLOGICAL EFFECTS OF DIETARY FATTY ACIDS

The effect of dietary fatty acids on lipid metabolism and physiology can be separated into three major categories: (1) the influence of dietary fat on de novo fatty acid synthesis and fatty acid oxidation; (2) the influence of dietary fatty acids on circulating lipoproteins, blood lipids, and cholesterol; and (3) the influence of dietary fatty acids on eicosanoid production and maintenance of physiological homeostasis. Each topic is discussed separately below.

A. De Novo Synthesis

The first category is well understood, and many of the biochemical mechanisms have been described (Volpe and Vagelos, 1973; McGarry and Foster, 1980; Wakil et al., 1983). The regulatory processes

are generally known and predictable. Dietary fatty acids suppress de novo fatty acid synthesis, and so does starvation. Although the two conditions are quite different physiologically, the regulatory processes are very similar. Both acetyl CoA carboxylase and fatty acid synthase are inhibited by excess acyl CoA, particularly palmitoyl CoA (Volpe and Vagelos, 1973; McGarry and Foster, 1980; Wakil et al., 1983; Large et al., 2004). As described earlier, after the hydrolysis of TGs in lipoproteins, the remnants are transported to the liver where they are converted to acyl CoA derivatives for further metabolism (Jelsema and Morre, 1978; Gurr and James, 1980). The excess acyl CoA produced in the liver suppresses de novo fatty acid synthesis by inhibiting both acetyl CoA carboxylase and fatty acid synthase. For some time it was thought that only PUFAs suppressed fatty acid synthesis *in vivo*, but Kelley et al. (1986) showed that SFAs also suppressed fatty acid synthesis in cultured rat hepatocytes. Hence, it is likely that all dietary fatty acids, except short-chain fatty acids absorbed through the portal vein, suppress de novo fatty acid synthesis. Furthermore, the effects of various dietary fatty acids on fat metabolism can be isomer specific as in the case of CLA. In this case, dietary *t*10, *c*12-CLA increased the relative proportion of 18:1n-9 and decreased that of 18:2n-6 in mouse liver lipids, while the *c*9, *t*11-CLA had the opposite effects (Kelley et al., 2004).

Starvation mobilizes fatty acids from adipose and peripheral tissues and transports them to the liver as FFA bound to albumin. These FFAs are converted to their acyl CoA derivatives in liver, and suppress de novo fatty acid synthesis, identical to dietary-derived acyl CoA derivatives. The release of the FFA from adipose tissue is a complex phenomenon caused by the drop in blood glucose after the liver glycogen stores are depleted by fasting. The drop in blood glucose levels causes a decrease in insulin levels and an increase in glucagon levels (Cahill, 1971). This reduction in insulin level stimulates adipocytes to hydrolyze their triacylglycerol stores to FFA while simultaneously turning off de novo fatty acid synthesis (Cahill, 1971; Beynen et al., 1979; Geelen et al., 1980).

B. Circulating Lipoproteins, Lipids, and Cholesterol

Dietary fatty acids have a profound effect on the lipids and lipoprotein levels in the circulation. Since circulating cholesterol levels and lipoproteins are intimately related to the pathology of CVD (Lindgren et al., 1951; Fredrickson et al., 1967; Goldstein et al., 1973, 1983; Brown and Goldstein, 1976; AHA, 2005; Breslow, 2006), there is great interest in this topic both in the scientific community and on the part of the general public, albeit on different levels of sophistication.

As the gut and the liver are the major sites of lipoprotein synthesis and catabolism, it is certainly not surprising that the transport system for delivery and removal of fatty acids to and from peripheral tissues responds to dietary fatty acids. It has been known for many years that diets high in fat and cholesterol tend to raise blood cholesterol (Anitschkow and Chalatov, 1913; Keys, 1975; Mattson and Grundy, 1985; Keys et al., 1986; Lands, 1986a). It was soon observed that isocaloric substitution of n-6 PUFA for SFAs caused a reduction in the plasma cholesterol level (actually a reduction in LDL and VLDL levels) even when cholesterol was present in the diet (Insull et al., 1959; Glueck and Connor, 1978; Horrobin and Manku, 1983; Mattson and Grundy, 1985). However, modulation of cholesterol synthesis is not the major mechanism by which PUFAs lower plasma LDL cholesterol (Mattson and Grundy, 1985). In addition, the observed increase in plasma cholesterol concentrations due to SFAs do not appear to be related to increased de novo synthesis; this has been confirmed by sterol balance studies as well as by studies using the more sensitive method of deuterium incorporation into newly synthesized cholesterol (Jones et al., 1998). Deuterium incorporation studies also revealed that cholesterol synthesis increases with PUFA intake. Thus, the lowering of plasma LDL with PUFA intake may be due to other mechanisms such as redistribution of cholesterol between plasma and tissue pools and upregulation of LDL receptors (Fernandez and West, 2005). Mustad et al. (1996) demonstrated in murine model that diet enriched with SFA markedly decreased LDL receptor protein levels in liver and a PUFA-enriched diet increased LDL receptor levels. Keys et al. (1965) and Hegsted et al. (1965) had shown that stearic acid was an exception to the rule that SFAs raise blood cholesterol levels, although the two equations that these investigators proposed did not specifically eliminate stearic acid. More recent studies indicate that compared with other SFAs,

stearic acid lowers LDL cholesterol. Although the effects of stearic acid on HDL cholesterol have been variable, but most of these studies suggest that stearic acid reduces the ratio of total to HDL cholesterol slightly when compared to palmitic or myrisitic acid. Without doubt the effects of stearic acid were more favorable than *trans* monounsaturated fatty acids (Mensink, 2005). Monounsaturated fats were considered neutral, that is, it did not raise or lower the plasma cholesterol level. However, more recent studies show that replacing SFAs with either monounsaturated fatty acids (MUFAs) or PUFAs lower total and LDL cholesterol (de Roos et al., 2001; Hodson et al., 2001; Gulesserian and Widhalm, 2002; Kratz et al., 2002; Montoya et al., 2002; Nicklas et al., 2002); but the effect on HDL is inconsistent. Some studies found no differences in the HDL concentrations between MUFA and PUFA diets (Montoya et al., 2002; Nicklas et al., 2002), whereas others found a higher level of HDL with MUFA diets (Hodson et al., 2001; Kratz et al., 2002). MUFAs really mean oleic acid unless some exotic oil, similar to high-eruric acid rapeseed oil, is being fed as a source of fat in the diet (Ackman, 1983). MUFAs have beneficial effects as an isocaloric substitution for lauric, myristic, or palmitic acids because the substitution has the same effect as removing the saturated fat from the diet. Either way, substituting MUFAs or simply removing the saturated fat from the diet would get the same cholesterol lowering (Grundy, 1991). Substituting PUFAs (again PUFA in the human diet usually means LA) will lower the plasma cholesterol level beyond the value observed when saturated fat is simply removed from the diet (Shore et al., 1983). Mattson and Grundy (1985) have shown that oleic acid lowered plasma cholesterol as much as LA, although Hegsted (1991) has questioned this claim.

A number of epidemiological studies indicate a positive association between the intake of *trans* fatty acids and the incidence of CVD (Ascherio et al., 1994; Bolton-Smith et al., 1996; Oomen et al., 2001; de Roos et al., 2003; Lichtenstein et al., 2003). CLA, a *trans* fatty acid, has gained significant attention in the past few years both in the research communities as well as in the general population because of its claimed health benefits of reducing obesity, particularly central obesity, improving lean body mass, and its potential anticancer effects. CLA isomers are formed by the shifting of the double bonds in LA and the two most abundant isomers are the *c*9, *t*10-CLA and *t*10, *c*12-CLA, formed primarily as a result of biohydrogenation in ruminant stomachs and industrial food processing, respectively. There have been a number of intervention studies using a mixture of CLA isomers. Because of the variable compositions of the CLA isomers used and the distinctly different effects of the individual isomers, results from these studies have been variable and were reviewed recently (Terpstra, 2004; Tricon et al., 2005). Results from human studies conducted with single isomers showed that the *c*9, *t*11-CLA did not have any adverse or beneficial effects on blood lipids, while the *t*10, *c*12-CLA increased TGs and the ratios of total cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol (Riserus et al., 2002, 2004; Burdge et al., 2004; Tricon et al., 2004). Overall, these studies have failed to see any beneficial effects from the use of these *trans* fatty acids and in contrast indicate detrimental health effects (insulin resistance, increased lipid peroxidation, and inflammation) particularly with *t*10, *c*12-CLA.

The role of dietary n-3 long-chain fatty acids in raising or lowering blood cholesterol levels is complex. Previous reports (Ahrens et al., 1959; Bang and Dyerberg, 1972; Harris and Connor, 1980) suggested that these compounds were similar to their n-6 counterparts in lowering plasma cholesterol. Other studies did not confirm that observation, particularly the effect of substituting polyunsaturated fatty acids for SFAs was considered (Phillipson et al., 1985; Simons et al., 1985; Bruckner et al., 1987). Long-chain, n-3 PUFAs may raise LDL levels in normolipemic individuals. This may be because, the main effect of these compounds is not on the plasma cholesterol levels but a reduction in TG and VLDL levels; this phenomenon was first reported by Harris and coworkers (Connor et al., 1981; Harris et al., 1984, 1988). Subsequent studies have shown that long-chain n-3 PUFAs reduce fasting as well as postprandial TGs in both normal and hypertriglyce(ri)mic human subjects (Harris, 1996). Increased postprandial TGs have been positively correlated to increased blood coagulation, reduced LDL particle size, and impaired endothelial functions, all of which are risk factors for CVD (Endres et al., 1989; Roche and Gibney, 1995; Jagla and Schrezenmeir, 2001; Silveira, 2001). Thus, TG lowering effect of n-3 PUFA can reduce the risk for CVD.

The reduction in VLDL levels, particularly in hyperlipemic individuals, explains why investigators first thought that long-chain n-3 PUFAs lowered plasma cholesterol levels. VLDL carries mainly TGs but does have about one-fifth as much cholesterol as LDL. Lu et al. (1999) reported that the marine omega-3 fatty acid supplementation in humans enhances the propensity of VLDL to be converted to LDL. This explains the rise in LDLs reported by some of the studies with fish oil supplementation. If an individual's VLDL is elevated, both the cholesterol and TG may be elevated as well. Normalizing one's VLDL could lower one's cholesterol level and raise one's LDL levels because of the conversion of VLDL to LDL, but the final result still may be a reduction in one's total plasma cholesterol level.

The role of elevated plasma LDL in the development of atherosclerosis has been studied extensively (Chapman et al., 1998; Staprans et al., 2005) and the effect of different types of dietary fats in modulating blood LDL levels has been the focus for several years now. Until recently, only LDL particles were thought to be the primary culprits in the development of atherosclerosis, however, it has now become clear that chylomicron remnants, especially oxidized chylomicron remnants are also highly atherogenic (Tomkin and Owens, 2001; Yu and Cooper, 2001; Wilhelm, 2003) and can penetrate artery walls like the LDLs and are retained in the subendothelial space (Proctor et al., 2002). Fatty acid composition of the chylomicron remnants influences their propensity to oxidative modification. Remnants enriched with n-6 PUFAs as compared to n-3 PUFA appeared more susceptible to copper-induced oxidation (Napolitano et al., 2004). Thus, reduction in oxidizability of chylomicron remnants by dietary n-3 PUFAs could be another mechanism by which the fish oils exert their cardioprotective effects. Consumption of ≈1g of fish oil for 12 weeks by normolipidemic and hyperlipidemic subjects has been shown to reduce plasma concentration of remnant-like particle-cholesterol (RLP-C) (Hamazaki et al., 2003). RLP-C are a subfraction of lipoproteins that are highly heterogeneous in size but normally of the size of large LDL particles in healthy individuals and are enriched in Apo E, Apo CIII, and ApoB-100; high RLP-C has been positively correlated with increased risk of atherosclerosis (Twickler et al., 2004). Thus, dietary fat can affect both the quantity and quality of circulating lipoproteins.

Recent comparative studies with EPA and DHA, the two main long-chain PUFAs found in fish oils, indicate that both these fatty acids reduce the TGs but DHA is more effective than EPA (Grimsgaard et al., 1997; Buckley et al., 2004). Effects of DHA on total, HDL and LDL cholesterol have been variable; increased LDL and HDL cholesterol was reported in some (Grimsgaard et al., 1997; Theobald et al., 2004; Geppert et al., 2006) but not in other studies (Mori et al., 2000; Buckley et al., 2004). A closer look at the distribution pattern of cholesterol indicates that DHA alters its distribution between different LDL and HDL subfractions. DHA but not EPA increased the HDL_2 cholesterol and also increased the LDL particle size in mildly hyperlipidemic men (Mori et al., 2000). Furthermore, it is only DHA that increased blood flow and reduced heart rate (Mori et al., 2000; Stark and Holub, 2004). Thus, the effects of EPA and DHA are similar in some aspects of lipid metabolism and yet distinct in others.

This subject is also confounded by the influence of caloric level and percent fat in the diet. Reducing total fat calories in the diet will usually lower the plasma cholesterol regardless of the type of fatty acids reduced. Replacing saturated fat with monounsaturated fat or polyunsaturated fat, either n-6 or n-3, will also lower plasma cholesterol levels, with the magnitude of the lowering being n-6 > monounsaturated > n-3. Similarly, removing all saturated fat from the diet will usually produce maximal lowering (Harris et al., 1983; Harris, 1989). Individuals eating a low-fat diet over a long period of time will invariably have lower plasma cholesterol levels than individuals eating a high-fat diet regardless of the type of fatty acid in the diet (Liebman and Bazzarre, 1983). More recent studies have cast doubt on this idea. Reports by Hayes and colleagues (Hayes et al., 1991; Hayes and Khosla, 1992; Khosla and Hayes, 1992) and Nelson et al. (1995) suggest that it is the ratio of the various fatty acids in the diet as well as the dietary amounts of each class of fatty acids that determine an individual's blood cholesterol level. Nelson et al. (1995) showed that normolipidemic individuals on either a low-fat (22% of calories from fat) or a high-fat diet (38% of calories from fat) showed no change in their blood cholesterol content as long as the ratios of the various fatty acids, saturated, monounsaturated, and polyunsaturated, were held constant.

Although AA is not a major component of the human diet, it is a normal constituent of the nonvegan human diet (Phinney et al., 1990). Lacto-ovo vegetarians would consume AA from egg yolks. Based on NHANES 1999–2000 data, the average intake of AA in the United States diets is 180 mg/day for men and 110 mg/day for women (Gebauer et al., 2006). O'Dea K and Sinclair (1985) reported that the Australian diet contained between 80 and 100 mg/day of AA. This fatty acid has a reputation of being a harmful substance if it is consumed in significant amounts. AA injected as the free acid intravenously in rabbits causes rapid disseminated intravascular coagulation and the death of the animals (Gerritsen and Cheli, 1983; Lefer et al., 1983). In animal studies, large amounts of AA in the ester form do not seem to cause significant harm to the animals in the short term (Weiner and Sprecher, 1984; O'Dea and Sinclair, 1985; Sinclair and O'Dea, 1987; Steel et al., 1990, 1993; Whelan et al., 1993; Mann et al., 1994). Seyberth et al. (1975) reported a human feeding study in which 6 g/day of AA were given to normal male subjects in the form of ethyl ester. Although the study was planned to continue for a month, it was terminated after 3 weeks due to markedly increased *ex vivo* ADP-induced aggregation (10%–60%) of platelets from the volunteers. Nelson et al. (1996a,b,c) reported results from a study in which 1.5 g of AA as TG was fed to normal male volunteers. At this level of intake dietary AA did not alter plasma lipoprotein levels, and there was no adverse effects noted during the 50 days of this study. Thus, it would appear that dietary AA, if fed as a TG, is not harmful to humans (or rats, see Koskelo et al., 1997) and it does not alter blood lipids at least in the presence of adequate amounts of LA.

The mechanism by which dietary fatty acids raise or lower blood cholesterol levels is fairly well understood now (Grundy, 1991). It would appear that regulation is partly achieved through the stimulation or suppression of LDL receptors (Spady and Dietschy, 1985; Fox et al., 1987; Nicolosi et al., 1990; Grundy, 1991). In an *in vitro* study with fibroblasts and HepG2 cells, it was demonstrated that 25-OH cholesterol reduced the protein and mRNA levels of low-density lipoprotein receptor (LDLr) and AA, EPA and DHA treatment increased LDLr by one to three fold (Yu-Poth et al., 2005). In an *in vivo* study in golden syrian hamsters, it was shown that compared to a diet enriched in butter, a diet high in canola oil or soybean oil had a four fold or eight fold higher LDLr expression, respectively. Different fatty acids differentially regulate expression of different genes involved in cholesterol and fatty acid synthesis and metabolism. Details of this will be discussed in the chapter by Sampath and Ntambi (Chapter 29).

It was suggested by Bonanome and Grundy (1988) that stearic acid is not hypercholesterolemic because it is converted rapidly to oleic acid by the active 9–10 dehydrogenase present in the liver. Although this is a plausible explanation, when deuterium-labeled stearic acid was fed to human volunteers, Emken (1981) found no significant conversion of dietary stearic acid to oleic acid. Therefore, it is questionable if conversion of stearic to oleic acid can explain the nonhypercholesterolemic character of stearic acid.

The mechanism by which long-chain, n-3 PUFAs reduce VLDL is now partially understood (Nestel et al., 1984; Wong et al., 1985; Wong and Nestel, 1987; Yamazaki et al., 1987; Wong and Marsh, 1988; Nestel, 1990; Yeo and Holub, 1990; Harris and Bulchandani, 2006). It appears that these compounds suppress both TG synthesis in the liver and gut and the assembly of nascent VLDL (Nestel et al., 1984; Yeo and Holub, 1990). Wong and Nestel (1987) and others (Nelson et al., 1988; Rustan et al., 1989) have investigated this phenomenon in considerable detail. It has been shown that PUFA-rich diets suppress the transcription of lipogenic genes by suppressing the transcription and reducing maturation of the protein, sterol response element binding protein (SREBP) (Fernandez and West, 2005). The role of SREBPs in lipogenesis and cholesterol metabolism is well established (Xu et al., 1999). It has been shown that n-3 PUFAs are more potent than n-6 PUFAs in suppressing SREBP-1 expression. Vasandani et al (2002) using LDL-receptor-deficient mice model showed that dietary n-3 PUFAs markedly decreased TG and cholesterol ester levels in the liver and reduced Apo B containing lipoproteins in plasma. On the basis of the current evidence, the mechanisms by which n-3 PUFA affect blood lipids has been summarized by Fernandez and West (2005) as follows: (1) suppress SREBP 1 expression and processing, thus leading to decreased lipogenesis and VLDL

secretion; (2) decreasing the LPL inhibitory ApoCIII levels and increasing LPL expression and facilitating enhanced hepatic clearance of lipoproteins; and (3) possibly increasing reverse cholesterol transport.

Results from earlier studies regarding the effects of *trans* fatty acids on blood lipids have been variable. Vergroesen (1972) and Vergroesen and Gottenbos (1975) found some elevation of plasma cholesterol after feeding 35% partially hydrogenated dietary fat containing *trans* fatty acids. Conversely, Matson et al. (1975) did not observe an elevation of plasma cholesterol in subjects fed diets containing 34% partially hydrogenated fat. Gottenbos (1983) reviewed this topic and concluded that *trans* fatty acids can raise plasma cholesterol levels and may be mildly atherogenic but have no effect on EFA metabolism. Additionally, dietary LA eliminated the hypercholesterolemic influence of dietary *trans* fatty acids consumed concurrently. Kritchevsky (1983) also concluded that *trans* fatty acids act metabolically much like SFAs and have no other significant adverse metabolic consequences. Emken (1991) demonstrated that *trans* fatty acids are metabolized much like their *cis* analogues and had no immediate adverse effects on lipid metabolism.

Results from recent studies indicate that *trans* fatty acids increase LDL and decrease HDL cholesterol (de Roos et al., 2003; Lichtenstein et al., 2003). Mensink and Katan (1990), in a carefully controlled human feeding study, compared the effects of diets containing either saturated (19%), monounsaturated (23%), or *trans* (11%) fatty acids on blood lipids. Polyunsaturated fat and the other dietary constituents were held constant. They found that *trans* fatty acids raised plasma cholesterol levels but less than the equivalent amount of saturated fat. They confirmed the work of Vergroesen (1972); however, they also reported that *trans* fatty acids lowered plasma HDL cholesterol levels, which SFAs do not, and raised plasma LDL levels in a quantity equivalent to SFAs, thus increasing the ratio of total cholesterol to HDL cholesterol. This increase in the ratio of total cholesterol to HDL cholesterol is a strong predictor of CVD (Stampfer et al., 1991). *Trans* fatty acids also increased blood levels of TGs as compared with the intake of other fats (Mensink et al., 2003), increased levels of Lp (a) (Ascherio et al., 1999), reduced particle size of LDL cholesterol (Mauger et al., 2003) and impaired endothelial function (de Roos et al., 2001), each of which may further increase the risk of CVD. These fatty acids have been shown to also promote inflammation by increasing plasma concentrations of tumor necrosis factor-α (TNF-α), interlukin-6 (IL-6), and C-reactive protein (Mozaffarian, 2006). These findings suggest an adverse effect of these *trans* fatty acids on one's cardiovascular risk profile (Grundy, 1990) beyond that suggested by the change in total plasma cholesterol levels.

In a recent analysis of the CVD incidence in a large-scale epidemiological study of professional nurses (Nurses Health Study) (Hu et al., 1997), it was estimated that a 2% reduction in energy intake from *trans* fatty acid would decrease the risk of developing CVD by 53%. In this study, the intake of *trans* fatty acids was estimated from the self-reported intake of margarine; margarine is one of the primary source of *trans* fatty acids in the U.S. diet. In a more recent meta-analysis (Mozaffarian et al., 2006) of four prospective cohort studies (Ascherio et al., 1996; Pietinen et al., 1997; Oomen et al., 2001; Oh et al., 2005) involving nearly 140,000 subjects, it was reported that a 2% increase in energy intake from *trans* fatty acids was associated with a 23% increase in the incidence of CVD. Thus, the consumption of even relatively small amounts of *trans* fatty acids may have major adverse health consequences, especially on cardiac health. The reader is pointed to recent reviews for more details on this subject (Blackburn and Khaodhiar, 2003; Mensink, 2005; Mozaffarian, 2006; Mozaffarian et al., 2006). Because of the proven adverse effects from consumption of *trans* fatty acids, Department of Agriculture food guide pyramid recommends limited intake of *trans* fatty acids; Dietary Guidelines Advisory Committee recommends consumption of *trans* fatty acids be kept below 1% of total energy intake (DHHS, 2005; Dietary Guidelines Advisory Committee, 2005). Recognizing the adverse health effects of *trans* fatty acids, Food and Drug Administration (FDA) ruled that, effective from January 1, 2006, the nutritional labels for all conventional foods and supplements must indicate the content of *trans* fatty acids (FDA, 2005). It is anticipated that adherence to these regulation regarding the consumption of *trans* fatty acids will reduce the risk for CVD.

C. Conversion to Eicosanoids

Eicosanoids are a key link between PUFAs and inflammation; they are generated from 20-carbon PUFAs (AA, EPA, DHGLA) released from membrane phospholipids by phospholipases, primarily phospholipase A2. Depending on the enzyme implicated (COX or LOX), eicosanoids produced include prostaglandins (PGs), thromboxanes (TXAs), leukotrienes (LTs), and HETEs. Different series of metabolites are formed depending on the nature of the 20-carbon precursor. When DHGLA (20:3n-6) is the substrate of COX, PGs of series 1 are formed while AA (20:4n-6) as substrate forms PGs and TXA of series 2 and EPA (20:5n-3) forms PGs and TXA of series 3 (Roland et al., 2004). See Figure 23.7 for a brief overview of the different types of eicosanoids produced by the three 20-carbon PUFAs. The different series of eicosanoids have distinct physiological effects. PGE_1 formed from DHGLA is antiinflammatory, while PGE_2 formed from AA is proinflammatory causing fever, increased vascular permeability and vasodilatation. LTB_4, also formed from AA, is a potent chemotactic agent for leukocytes, enhances generation of reactive oxygen species, and increases production of inflammatory cytokines such as TNF-α, interleukin-1 (IL-1), and IL-6. PGE_3 formed from EPA is mildly antiinflammatory and immune enhancing.

Lands (1986a) speculated that excess dietary n-6 fatty acids are the major cause of many chronic diseases in the Western world. Production of AA-derived eicosanoids increase during inflammatory conditions and elevated levels of these eicosanoids are observed in blood and tissues from patients with acute and chronic inflammatory conditions (Calder, 2006). There has been a great increase in n-6 PUFAs in the diets of the Western world in the past 100 years corresponding to the rise in the debilitating chronic diseases, such as heart disease, cancer, and autoimmune diseases. Lands' (1986a) view is that current levels of linoleate in the Western diet are much higher than those that would be obtained on a diet not strongly influenced by agricultural oilseed production. His conjectures seem to be true; it has been shown that the membrane phospholipids of inflammatory cells taken from people eating a Western-type diet typically contain approximately 20% of fatty acids as AA while that of other 20-carbon PUFAs such as DHGLA is only about 2% and that of EPA is <1% (Calder, 2006). Our current Western diet evolved quite recently with the development of efficient

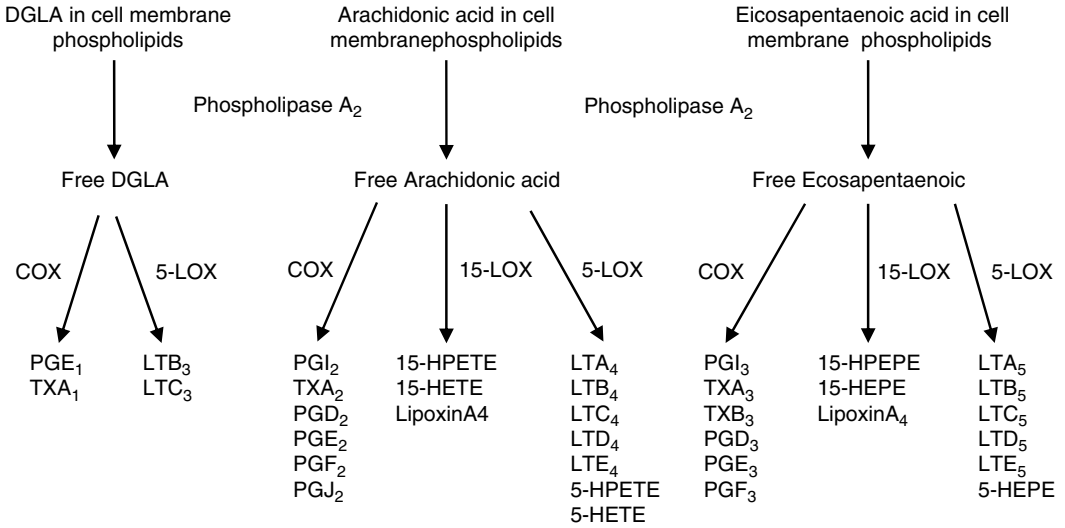

FIGURE 23.7 Synthesis of eicosanoids from the three different 20 carbon fatty acids, arachidonic acid, Dihomo-gamma-linolenic acid and eicosapentaenoic acid; COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane. Adapted from Calder (2006).

seed processing methods in the late nineteenth and early twentieth centuries. Prior to that time the Western diet generally had lower fat content, lower linoleate content, and perhaps a higher n-3 PUFA level. Lands hypothesized that the increase in linoleate levels will lead to a concomitant increase in AA levels and this presumably would make more AA available for eicosanoid production.

Zollner et al. (1979), Nugteren et al. (1980), Adam et al. (1980, 1982), and Adam and Wolfram (1984), all suggested that diet could alter eicosanoid production rates. Adam et al. (1982) used two different levels of linoleate (0% and 20%) in the diet of human volunteers to show a difference in the excretion of tetranorprostanedioic acid, a product derived from E and F series of PGs (PGE and PGF). Ferretti and coworkers (1989) evaluated the effects of moderate dietary changes on eicosanoid production. They found that less eicosanoid (PGE-M) was excreted in the urine by subjects who consumed a diet low (6 en%) in n-6 PUFAs as compared to subjects eating a diet high (9 en%) in LA. In another study, increasing the LA content of diet from 3 en% to 8.3 en% increased urinary PGE_2 by 40% (Blair et al., 1993). Kelley et al. (Kelley et al., 1998) reported that supplementing the diets of healthy men with 1.5 g of AA per day for 7 weeks resulted in increased production of AA-derived PGE_2 and LTB_4 by endotoxin-stimulated mononuclear cells. Thus, moderate increase in the consumption of both LA and AA increases the production of PGE_2 and LTB_4.

Consumption of a diet rich in n-3 PUFAs such as EPA and DHA, typically found in fish and fish oil liquids, has been shown to reduce production of inflammatory eicosanoids and reduce the risk of inflammatory diseases. Increased consumption of n-3 PUFAs by humans results in increased proportions of these fatty acids in the phospholipids of inflammatory cell (Gibney and Hunter, 1993; Healy et al., 2000; Thies et al., 2001; Calder et al., 2002). This incorporation of n-3 PUFAs in human inflammatory cells occurs in a dose response fashion and is partly at the expense of AA (Calder et al., 2002), thereby reducing the amount of substrate available for eicosanoid synthesis from AA. This is evident from the fish oil supplementation studies in humans, which report decreased production of AA-derived eicosanoids such as PGE_2, TXB_2, LTB_4, 5-HETE, and LTE_4 by inflammatory cells and an increase in EPA-derived eicosanoids such as LTB_5, LTE_5, and 5-hydroxyeicosapentaenoic acid (Calder, 2006). Ferretti et al. (1991) reported that total eicosanoid production as measured by PGE-M in the urine was reduced in a group of subjects consuming salmon diet compared to the group consuming a diet without salmon. In this study, the level of linoleate in the diet and percent calories from fat were held constant in both the groups. Ferretti et al. (1998) reported that normal subjects consuming 6 g/day of DHA in the absence of EPA showed a reduction in the excretion of $TXAB_2$, but no reduction in the excretion of prostacyclin metabolites. In the same study, *ex vivo* secretion of PGE_2 and LTB_4 by mononuclear cells stimulated with LPS was reduced (Kelley et al., 1999), however, bleeding times, *in vitro* coagulation, and platelet aggregation were unaffected (Nelson et al., 1997a). These authors (Nelson et al., 1997b) did report that there was a marked increase in both the EPA and DHA content of both the plasma and platelets in their volunteers with a reduction in the amount of n-6 fatty acids in these tissues.

The switch from AA-derived eicosanoids to EPA-derived eicosanoids as seen after a high n-3 PUFA diet is important because eicosanoids formed from AA as substrate have highbiological activity and are mostly proinflammatory and those formed from DHGLA and n-3 PUFAs such as EPA and DHA have less biological activity and are antiinflammatory. For example, EPA-derived LTB5 is 10- to 100-folds less potent as a neutrophill chemotactic agent then the AA-derived LTB_4 (Goldman et al., 1983), while PGE_3 (from EPA) is less potent inducer than PGE_2 (from AA) of COX-2 gene expression in fibroblasts (Bagga et al., 2003). N-3 PUFAs alter eicosanoid synthesis not only at the substrate level but also at the level of gene expression. N-3PUFAs have been shown to reduce expression of COX-2, 5-LOX, and 5-LOX activating protein in chondrocytes (Curtis et al., 2000, 2002). EPA and DHA have been shown to inhibit toll-like receptor 2 mediated up-regulation of COX-2 in monocytes (Lee et al., 2003).

Recent research suggests that EPA and DHA, in addition to modulating eicosanoid production by acting as a substrates to COX and LOX enzymes, also produce a novel group of mediators termed E-series resolvins formed from EPA and D-series resolvins formed from DHA by COX-2

(Serhan, 2005b). Both E- and D-series resolvins seem to have antiinflammatory effects. In addition to resolvins, other DHA derived mediators called docosatrienes and neuroprotectins, also produced by COX-2, have been identified and appear to be antiinflammatory (Serhan, 2005a).

Though most of the AA-derived eicosanoids are proinflammatory, recent studies have suggested that some of the AA-derived eicosanoids may have both pro- and antiinflammatory effects and others, such as PGI_2, are mostly antiinflammatory. For example, PGE_2, which is pro-inflammatory, also inhibits 5-LOX and so decreases the production of inflammatory 4-series LTs and induces 15-LOX, which promotes formation of lipoxins that have been found to be anti-inflammatory (Calder, 2006). In a crossover study, Ferretti et al. (1997) reported that feeding 1.5 g/day of AA to a group of normal male volunteers was associated with increased production of thromboxane A_2 and PGI_2 (prostacyclin) compared to when the subjects were consuming 100 mg/day of AA. Excess thromboxane production is considered undesirable because it enhances platelet aggregation, however, the concomitant rise in PGI_2, a thromboxane inhibitor, may counterbalance the effect of thromboxane. In fact, this study did report that *ex vivo* platelet aggregation in response to collagen and ADP was not altered after increased AA consumption. Thus, production of eicosanoids with opposing effects may be one of the mechanisms by which body modulates proinflammatory effects of AA-derived eicosanoids. Thus, both the absolute amounts and the ratio of n-6 and n-3 PUFAs in diet may influence the production of inflammatory eicosanoids.

VII. SUMMARY AND CONCLUSIONS

Dietary fats have profound effects on lipid metabolism. The inhibition of endogenous fatty acid synthesis by exogenous dietary fat is now well understood. However, there is some controversy about whether dietary saturated fat is effective in suppressing de novo fatty acid synthesis, but the suppression of liver fatty acid synthesis by dietary PUFAs, particularly LA is well established.

Depending on their degree of unsaturation and chain length, dietary fatty acids can affect blood lipids and lipoprotein levels. Both SFAs (12:0–16:0) and dietary cholesterol increase blood cholesterol, and PUFAs reduce it. *Trans* fatty acids, even though are polyunsaturated, increase LDL and decrease HDL cholesterol, possibly because of the presence of *trans* bonds affecting metabolism. MUFAs, mainly oleic acid (18:1n-9) reduce total and increase HDL cholesterol. Most studies with long-chain n-3 PUFA did not find a change in total cholesterol, but both the LDL and HDL cholesterols were increased and TGs were reduced in those studies. Studies with purified DHA and EPA supplementation found both fatty acids were effective in reducing plasma TGs, although some found DHA to be more effective than EPA. These studies also indicated that it is only DHA that reduced the number of small dense LDL particles and increased the concentration of HDL-2, increased blood flow, and reduced heart rate.

Increased consumption of n-6 PUFAs has been found to enhance the production of inflammatory eicosanoids such as PGE_2 and LTB_4, while EPA and DHA reduced the tissue concentration of AA and production of inflammatory eicosanoids. N-3 PUFAs have also been found to reduce the expression of genes for inflammatory cytokines (IL-1β, IL-6, and TNF-α) and have antithrombotic and antiarrhythmic effects. Despite the high degree of unsaturation and susceptibility to oxidation, EPA and DHA have been reported by some investigators to reduce oxidative stress. Evidence from a number of epidemiological, prospective, and intervention studies clearly show that increased intake of long-chain n-3 PUFAs reduces the risk for CVD. Because of limited elongation of ALA to EPA and DHA, its health effects are not as dramatic as those of the long-chain n-3 PUFA. Despite the knowledge of the many health benefits of n-3 PUFAs, their consumption remains at a minimal level in the United States. On the other hand, consumption of proinflammatory n-6 PUFAs has markedly increased. The reduced intake of n-3 PUFAs and increased intake of n-6 PUFA over the past several decades may be the major reason for the increase in inflammatory diseases. Thus, an increase in the consumption of n-3 PUFAs and a decrease in the consumption of n-6 PUFAs are needed to balance the ratio between the consumption of these two groups of fatty acids. Reduction in the consumption of

trans fatty acids and SFAs will also reduce the risk for CVD. Further studies are needed to determine the dose dependent effects of n-3 PUFAs, interaction between n-3 and n-6 PUFAs, and the underlying mechanisms.

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24 Dietary Fatty Acids and Minerals

Elizabeth A. Droke and Henry C. Lukaski

CONTENTS

I. INTRODUCTION

The absorption of dietary minerals is determined by nutritional needs of the organism, by the amount present in the diet, and by factors influencing the bioavailability and utilization of the mineral. Whereas nutritional needs tend to modulate homeostatic mechanisms of absorption, the bioavailability of minerals is principally influenced by exogenous factors. Factors influencing mineral bioavailability can be grouped as to the site at which they occur and include luminal, mucosal, and postabsorptive events (Rosenberg and Solomons, 1984). Luminal events refer to the dissociation of the mineral from the chemical matrix with which it was associated in the food and possible interactions with factors that may enhance or reduce its solubility. Mucosal actions include uptake of minerals at the mucosal membrane, which may or may not include receptors. Postabsorptive transport of minerals away from the intestinal epithelium to body tissues and organs involves the participation of binding or transport proteins. Each of these factors or processes depends on nutrients and chemical compounds in the diet that directly or indirectly affect absorption and utilization of minerals. Other factors, such as enteral recycling and hormonal influences, may also play a role.

Many dietary components have been shown to influence mineral bioavailability in animals and humans (Solomons and Rosenberg, 1984). Understanding the influence of dietary components on

mineral absorption and utilization is critical to developing recommendations for the intake of minerals. Dietary components that have been evaluated for their impact on mineral bioavailability include protein, carbohydrate, fiber, organic acids, and other minerals. One dietary component, fat or fatty acids, has received minimal attention. One reason for the lack of evidence regarding the effect of fat on mineral bioavailability may be that there is little *in vitro* physicochemical evidence to suggest fatty acids will bind minerals, because stability constants are negligible or nonexistent (Sillen and Martell, 1964; Perrin, 1979; Martell and Smith, 1982). However, because dietary fat represents a significant fraction of daily energy intake in the United States, with estimates ranging from 30% to 40% of energy intake (Wright et al., 2004), and because *in vivo* physiological interactions between fatty acids and minerals have been reported (Simpson and Peters, 1987a,b; Simpson et al., 1988), there is increasing interest in examining the effects of dietary fatty acids on the bioavailability of minerals.

The purpose of this chapter is to summarize research findings on the effects of dietary fat on the bioavailability of some essential minerals, including calcium and magnesium and the trace minerals, copper, zinc, and iron. This chapter will also integrate the experimental findings on bioavailability with current understanding of the mechanisms of mineral absorption and end points of mineral utilization. In addition to the importance that interactions between dietary fat and minerals may have in the determination of dietary mineral intakes, knowledge of these interactions may be useful in the prevention and/or therapy of various conditions such as osteoporosis (Kruger and Horrobin, 1997).

II. EFFECTS OF DIETARY FATTY ACIDS ON MINERAL BIOAVAILABILITY AND UTILIZATION

A. Calcium

a. Animal Studies

i. Absorption

Apparent calcium absorption is defined as the difference between calcium intake and fecal calcium losses. The effect of fat on apparent calcium absorption in laboratory animals has been extensively studied; however, results have been equivocal. Essential fatty acids (EFAs) (as reviewed by Kruger and Horrobin, 1997) and arachidonic acid (Song et al., 1983) have been reported to increase calcium absorption. Tuna oil, high in docosahexaenoic acid (DHA; n-3), has also been shown to increase calcium absorption in young growing male rats in comparison with rats fed corn oil (linoleic acid; n-6) or evening primrose oil, which is also high in linoleic acid (25.4 ± 2.5 vs. 18.4 ± 1.8 and 18.1% ± 2.1%; respectively, for tuna oil, corn oil, and evening primrose oil) (Kruger and Schollum, 2005). Conjugated linoleic acid (CLA) fed to 4-week-old male rats for 8 weeks at 10 g CLA/kg diet, increased the net fractional (%; $p < .01$) and absolute (mg; $p < .05$) absorption of calcium when rats were fed a n-3 polyunsaturated fatty acid (PUFA)rich diet (menhaden oil–safflower oil) but not when fed an n-6 PUFA-rich diet (soybean oil) (Kelly et al., 2003). However, in adult, ovariectomized rats, intestinal calcium absorption was unaffected by CLA (Kelly and Cashman, 2004). In contrast, other studies have demonstrated decreased calcium absorption with diets containing 20%–25% dietary fat as corn oil (high in linoleic acid; n-6) (Kane et al., 1949), tripalmitin or tristearin (Nordin, 1968; Tadayyon and Lutwak, 1969), or cottonseed oil (Knudson and Floody, 1940), while others found no effect with 20% lard (Beadles et al., 1951) or 5% peanut oil (Calverly and Kennedy, 1949). These differences among studies may be the result of varying concentrations of n-3, n-6, and n-9 fatty acids. This possibility is further supported by the findings of Claassen et al. (1995) in which γ-linolenic acid (GLA, n-6) and eicosapentaenoic acid (EPA, n-3) were fed to male rats at a ratio of 3:1 (GLA:EPA), increased calcium absorption (mg/24 h) by 41.5% compared with the control group that was fed linoleic acid (n-6) and α-linolenic acid (ALA, n-3) at a ratio of 3:1.

The lack of a consistent effect of dietary fat on calcium absorption may be due to other factors besides the type and amount of dietary fat. One of these factors may be the age of the animals used. Calcium-binding ligands in the small intestine are different in young vs. older rats (Song et al., 1983). Kaup et al. (1990) observed that calcium absorption decreased as rats aged from 2 to 8 months and that increased butterfat ingestion (5% vs. 20%) had no effect on apparent calcium absorption in young rats but decreased it in mature rats. Experimental conditions in several studies were also optimized to evaluate the effect of dietary fat on calcium retention and utilization (e.g., low-dietary calcium, low-fat, or fat-free control diets) (Nordin, 1968; Tadayyon and Lutwak, 1969), further complicating the determination of the effect of dietary fat on calcium absorption.

ii. Medium-Chain Triglycerides and Absorption

Medium-chain triglycerides (MCT) fed at 11% of metabolizable energy intake (5.66% wet weight; 43% of total fat) to dogs did not significantly affect absorption of calcium or magnesium (Beynen et al., 2002). The authors suggested the level of calcium in the diets fed to the dogs may have been great enough to mask or prevent an observed effect of MCT on mineral absorption.

iii. Retention/Tissue Mineral Concentrations

The effects of dietary fat on calcium retention and tissue mineral concentrations appear to be related to the ratio of n-6:n-3, the amount of dietary fat, and the level of calcium intake. French and Elliot (1943) reported a decrease in calcium retention as fat increased from 5% to 45% oleo oil. A decrease in calcium retention has also been observed with 5% coconut oil or cottonseed oil (Calverly and Kennedy, 1949), or 20% cocoa butter (Beadles et al., 1951). Research with turkey poults demonstrated that type of dietary fat (5% of either tallow, corn oil, soybean oil, animal–vegetable blend fat or canola oil) had no effect on apparent retention of calcium (Leeson and Atteh, 1995). In contrast, when palmitic acid, oleic acid, or a 50:50 mixture of these fatty acids was fed to the turkey poults, a significant reduction in apparent calcium retention did occur (Leeson and Atteh, 1995). However, more recently, increased consumption of n-3 fatty acids increased calcium balance (mg/24 h) by 41.5% in rats (Claassen et al., 1995). In line with this, urinary excretion of calcium has been reported to be reduced by EFAs (Kruger and Horrobin, 1997) and negatively correlated with n-3 fatty acid levels in the diet (Claassen et al., 1995). In other research with mice, a 5% addition (w/w) of either corn oil (predominantly linoleic acid; n:6) or olive oil (predominantly oleic acid; n:9) resulted in increased liver and spleen calcium concentrations (Milin et al., 2001) compared with the control diets (normal fat content). Thymus calcium concentrations were also increased in mice fed the higher corn oil diet. However in a study with young growing rats, the type of dietary fat (safflower oil, flaxseed oil, olive oil, or beef tallow), varying in concentrations of n-3, n-6, and n-9 fatty acids, had no effect on plasma and liver calcium concentrations in the presence of adequate dietary calcium (Shotton and Droke, 2004). Another evidence suggests the type of dietary fat has an inhibitory effect on calcium metabolism when calcium intake is low (<0.4% of the diet by weight) (Nordin, 1968).

iv. Calcium Utilization

EFAs are critical for maintenance of skeletal health as demonstrated by the severe osteoporosis that develops in EFA-deficient animals (Kruger and Horrobin, 1997). EFAs enhance the synthesis of collagen in bone and increase deposition of calcium into bone (Kruger and Horrobin, 1997). Another evidence also indicates that the ratio of n-6:n-3 EFAs is important in the effect of dietary fat on calcium utilization in bone (Claassen et al., 1995), thus suggesting dietary fats with different ratios of n-6:n-3 will have varying effects on bone calcium metabolism.

Utilization of calcium in bone (i.e., prevention of rickets, increased bone calcification and ash) was increased with vitamin D deficient diets containing 10%–11% lard or olive oil (McDoughall, 1938;

Jones, 1940) or 5%–20% cottonseed oil (Knudson and Floody, 1940) in comparison with the low-fat or fat-free control diets. The femur calcium content of rats receiving 25% triolein or 5% fat as triolein, tripalmitin, or tristearin was also reported to be greater than that of the animals receiving the fat-free diet, and this was greater than those fed 25% tripalmitin or tristearin (Tadayyon and Lutwak, 1969). The femur calcium content also increased when the 25% tristearin diet was supplemented with 5% triolein. Research by Schlemmer et al. (1999) demonstrated that a diester-containing GLA and EPA increased femur calcium content to sham levels and potentiated the effect of an estrogen implant in ovariectomized rats. Other research also suggests n-3 fatty acids (i.e., EPA) increase bone calcium (Claassen et al., 1995).

In contrast, other evidence indicates fats have no or an inhibitory effect on calcium utilization. Evidence from turkey poults fed palmitic acid, oleic acid, or a 50:50 mixture of the fatty acids indicates that even though reduced mineral retention was observed with these fatty acids, this did not result in changes in concentrations of bone ash or bone calcium concentrations (Leeson and Atteh, 1995). Femoral bone mineral density (BMD) in adult ovariectomized rats was also unaffected by CLA (2.5, 5, or 10 g CLA/kg diet) (Kelly and Cashman, 2004). In other work, as fat intake increased in excess of 10% of daily energy intake, bone calcium content began to decrease in animals fed diets containing cottonseed oil (Knudson and Floody, 1940). In broiler chickens fed 8% palmitic or stearic acid, bone ash and bone calcium concentrations were significantly reduced whereas bone ash and bone calcium concentrations were unaffected by oleic acid (Atteh and Leeson, 1983).

In a more recent study to determine whether EPA could prevent the deterioration of bone mass that occurs with estrogen deficiency, Poulsen and Kruger (2004) found that in ovariectomized rats, 1.0 g EPA/kg of body weight decreased ($p < .01$) BMD. The authors proposed that an increase in lipid peroxidation may have decreased intestinal calcium absorption, thus stimulating parathyroid hormone-mediated bone resorption. This finding was supported by later research with young male growing rats in which tuna oil (4% + 1% corn oil; rich source of DHA) appeared to be more effective in improving bone mass than fish oil (4% + 1% corn oil; rich source of EPA) (Kruger and Schollum, 2005). Total calcium per bone was 172.6 ± 5.2 mg in the tuna oil-fed group vs. 152.4 ± 4.4 mg in the fish oil-fed group. This possible fatty acid-dependent effect on bone resorption is further supported by the finding that CLA (5 and 10 g/kg diet) reduced ($p < .001$) urinary markers of bone resorption in ovariectomized rats (Kelly and Cashman, 2004). However, this effect of CLA may be age- and gender-specific because in contrast to the results with the ovariectomized rats, young, growing male rats fed CLA (10 g/kg diet) did not alter concentrations of osteocalcin, a marker of bone formation, or insulin-like growth factor-I, a mediator of bone metabolism (Kelly et al., 2003). In this study with male rats, urinary markers of bone resorption were not significantly affected by CLA (10 g/kg diet), but the markers were greater in rats consuming an n-6 PUFA-rich diet (soybean oil) compared with an n-3 PUFA-rich diet (menhaden oil–safflower oil) suggesting n-6 fatty acids may increase bone resorption.

v. Calcium Soap Formation

The previous findings on calcium absorption, retention, and utilization were complemented by the recognition that dietary calcium per se may influence dietary fat absorption. The presence of ionized calcium in the intestine determines the extent of soap formation, although the amount ultimately excreted in the feces depends on the solubility of the soap formed, which is inversely related to chain length and degree of unsaturation. When a diet containing a relatively high-calcium intake (60–70 mg/day) was fed to rats, the formation of oleate, palmitate, and stearate soaps was found to be 90%, 38%, and 25%, respectively. Conversely, when a relatively low-calcium diet was used (13.5–41.4 mg/day), the absorption of palmitate and stearate was increased to 65% and 45%, respectively (Boyd et al., 1932). The fact that oleate soaps were used preferentially to the soaps of the saturated fatty acids indicates that the melting point is an important factor influencing the absorption of fats and their soaps. Gacs and Barltrop (1977) observed that the absorption of calcium was

inversely correlated with the chain length of the fatty acid, varying from 1% for calcium stearate to 60% for calcium hexanoate. Increasing the degree of unsaturation of the fatty acid was accompanied by increased calcium absorption. The degree of calcium-soap formation and the inhibition of calcium absorption were well correlated ($r = .82$, $p < .01$). No soap formation was noted when fats were given in the form of triglycerides. Other evidence from broiler chickens also supports the formation of calcium soaps and a reduction in calcium retention when palmitic and stearic acids (8%) are fed (Atteh and Leeson, 1983). The formation of calcium soaps has been suggested to be a normal part of lipid digestion (Patton and Carey, 1979). In summary, studies in animal models, although more inconsistent than suggestive, indicate two trends. First, calcium absorption and utilization are impaired when fat intakes exceed 10% of the energy intake. Second, saturated fatty acids reduce calcium utilization.

b. Human Studies

i. Absorption

Early studies in children consuming a mixed diet indicated a positive relationship between fat intake and apparent calcium absorption. Holt et al. (1920) reported calcium absorption of 40.4% when the intake exceeded 30 mg calcium/kg body weight, but when the intake was less, the absorption averaged only 20.3%. The greatest absorption of calcium occurred when the dietary fat intake exceeded 3 g/kg body weight and when there was an adequate intake of 300–500 mg calcium/kg body weight. The principal source of dietary fat was milk and butter. The excretion of calcium in stools was not related to the excretion of total fat but showed a minor relation to the excretion of fat as soap. Holt and Fales (1923) subsequently studied seven children aged 2–6 years who were fed diets containing fat at two levels (high fat: 30–65 g/day; and low fat: 5–8 g/day) with a constant calcium intake (1.7–1.9 g/day). Calcium absorption was markedly reduced when the low-fat diet was consumed. The composition of the high-fat diet was mostly saturated fat and the low-fat diet was principally unsaturated fat. Impaired calcium absorption was associated with the increased presence of calcium soaps in the stool. Other research also indicates that the apparent absorption of calcium may be improved by increasing the dietary fat content (Kies, 1985, 1988).

ii. Medium-Chain Triglycerides and Absorption

Similar to the findings of Beynen et al. (2002) with dogs, Haderslev et al. (2000) observed no changes in the absorption of calcium and magnesium with MCT (mixture of long- and medium-chain fatty acids totaling 50% fat in diet) in patients with intestinal resections. In contrast to these findings with adults, MCT-containing formulas (first, MCT, corn oil, and coconut oil, 40:40:20; second, MCT and corn oil, 80:20) fed to premature infants with birth weights less than 2000 g, significantly increased calcium absorption compared with the control formula containing corn oil, oleo, and coconut oil (39:41:20) (Tantibhedhyangkul and Hashim, 1978).

iii. Retention

Mallon et al. (1930) studied the calcium retention of two college-aged women consuming 450–500 mg calcium/day who were fed high- and low-fat diets based on milk products for 18 days. They found no significant change in the calcium balance or fecal calcium excretion during two consecutive 3-day balance periods, and they concluded that fat per se did not affect calcium retention. Aub et al. (1937) observed that the addition of 200 g fat to the diet of two healthy adults did not increase calcium excretion. Steggerda and Mitchell (1951) observed no effect on the calcium balance or fecal losses of calcium from milk fat (5–160 g/day) fed at 1%–32% of daily energy intake to 13 men. Fuqua and Patton (1953) studied nine college-aged women who consumed diets containing 600 mg calcium and

supplying 45, 91, and 135 g of fat (19%, 39%, and 58% energy as fat). Mean calcium balances were not significantly influenced by fat intake, but they were highly variable. In a study of 12 men, van Dokkum et al. (1983) reported no change in calcium retention with an increase in linoleic acid from 4% to 16% of energy intake when total fat intake was constant at 42% of energy. In very low birth weight infants, consumption of a long-chain PUFA supplemented formula (egg-lipid extracts and evening primrose oil; 5 g/100 kcal) had no effect on calcium balance (Martinez et al., 2002).

In contrast, Basu and Nath (1946) studied the mineral metabolism of four young men fed diets containing 187–512 mg calcium/day and in which fat was provided principally by mustard oil, coconut oil, groundnut oil, sesame oil, or butterfat. The control was a fat-free diet. The addition of each of the fats, except coconut oil, slightly decreased the excretion of fecal calcium. With coconut oil, there was an increase in the fecal calcium excretion.

Dietary fat is principally in the form of mixed-triglycerides, with different n-3, n-6, and n-9 fatty acids in the *sn*-1, *sn*-2, and *sn*-3 positions. This effect of dietary fat on calcium absorption and retention may be due in part to the types of fatty acids present within the triglycerides and their structural positions. Palmolein (PO) is used in infant formulas to match the palmitic acid content of human milk; however, evidence indicates that this can result in reduced calcium absorption in infants fed these formulas (Nelson et al., 1998), suggesting the structural position of the palmitic acid within a triglyceride may be important. In healthy term infants (5 weeks of age), fecal calcium excretion was significantly decreased when infants were fed a formula that more closely resembled human milk (24% palmitic acid with 66% esterified in the *sn*-2 position) than when they were fed formulas containing less esterified palmitic acid in the *sn*-2 position (24% palmitic acid, 39% esterified in *sn*-2; or, regular formula, 20% palmitic acid, 13% esterified in *sn*-2) (Carnielli et al., 1996). Calcium retention tended ($p = .10$) to be greater in those infants fed the formula with 66% palmitic acid esterified in the *sn*-2 position. Carnielli et al. (1996) also evaluated the correlation between the excretion of calcium and fatty acids. Positive correlations were found between palmitic acid ($r = .84$), oleic acid ($r = .60$), and linoleic acid ($r = .51$), and calcium excretion suggesting differing effects of saturated vs. unsaturated (e.g., n-6, n-9) fatty acids on calcium excretion.

iv. Calcium Utilization

It is unclear whether usual intakes of dietary fat independently affect calcium utilization in humans. Excessive intakes of fat in pathological conditions that result in steatorrhea can negatively influence human calcium utilization (Aub et al., 1937). The type of fat can also influence calcium utilization in bone. Healthy term infants were fed formulas varying in PO for the first 6 months of life and bone mineral content (BMC) and BMD were measured by dual x-ray absorptiometry at baseline, 3 and 6 months of age (Koo et al., 2003). The formula containing PO (45%/20%/20%/15% oil; PO/coconut/soy/high-oleic sunflower oils; ~22.1% palmitic acid) resulted in lower ($p < .001$) BMC and BMD when compared to a formula without PO (40%/30%/30% oil; high-oleic safflower/coconut/soy oils; 8.2% palmitic acid) fed to another group of infants. These findings suggest that not only the amount, but also the type of fat and positioning of fatty acids within triglycerides can influence calcium utilization.

Bone density improved in elderly women (mean age 79.5) fed GLA and EPA (Kruger et al., 1998). Recently, dietary intake of CLA (63.1 ± 46.8 mg, mean ± SD) was found to be a significant ($p = .040$; $r^2 = .286$) predictor of Ward's triangle BMD in healthy postmenopausal women (Brownbill et al., 2005). Years since menopause, lean tissue, energy intake, intakes of calcium, protein, fat and zinc, as well as current and past physical activity were all accounted for in their multiple regression model.

c. Mechanisms for Dietary Fat-Induced Alterations in Calcium Absorption and Utilization

Several mechanisms appear to be involved in the effects of dietary fat on calcium absorption and utilization. Song et al. (1983) demonstrated the existence of calcium-binding ligands in the small intestine of rats that appear to be age dependent. Jejunal transport processes may also be altered

by small changes in the percentages of EFAs and non-EFAs, without changes in total fat (Thomson et al., 1986). Haag et al. (2003) reported that, in *in vitro* models, the n-3 fatty acids, DHA and EPA, affected Ca^{+2}- and Na^{+},K^{+}-ATPases important in the intestinal absorption of calcium. In Caco-2 cells, 50 μM of the *trans*-10,*cis*-12 isomer of CLA increased paracellular epithelial transport whereas the *cis*-9,*trans*-11 isomer had no effect (Roche et al., 2001). Jewell and Cashman (2003) confirmed these findings and observed that only paracellular, not total transepithelial or transcellular transport, was affected by 80 μM of *trans*-10,*cis*-12 isomer of CLA. EFAs may also increase calcium absorption by enhancing the effects of vitamin D on calcium uptake and use (Kruger and Horrobin, 1997).

Dietary fat may also impact bone quality and quantity through effects on inflammatory cytokines (Kettler, 2001). Sources of n-3 fatty acids such as fish oil (i.e., EPA and DHA), flaxseeds, and flaxseed oil (i.e., ALA) can suppress production of the inflammatory cytokines, IL-1, IL-6, and TNF-α (Meydani et al., 1991; Blok et al., 1996; Caughey et al., 1996; Kettler, 2001). This reduction in cytokine production has been observed with a dietary intake of 1–5 g/day of EPA and/or DHA from fish or fish oil (Endres et al., 1989; Meydani et al., 1991, 1993) as well as from a dietary intake of 14 g/day of ALA from flaxseed oil or from fish oil added to a high-ALA diet (Caughey et al., 1996). The inflammatory cytokines increase the formation and activity of osteoclasts (Manolagas and Jilka, 1995) that are involved in bone resorption. Decreases in the production of inflammatory cytokines should decrease bone resorption and, thus, bone loss.

B. Magnesium

Scientific interest in the interaction between dietary fat and magnesium has been minimal in contrast to dietary fat and calcium. In fact, much of what has been reported about magnesium bioavailability relative to fat has been the beneficiary of research on calcium and fat interactions. In a review of the early literature, Seelig (1964) concluded that the available evidence was insufficient to define the effect of fat on magnesium metabolism. More recent experimental data may improve our understanding of this relationship.

a. Animal Studies

i. Absorption and Retention/Tissue Mineral Concentrations

Tadayyon and Lutwak (1969) studied the effects of dietary triglycerides on magnesium metabolism in weanling rats. Magnesium absorption was significantly correlated ($r = .43$; $p < .1$) with fat absorption. Animals receiving either a fat-free diet or 25% triolein excreted the least magnesium in feces. At 5% intake, triolein, tripalmitin, and tristearin had similar effects on magnesium absorption and resulted in higher absorption of magnesium than with 25% tripalmitin or tristearin. The magnesium content of the femur was highest in the groups fed 5% or 25% triolein and lowest in the group fed 25% tripalmitin. In contrast, Watkins et al. (1992) found no effect of varying dietary fat content (1%, 5%, or 10%) on magnesium absorption. In turkey poults, retention of magnesium was less with more saturated fats, and palmitic acid consumption significantly decreased bone magnesium content (Leeson and Atteh, 1995). Kaup et al. (1990) observed a variable effect of fat on magnesium absorption in young and mature rats. Magnesium absorption was consistently greater among young rats fed high-butterfat (20%) diets vs. low-butterfat (5%) diets. Magnesium absorption, however, tended to be reduced among mature rats fed more fat.

ii. Magnesium Soap Formation

The interactions between fatty acids and magnesium are generally accepted to occur within the intestinal lumen where soaps are formed (Boyd et al., 1932). This soap formation results in insoluble complexes that are not absorbed but are excreted in the feces (Gacs and Barltrop, 1977). The consistent observation that fecal magnesium losses are related to fecal fat excretion suggests that soap formation is the principal mechanism by which fats and minerals interact.

b. Human Studies

i. Absorption, Retention, and Utilization

In a study of young men (van Dokkum et al., 1983), magnesium retention was not affected by increasing dietary linoleate intake from 4% to 16% of energy intake (total fat intake was constant at 42% of energy intake). Similarly, reducing total fat intake from 42% to 22% with a constant linoleate intake of 18% did not affect magnesium retention. These findings are consistent with the conclusion of Seelig (1964) that there is little evidence to support a definite effect of dietary fat on magnesium absorption. However, as described in the above section on animal studies, other evidence suggests diets high (>20%) in saturated dietary fat tend to reduce magnesium absorption in mature animals. In premature infants, however, magnesium absorption was increased when the infants were fed an MCT-containing formula (MCT and corn oil, 80:20) in comparison with the control formula (corn oil, oleo, and coconut oil, 39:41:20) (Tantibhedhyangkul and Hashim, 1978).

C. Copper and Zinc

In contrast to macrominerals, such as calcium and magnesium, that are required in amounts of hundreds of milligrams per day, the daily human requirements for trace elements, such as cooper, zinc, and iron, are estimated to be in amounts ranging from about 3 to 15 mg (National Research Council, 2001). Factors affecting the bioavailability of these trace substances have been intensively studied (Halsted et al., 1974; Mason, 1979). However, the influence of absolute or relative amounts of fat has only recently come under investigation as a putative factor affecting copper and zinc utilization.

a. Animal Studies

i. Absorption

The effects of the type of dietary fat (coconut or safflower oil; 10% by weight) on copper and zinc absorption was studied in weanling, male Sprague-Dawley rats fed semipurified diets containing adequate amounts of copper and zinc (Lukaski et al., 1986). Absorption was estimated by determination of the remaining radioactivity by whole-body counting after labeling each animal with ^{67}Zn. The safflower oil-based diet was associated with a slightly depressed absorption of copper (35% vs. 39%) and zinc (79% vs. 84%).

In a rat model using *in vivo* jejunal perfusion, 1.0 mM of palmitate or stearate were observed to suppress copper absorption rates compared with controls (infusate without fatty acid or triglycerides; control: 104.4 ± 8.8 vs. palmitate: 12.5 ± 17.6 pmol/min × cm, $p < .01$; control vs. stearate: 37.2 ± 25.6 pmol/min × cm, $p < .05$) (Wapnir and Sia, 1996). Copper absorption was unaffected by medium-chain free fatty acids such as caprylate and caproate, or was it affected by an emulsion of medium- or long-chain triglycerides (1.0 or 2.5 mM). Earlier research indicated that 1 mM of palmitic acid significantly enhanced zinc absorption (Wapnir and Lee, 1990). The results of these studies suggest long-chain free fatty acids may impair copper absorption whereas zinc is increased by saturated fatty acids.

In contrast, n-3 fatty acids and palmitate have been reported to enhance absorption of zinc (Lee and Wapnir, 1993). In rats with or without experimental osmotic diarrhea, 1 mM stearate enhanced zinc absorption, whereas 1 mM palmitate was effective only in normal rats (Lee and Wapnir, 1993). The authors suggested that saturation and a longer-chain length were positive factors in the enhancement of mineral absorption.

ii. Retention/Tissue Mineral Concentrations

The effects of dietary linoleic acid on the tissue status of zinc and copper were examined in adult male Fisher 344 rats (Koo and Ramlet, 1984). One group received a diet containing 4% hydrogenated

coconut oil (about 0.8% linoleate) and the other received a diet containing 3.4% nonhydrogenated coconut oil plus 0.6% linoleic acid. The linoleate contents of the diets were 0.8% and 2.55%, respectively. After 6 weeks, the high-linoleate diet resulted in a significant depression in serum zinc (23.7 vs. 21.4 μmol/L) and a slight decrease in serum copper concentration (20.5 vs. 19.7 μmol/L). Liver and tibia wet weights were similar between the groups. The higher linoleate diet was associated with a significantly depressed zinc content of the tibia (145 vs. 156 μg/g wet weight) and copper content of the tibia (0.20 vs. 0.33 μg/g wet weight). Zinc and copper content of the liver were not significantly affected by linoleate intake.

The effects of the type of dietary fat (coconut or safflower oil; 10% by weight) on copper and zinc retention were studied in weanling, male Sprague-Dawley rats fed semipurified diets containing adequate amounts of copper and zinc (Lukaski et al., 1986). Retention was estimated by determination of the remaining radioactivity by whole-body counting after labeling each animal with ^{65}Zn. The safflower oil-based diet was associated with a slightly depressed half-life of copper (10 vs. 11 days) and zinc (82 vs. 85 days). Safflower oil marginally depressed hepatic copper content (10.9 vs. 11.2 μg/g dry weight), but significantly decreased liver zinc (287 vs. 381 μg/g dry weight). Lynch and Strain (1989) also reported an effect of type of dietary fat on hepatic copper. They studied weanling, male Wistar rats fed diets containing 20% by weight of either coconut or safflower oil with two different copper contents (0.4 and 11 ppm) for 56 days. In the rats fed with adequate copper diets, the liver copper content was significantly less (7.5 vs. 18.2 μg/g wet weight) when safflower oil was the fat source. Similarly, safflower oil significantly decreased hepatic copper in the rats fed with copper-deficient diet (7.5 vs. 8.1 μg/g wet weight). In addition to the evidence for an effect with the type of dietary fat, Wapnir and Devas (1995) observed that a high-fat diet (45% as corn oil and hydrogenated vegetable oil) decreased plasma copper in rats fed either a normal- or low-copper diet. Tallman and Taylor (2003) also demonstrated that mice fed a high-fat diet (39% kcal from lard and 16% kcal from soybean oil) had significantly increased fat pad weights and lower adipose zinc concentrations than those mice fed a low fat diet (16% kcal from soybean oil). The adipose tissue zinc concentration of the high-fat fed mice was 192 ± 14 nmol/g vs. 241 ± 18 nmol/g in the low-fat-fed mice. The findings of these animal studies indicate that the consumption of a diet consisting of predominantly PUFAs can depress zinc and copper status and that the dietary fat content may also influence tissue distribution of trace minerals.

b. Human Studies

i. Absorption and Retention

The influence of the type and amount of dietary fat on human trace element metabolism has not been intensively investigated. Three highly trained endurance cyclists lived in a metabolic unit and consumed diets made of conventional Western foods for 3 months (Lukaski et al., 2001). The diets, which were high (45%–55%) in carbohydrate and saturated or polyunsaturated fat, were presented in random order for about 28 days each; the effects of the type and amount of dietary fat were evaluated by the chemical balance technique. Zinc and copper balance data were expressed as the values of two consecutive 6-day balance periods at the end of each dietary period. Zinc retention was significantly affected by the type of dietary fat (Table 24.1). Although a small difference in average zinc intake occurred, probably the result of changes in food used to accommodate the required changes in carbohydrate and fat composition of the diets, the zinc balance was significantly decreased by polyunsaturated fat as compared to saturated fat or carbohydrate. Relative zinc losses in the feces increased when polyunsaturated fat was consumed. The difference of approximately 5 mg in zinc retention exceeds the difference of 1.5 mg in zinc intake. There was no significant effect of dietary fat on the copper excretion and balance. Interestingly, the copper balance was positive although not different from 0, only when saturated fat was consumed.

Because of the differences in the calculated linoleate intake between the polyunsaturated and saturated fat diets, it was possible to evaluate zinc and copper balances relative to intake of this fatty

TABLE 24.1
Summary of Two 6-Day Balance Periods in Three Cyclists Consuming Diets High (44%–55%) in Carbohydrate (CHO), Saturated Fat (SATF), and PUFA

		Feces		Urine		
Diet	**Intake (mg)**	**mg**	**% Intake**	**mg**	**% Intake**	**Balance**
Zinc						
CHO	23.1 ± 0.9[a]	19.5 ± 1.0[a]	85 ± 3[a]	0.9 ± 0.2	4 ± 0.6	2.7 ± 0.7[a]
PUFA	25.8 ± 1.0[a]	24.4 ± 1.1[b]	95 ± 4[b]	0.8 ± 0.2	3 ± 0.5	0.6 ± 0.9[b]
SATF	27.3 ± 0.9[b]	20.5 ± 1.4[a]	75 ± 3[a]	0.9 ± 0.2	3 ± 0.6	5.8 ± 0.8[a]
Copper						
CHO	2.8 ± 0.2[a]	2.7 ± 0.2	98 ± 12	0.1 ± 0.1	3 ± 0.2	–0.03 ± 0.3
PUFA	2.3 ± 0.2[b]	2.2 ± 0.1	99 ± 4	0.1 ± 0.01	4 ± 0.4	–0.06 ± 0.1
SATF	2.3 ± 0.2[b]	2.1 ± 0.2[e]	94 ± 3	0.1 ± 0.01	4 ± 0.2	0.04 ± 0.1
Iron						
CHO	44.2 ± 2.2	30.1 ± 1.2[a]	69 ± 4[a]	0.1 ± 0.01	0.2 ± 0.01	14.0 ± 2.6[a]
PUFA	39.7 ± 3.1	37.9 ± 1.3[b]	96 ± 6[b]	0.1 ± 0.01	0.3 ± 0.03	1.7 ± 2.8[b]
SATF	46.3 ± 2.4	33.3 ± 1.9[a]	72 ± 7[a]	0.1 ± 0.01	0.2 ± 0.02	13.0 ± 3.9[a]

Values are mean ± SE.

[a,b] Values with different superscripts in same column are different ($p < .05$).

Source: Adapted from Lukaski, H.C., et al. (2001). *Int. J. Sport Nutr. Exerc. Metab. 11*: 186–198.

TABLE 24.2
Effects of Linoleate Intake on Zinc, Copper, and Iron Retention in Three Cyclists

Linoleate Intake (g/day)	**Zinc Retention (mg/6 day)**	**Copper Retention (mg/6 day)**	**Iron Retention (mg/6 day)**
≤ 13	4.3 ± 1.0	0.01 ± 0.1	13.5 ± 2.1
≥ 140	0.6 ± 0.7	– 0.06 ± 0.09	1.8 ± 1.9
$p =$	.05	.75	.009

Values are mean ± SE.

Source: Adapted from Lukaski, H.C., et al. (2001). *Int. J. Sport Nutr. Exerc. Metab. 11*: 186–198.

acid. Table 24.2 shows the effects of high- and low-linoleate intakes on zinc and copper balances. Low daily intakes of linoleate (about 13 g or less) were associated with a significantly greater zinc balance than higher intakes (140 g or more). Zinc retention was inversely and significantly related ($r = –.49$; $p < .5$) to linoleate intake. The copper balance was not affected by linoleate intake.

Boeckner and Kies (1986) observed that zinc retention in adolescents tended to be less with high- vs. low-fat diets; however, the amount of dietary zinc likely affected this association.

c. Mechanisms for Dietary Fat-Induced Alterations in Copper and Zinc Absorption and Utilization

Experimental data from rats indicate that although absorption and retention of copper and zinc determined by using radioisotopes are not significantly influenced by the type of fat, tissue pools of

these trace elements are decreased by polyunsaturated fat (Koo and Ramlet, 1984; Lukaski et al., 1986; Lynch and Strain, 1989). These findings suggest that dietary fat influences copper and zinc redistribution in the body. The mechanism underlying these changes in tissue redistribution may be alterations in transport proteins, such as zinc transporters. Recent evidence indicates that rats raised and/or maintained on a diet deficient in n-3 PUFA (7% safflower oil, 6.7% palmitic acid, 2.6% stearic acid, 16.7% oleic acid, 71.9% linoleic acid, and 0.2% ALA of dietary fat with no long-chain n-3 PUFA) had significantly decreased plasma zinc concentrations as well as increased expression of *ZnT3* in the brain compared with rats on a control diet with n-3 PUFA (5.84% safflower oil and 1.16% flaxseed oil; 6.5% palmitic acid, 2.3% stearic acid, 15.0% oleic acid, 63.0% linoleic acid, and 11.4% ALA of dietary fat with no long-chain n-3 PUFA) (Jayasooriya et al., 2005). Similar to the findings in rats, data from men indicate that retention of zinc is significantly reduced when a diet high in polyunsaturated fat is consumed (Lukaski et al., 1992). This impairment is related to the increased fecal excretion of zinc. These findings in animals and humans suggest that fats may act at either the intestinal lumen or the cell membrane to exert the observed effects.

Although intensively studied, little is known about the factors that regulate copper and zinc absorption (Cousin, 1985). Copper and zinc are taken up by brush-border membrane transport systems at the mucosal cell. These systems are not well understood, but they are thought to involve a carrier-mediated transport protein. Whether dietary fat directly affects the structure and function of these transport systems is not known. However, it is generally accepted that changes in dietary fat intake can significantly influence membrane fluidity, and thereby significantly affect cellular functions, including carrier-mediated transport and membrane-bound enzyme activities (Spector and Yorek, 1985).

D. Iron

The importance of iron in maintaining health and optimizing biological function has been long recognized by nutritionists (Dallman, 1986). However, there has been intensive research to extend our understanding of the dietary factors affecting the availability of iron for absorption and utilization (Bowering et al., 1976). The effects of micronutrient factors, such as other minerals, ascorbic acid, and phytic acid, have been studied in detail (Hallberg, 1981), but the influences of macronutrients, such as protein, carbohydrate, and fat, are less well understood.

a. Animal Studies

i. Absorption

Amine and Hegsted (1975) reported an effect of dietary fat on iron absorption. In one experiment, iron absorption was determined by using ^{59}Fe in adult, iron-deficient, female rats fed diets containing varying amounts of coconut or corn oil. Diets high in fat (30% vs. 5% or 15%) apparently promoted iron retention. Iron absorption was greater in diets in which fat was supplied as coconut oil than those in which fat was provided as corn oil. The difference between the oils was greatest when fat in the diet was low (5% coconut oil: 38% ± 3% ^{59}Fe retained vs. 5% corn oil: 25% ± 2% ^{59}Fe retained, $p < .05$).

The effect of changing the amount and type of dietary fat on iron absorption in weanling male, iron-deficient rats fed varying amounts of heme and nonheme iron was studied by Bowering et al. (1977). Changes in the fat content included an increase from 5% to 20% of the diet and exchange of lard for corn oil. Increasing the fat content and changing to a more saturated fat source were associated with small but significant increases in iron absorption. The enhancing effect on iron absorption observed with changing the type of dietary fat was observed only when ferrous sulfate, and not heme iron, was fed at a suboptimal (15 ppm) amount in comparison to adequate (25 ppm) or luxuriant (350 ppm) amounts.

Factorial studies were undertaken to examine the effects of dietary iron intake (10 vs. 35 ppm), fat intake (5% vs. 35%), and type of fat (safflower oil vs. coconut oil) on heme and nonheme iron

absorption (Johnson et al., 1987). Rats were made moderately anemic by feeding an iron-deficient diet and were then fed one of the eight experimental diets. Iron absorption was determined by feeding a ^{59}Fe test meal and by determining the remaining radioactivity in each animal by using whole-body counting for the following 5 weeks. Unlike in humans, nonheme iron was better absorbed than heme iron regardless of other dietary factors. Both heme and nonheme iron absorption was greater when high (30%) rather than low (5%) dietary fat was fed.

Other evidence also indicates a higher amount of dietary fat (15% or 30% of dietary energy vs. 7% or 14% of dietary energy) can enhance iron absorption in rats fed low dietary iron (10 μg Fe/g diet) (Droke and Lukaski, 1996). The type of fat (unsaturated vs. saturated) had an effect on iron absorption; absorption was significantly enhanced when rats were fed 7% or 15% stearic acid (fatty acid form), cocoa butter, or beef tallow than when rats were fed safflower oil. No differences in absorption was observed between stearic acid, cocoa butter, or beef tallow. Iron absorption was also significantly greater in rats fed 7% or 15% cocoa butter or 15% beef tallow than in rats fed 7% safflower oil, suggesting a more saturated fat source, regardless of the fat content of the diet, may increase iron absorption in iron-deficient animals (Droke and Lukaski, 1996).

ii. Retention/Tissue Mineral Concentrations

The importance of the interaction between dietary fat and iron was highlighted by Kaufman et al. (1958). In adult male rats, the liver iron concentration decreased significantly from 88.6 to 15.7 mg/100 g when dietary fat (lard) was reduced form 30% to 10% and protein (casein) was maintained at 10%. When the diet was supplemented with additional iron (2% as ferric citrate), the liver iron also increased when fat was high (88.6–113.5 mg/100g) or low (15.7–22.5 mg/100 g). Thus, both fat and iron increased the hepatic iron content in iron-adequate rats. Liver iron in weanling male rats was increased to the greatest extent when the rats were fed an iron-adequate diet (35 μg/g) containing 15% fat (Droke and Lukaski, 1996). The lowest amount of liver iron was observed in rats fed iron-deficient diets (10 μg/g) containing 7% or 15% fat. An iron-adequate diet containing 7% fat resulted in liver iron concentrations that were intermediate between both the deficient diets and an adequate diet containing 15% fat. However, in later research, liver iron concentrations were unaffected by safflower oil, flaxseed oil, olive oil, or beef tallow (Shotton and Droke, 2004).

iii. Iron Utilization

The development of iron deficiency has been promoted by feeding diets low in iron and/or high in polyunsaturated fat (Amine et al., 1976; Rao et al., 1983; Droke and Lukaski, 1996). In contrast, feeding fat-free or saturated fat diets has been used to inhibit the development of iron deficiency in animals and fowl (Rao et al., 1980, 1983).

Factorial studies were undertaken to examine the effects of dietary iron intake (10 or 35 ppm), fat intake (5% or 35%), type of fat (safflower or coconut oil), and iron source (heme or nonheme) on indices of iron status (Johnson et al., 1987). Rats were made moderately anemic by feeding an iron-deficient diet and were then fed 1 of the 16 experimental diets. Rats fed both heme or nonheme iron had significantly greater hemoglobin, change in hemoglobin, and liver iron content when fed coconut oil compared to safflower oil. Rats fed nonheme iron had greater liver iron, but not hemoglobin or change in hemoglobin, when fed high- rather than low-dietary fat. Rats fed heme iron had greater hemoglobin, change in hemoglobin, and liver iron when fed high- rather than low-fat diet.

Later research (Droke and Luksaki, 1996) using the same model as Johnson et al. (1987) demonstrated that the effects of dietary fat on iron utilization (i.e., regeneration of hemoglobin) were observed only in rats fed low-dietary iron (10 μg/g). In addition, iron utilization was the greatest when rats were fed 15% stearic acid than when they were fed diets containing 7% or 15% safflower oil, cocoa butter, or beef tallow. Similar to these findings, suckling and weanling rats fed more saturated fat (coconut oil, human milk fat) had a greater percentage of ^{59}Fe in the blood (suckling rats), a greater hemoglobin regeneration (weanling), and significantly higher retention of ^{59}Fe (weanling)

(Pabon and Lonnerdal, 2001). Subsequent research (Shotton and Droke, 2004) evaluated the effects of fat source (safflower oil, flaxseed oil, olive oil, or beef tallow; 30% of dietary energy) in combination with different concentrations of dietary iron (10, 35, or 100 μg/g) on iron utilization. As in the previous research, the male weanling rats were initially fed a low-iron safflower diet to promote iron depletion. The results indicated that iron status and iron utilization (hemoglobin regeneration) were not significantly affected by an interaction between dietary fat source and iron concentration. However, rats fed low iron tended to have an increase in iron utilization when they were fed either olive oil or beef tallow. These conflicting results between studies may be due to differences in experimental design such as duration of feeding and dietary fat content.

These findings strengthen the growing evidence that the amount of dietary fat and its degree of saturation can affect the utilization of dietary iron. In general, the findings indicate that increasing amounts of saturated fat enhance and increasing amounts of unsaturated fat inhibit dietary iron absorption and utilization in rodent models.

b. Human Studies

i. Absorption and Retention

In a study of competitive male cyclists (Lukaski et al., 2001), the effects of the type and amount of fat on iron retention were also examined. Iron retention was significantly affected by the type of fat consumed (see Table 24.1), and it was either significantly decreased by polyunsaturated fat or enhanced by saturated fat. On the basis of the apparent increase in fecal iron excretion, unsaturated fat apparently impairs iron absorption. High intakes of linoleate were associated with a reduced iron retention (see Table 24.2). The iron balance was inversely and significantly related ($r = .64$; $p < .004$) to linoleate intake.

Similar findings were reported by van Dokkum et al. (1983), who examined the effects of changing the total fat intake and the amount of dietary linoleic acid on iron balance and blood biochemical indices of iron status in 12 men fed experimental diets for 28-day periods. Increasing the total fat intake from 22% to 42% of energy did not affect the iron balance. However, increasing the linoleic acid intake from 4 to 16%, while the total fat intake remained at 42%, resulted in a significant decrease in iron retention from 3.3 to 2.3 mg/day. At the same time, hemoglobin concentrations declined slightly from 9.6 to 9.1 mmol/L and the hematocrit decreased from 48% to 46%.

c. "Meat Factor"

Meat is another factor known to influence iron absorption. It is well recognized that heme iron, found primarily in meat or muscle, is better absorbed by humans than nonheme iron (Hallberg, 1981). Furthermore, meat or meat products facilitate nonheme iron absorption. Efforts to identify the factor or factors in meat that promote nonheme iron absorption have been extensive (Cook and Monsen, 1976; Hazell et al., 1978; Bjorn-Rasmussen and Hallberg, 1979; Hallberg and Rossander, 1982; Layrisse et al., 1984). Some investigators concluded that the active factor in meat that promotes nonheme iron absorption is an amino acid, such as glutathione or histidine, or small peptides, such as glutathione, but the effects of these and other components of meat on iron absorption have not always been consistent. Zhang et al. (1990) proposed that the "meat factor" must be a chemical compound, which binds iron through an action or mechanism that is different than chelation with an amino acid or peptide.

A candidate for the role of the meat factor is a fat or fatty acid. Mahoney et al. (1980) reported a relationship between a fat effect and the meat effect on iron absorption. They demonstrated that animals fed beef fat, compared to turkey fat, corn oil, or pork fat, were most efficient at converting iron from turkey meat into hemoglobin. One of the principal differences between beef fat and the other fats used in their study is that beef fat contains about 19% stearic acid (18:0), which is 10 times more than the stearic acid content of corn oil, 3.0 times more than that of turkey fat, and 1.5 times

more than in lard (Mahoney et al., 1980). The findings suggest dietary stearic acid may be important in facilitating iron absorption.

Johnson et al. (1992) investigated the effects of stearic acid on iron utilization in rats. Anemic, male rats were fed diets containing stearic acid plus safflower oil (22% stearate + 2% safflower oil and 20% stearate + 4% safflower oil) or safflower oil (24%) and low (10 ppm) or high (39 ppm) iron as ferrous sulfate. The repletion of hemoglobin, hematocrit, liver iron, and absorption of ^{59}Fe were assessed. Compared to safflower oil, stearic acid had a significant positive effect on the repletion of hemoglobin, hematocrit, and liver iron. The effect was greatest when dietary iron was low.

In another experiment (Lukaski et al., 1992), rats were fed low-dietary iron (10–11 ppm) and 24% safflower oil, 20% stearic acid + 4% safflower oil, 3.2% stearic acid + 20.8% safflower oil, or 20% beef tallow + 4% safflower oil. The 20% beef tallow provided 3.2% stearic acid. Rats fed beef tallow had significantly greater hemoglobin (6.9 vs. 5.6 g/L) and hematocrit (2.15% vs. 18.1%) repletion than did rats fed safflower oil, although the degree of repletion was less than that observed in rats fed 20% stearic acid (8.2 g/L and 25.98%). There was no difference in iron repletion of rats fed 3.2% stearic acid and rats fed beef tallow. Thus, stearic acid apparently increases iron utilization in rats fed nonheme iron.

Another experiment was designed to distinguish between the effects of meat protein and meat fat on iron utilization (Lukaski et al., 1992). Anemic rats were fed diets low or adequate in iron with either casein or beef (prime rib or fat-extracted beef) as the protein source and safflower oil, tallow, or stearic acid as the fat source. Dietary iron was low or adequate. Animals fed diets with tallow or stearic acid had the highest circulating hemoglobin regardless of the amount of iron in the diet. In addition, rats fed prime rib had reticulocyte counts three to five times greater than rats fed casein or lean beef with safflower oil. These findings indicate that beef fat enhances the utilization of ion for hemoglobin and red blood cell production.

The effects of combinations of various protein (lean beef, skim milk, and egg white) and fat (beef fat, milk fat, and partially hydrogenated vegetable fat) sources on iron absorption in iron-adequate weanling rats have been examined (Kapsokefalou and Miller, 1993). Whole-body retention of ^{59}Fe was similar for lean beef plus tallow as compared to lean beef plus vegetable shortening (78% vs. 70%) but was significantly less with egg plus shortening (78% vs. 57%). Overall, there was no effect of fat type within a specific protein group, but beef was a significantly better protein source for promoting iron absorption. The authors concluded that the fat source (e.g., tallow) played an important role in facilitating the ability of lean beef to promote nonheme iron absorption.

A novel method has been used to examine the effects of dietary fat on *in vivo* mucosal iron kinetics. This experimental approach uses two radioisotopes of iron to estimate *in vivo* iron absorption and plasma iron kinetics into tissues (Nathanson et al., 1984). In mature dogs made iron deficient by consumption of an iron-deficient diet and serial phlebotomy, stearic acid (20%) significantly increased iron absorption (50% vs. 21%), hemoglobin (25 vs. 6 g/L), and erythrocyte volume (83 vs. 32 mL) regeneration (Lukaski et al., 1993). This beneficial effect of stearic acid was the result of a significantly increased rate of transfer of iron from the mucosal cell to the plasma. Beef tallow, a typical source of dietary stearic acid, was shown to promote iron absorption and utilization as compared to safflower oil (McLaren et al., 1993).

These experiments were repeated with reduced amounts of fat (10%). It was found that stearic acid and beef tallow similarly enhanced iron absorption and utilization in anemic dogs (McLaren et al., 1997). Therefore, these findings support the hypothesis that dietary stearic acid may be one of the chemical factors in meat that promotes nonheme iron absorption and utilization.

d. Mechanisms for Dietary Fat-Induced Alterations in Iron Absorption and Utilization

Despite increasing evidence that dietary fat can influence iron absorption and retention (van Dokkum et al., 1983; Johnson et al., 1987, 1992; Lukaski et al., 1993, 2001; McLaren et al., 1993), there is a

paucity of information about the mechanism of this action. The mechanism(s) of these effects may be related directly to changes occurring within the lumen of the intestine or indirectly due to alterations in the integrity of enterocyte membranes and/or paracellular transport. In the early 1990s, it was proposed that bioavailable lipophilic complexes between ferrous iron and free fatty acids could be formed and taken up by mucosal cells (Kapsokefalou and Miller, 1993). This is supported by observations that saturated fat, particularly stearic acid, appears to promote iron uptake. Research involving chemical analyses of combustion products of tobacco smoke demonstrated that stearic acid has the capacity to affect the reduction of ferric to ferrous iron, to bind the resulting ferrous iron, and to transport the iron within the pulmonary macrophage (Qian and Eaton, 1989). Such enhancement of iron uptake by stearic acid may be related to the formation of stable monolayer stearate iron films (Wheeler et al., 1971). Monolayer films of anions may function as ionophores in the translocation of cations across biological membranes (Patel and Cornwell, 1977).

Fatty acids have been determined to participate in the uptake of iron at the mucosal membrane. Isolated brush-border membrane vesicles have been reported to have a high concentration of nonesterified fatty acids (Simpson and Peters, 1987a,b). It was demonstrated by using an *in vitro* brush-border membrane preparation that the major iron-binding components were associated with free fatty acids and that oleic and stearic acids show iron-binding capacities (Simpson and Peters, 1987a,b; Simpson et al., 1988). Abnormal fatty acid changes in cellular membranes were later shown to occur with iron deficiency (Tichelaar et al., 1997) and the authors suggested additional n-3 and n-6 fatty acids may be necessary to correct the changes occurring with iron deficiency. Later research by Pabon and Lonnerdal (2001) suggested the increase in absorption observed with saturated fats may have been due to changes in the fatty acid composition of the intestinal mucosa as significant positive correlations were found between dietary oleic and linoleic acids and intestinal mucosa concentrations of these fatty acids (oleic: $r = .95$, $p < .05$; linoleic: $r = .97$, $p < .05$).

Recently, research with Caco-2 cells provided support for the findings of Pabon and Lonnerdal (2001) by demonstrating a significant increase in ^{59}Fe uptake with 1 mM of oleate, palmitate, or stearate (Droke et al., 2003). Transport of ^{59}Fe by the Caco-2 cells was also significantly enhanced by 1 mM of palmitate or stearate. Palmitate increased transport to a greater extent than stearate, which could have been due to fatty acid metabolism within the cells and the elongation of palmitic acid to stearic acid. Overall, the results suggested that the fatty acids affected iron uptake to a greater extent than iron transport. These effects may have been the result of changes in monolayer integrity and paracellular transport that were observed with the cells, suggesting possible alterations in tight junctions.

III. CONCLUSION

Experimental evidence in animals and humans indicates that dietary fat may be important in mineral metabolism. The consensus is that dietary fat does significantly influence calcium and magnesium metabolism in healthy people with usual fat intakes, and there is accumulating evidence that fat may impact trace mineral metabolism.

Polyunsaturated fat may adversely affect the absorption and utilization of copper and zinc in animals. It apparently reduces the absorption of zinc in humans. Although the fecal excretion of zinc is increased with polyunsaturated fat, the mechanism of action is not known, it may involve alterations in the intestinal milieu that inhibit zinc-membrane receptor dynamics, or changes in cellular membrane receptor function by altering membrane fluidity.

The most striking effect of dietary fat on trace mineral metabolism is the finding of the enhancement of iron uptake and utilization by saturated fat, specifically stearic acid. The effects are prominent when dietary iron is limiting, and they thus indicate a novel role in promoting and adequate iron status in humans. The practical importance of using stearic acid to facilitate iron absorption in humans is that it has a minimal impact on serum cholesterol concentrations (Keys et al., 1965; Bonanome and Grundy, 1988) and it does not adversely effect platelet function and clotting (Schoene et al., 1993).

IV. DIRECTIONS FOR FUTURE RESEARCH

Additional research is needed to determine the mechanisms through which dietary fat and fatty acids affect mineral metabolism. It remains to be determined which intakes of a fat and which fatty acids significantly change mineral absorption and utilization, and if this results in a significant accumulation of the minerals. The use of new experimental approaches, including cell culture techniques and cell biology tools, is recommended to further delineate physical and chemical interactions between fatty acids and minerals at the cellular level. The use of microarray DNA technology to study gene expression in enterocytes may also provide valuable clues regarding the mechanism(s) for the effects of dietary fat on mineral absorption and utilization. This information will be useful in understanding the factors that affect the bioavailability of minerals in the diet to optimize human health and function.

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25 Interaction of Dietary Fatty Acids, Carbohydrates, and Lipids on Carbohydrate Metabolism

Béla Szepesi

CONTENTS

I. INTRODUCTION

The effects of dietary carbohydrates differ qualitatively and quantitatively from the effects of other nutrients. These differences between carbohydrates and other nutrients should be well understood before the various roles of carbohydrates and their effects of human health are considered. The first of these differences relates to the requirement for carbohydrate in the food. Whereas vitamins, minerals, proteins, individual amino acids, or polyunsaturated fatty acids (PUFAs) can be shown to be required for minimum human health, there is no absolute requirement for the intake of dietary carbohydrate. Apparently, human beings are able to maintain at least a minimal health status with a relatively wide range of consumption of carbohydrates with respects to both amount and type.

The second of these differences relates to the magnitude of the responses involved. Deficiencies in vitamin, mineral, protein, or PUFA consumption lead to often clear-cut and large physiological changes demonstrable over a relatively short period of time, and overconsumption of some of these substances again can lead to relatively large changes, which also are demonstrable. Overconsumption of saturated fatty acids and cholesterol, for example, has been recognized as a risk factor in arterial and heart diseases. The effects of such overconsumption are readily apparent on blood lipids and can be demonstrated in animal models and human beings in a matter of weeks. Changes induced by carbohydrates are smaller and are more difficult to demonstrate. The smaller magnitude in response to dietary carbohydrates imposes problems in demonstrating such changes in a statistically significant manner (Szepesi and Michaelis, 1986). Scientists and nonscientists who are used to the magnitude of changes that are seen with nutrient deficiency or excess in the case of vitamins, minerals, protein,

and lipids find it difficult to accept the biological significance of changes seen with the consumption of carbohydrates. Statistical constraints imposed on research with carbohydrates would require experimenters to use a large number of experimental subjects. This is often difficult, especially in human studies. Therefore, experimenters have opted to enlarge the response to carbohydrates by (1) selecting more responsive individuals and/or (2) increasing the amount of carbohydrate fed. By such techniques, the magnitude of the response can be enlarged and the time required to observe the response shortened. However, although such techniques are readily accepted with studies of other nutrients (including the feeding of large amounts of cholesterol to vegetarian rabbits to show arterial damage), the feeding of large amounts of simple sugars to either animals or humans has been criticized on numerous occasions (Bossetti et al., 1984).

The third difference relates to the interactive nature of the carbohydrate effects. Cohen and coworkers (Cohen et al., 1970; Yanko et al., 1972; Rosenmann et al., 1974) championed the hypothesis that the induction of type II diabetes in rats requires both a dietary vector (simple sugar) and heredity. Berdanier (1974) elucidated the concept of "carbohydrate sensitivity" (a subpopulation that has a greater response to carbohydrate) with the BHE rats. This concept was later generalized by Reiser and coworkers (Reiser et al., 1981, 1989; Hallfrisch et al., 1983) to humans. In these and numerous other studies, it was recognized that dietary carbohydrate strongly interacts with genetic predisposition. Dietary carbohydrates strongly interact with lipids as well. In the recommendations of the Senate Select Committee on Nutrition (U.S. Senate, 1973), recommendations were made to reduce the intake of dietary saturated fat, cholesterol, and simple sugars. The interactive nature of dietary lipid and carbohydrate was emphasized by an U.S. Food and Drug Administration (FDA) review that concluded that dietary sucrose is not an independent risk factor (Sugar Task Force, 1986). This chapter focuses primarily on the interaction of dietary carbohydrate and lipid.

II. GENERAL PHYSIOLOGICAL INTERACTIONS

A. Dietary Carbohydrate, Blood Lipids, and Lipoproteins

There is a well-known species difference in the utilization of glucose as a precursor of fatty acid synthesis. This difference should be kept in mind when evaluating the effect of dietary carbohydrates on blood lipids. In the pig, conversion of glucose to lipid takes place primarily in the adipose tissue; therefore, dietary carbohydrate should have very little effect on blood lipids. In humans, glucose is converted to fatty acids and cholesterol primarily in the liver and transported to the adipose tissue via the blood; thus dietary carbohydrate is expected to influence blood lipids. The rat uses glucose for lipid synthesis in both organs (Gandemer et al., 1983). The pig, then, is not appropriate as a model to study carbohydrate effects on blood lipids. Although one would not expect the rat to be used as a model of carbohydrate effects on blood lipids because of the relatively high-density lipoprotein (HDL), a number of studies indicate that even the rat responds to carbohydrate feeding. Feeding of sucrose (as compared to glucose) has been shown to increase triacylglycerol synthesis (Boogaerts et al., 1984), the plasma concentration of triacylglycerol, free cholesterol, cholesterol ester, and apolipoproteins (Boogaerts et al., 1984; Hostmark et al., 1984) as well as apolipoprotein synthesis (Boogaerts et al., 1984). Plasma triglyceride, very low density lipoprotein (VLDL) triglycerides, and total blood cholesterol were shown to be increased in Sprague-Dawley and lean Zucker rats, but not in obese Zucker rats (Sheehan et al., 1984). Other formulations of dietary sucrose produced elevated plasma triglyceride in the obese Zucker rat, and this was accompanied by an elevation of postheparin lipolytic activity (Basilico et al., 1984). Holtzman rats were shown to have increased liver lipids when fed a sucrose-glycerol diet (fat free) (Narayan, 1986). Dietary fructose by itself also elevates plasma triglyceride and increases insulin resistance (Thorburn et al., 1989). The two effects may be strongly connected (Haito et al., 1984). Postabsorptive serum cholesterol and phospholipid increase with age in the rat; food restriction reduces this trend (Liepa et al., 1980). The rat can therefore be used as an appropriate subject for study, but one must be careful in generalizing the phenomena observed in the rat to human beings.

A strong interaction between dietary carbohydrate and dietary cholesterol has been reported in cynomolgus monkeys (*Macaca fascicularis*) (Srinivasan et al., 1983, 1984). In the first study, sucrose produced an increase in total plasma cholesterol in the absence of added dietary cholesterol; this difference disappeared when cholesterol was added to the diet (Srinivasan et al., 1983). In the second study, in the absence of added dietary cholesterol, there was no carbohydrate effect on plasma cholesterol, and blood cholesterol in starch-fed rats was actually lower than in sucrose-fed rats when cholesterol was added to the diet (Srinivasan et al., 1984). Reports of contradictory trends are not uncommon in this field and are especially acute when considering human studies. The effect of dietary carbohydrates on blood lipids has been reviewed in detail (Sugar Task Force, 1986). Only a few relevant papers will be reviewed here to illustrate the controversy.

Dietary sucrose (Yudkin et al., 1980; Reiser et al., 1981; Hallfrisch et al., 1983; Yudkin et al., 1986) and fructose (Hallfrisch et al., 1983; Reiser et al., 1989) have been shown to increase blood lipids in hyperinsulinemic or even normal men. Some of the changes noted in other studies (Porikos and Van Italie, 1983) can be attributed to the ease of obtaining excessive calories when consuming sucrose, but a sucrose effect per se was also shown (Porikos and Van Itallie, 1983). Blood lipids were shown to increase when a high-carbohydrate, low-fat diet was fed to hyperglyceridemic men (Liu et al., 1983). Even in long-distance runners, a 30% increase in triglycerides was shown when such athletes were fed a high-carbohydrate diet (Thompson et al., 1984). The sucrose effect is reduced with the inclusion of dietary fiber (Albrink and Ullrich, 1986), exercise (Ullrich and Albrink, 1986), and nibbling (Gwinup et al., 1963). There are even more studies that show that inclusion of sucrose or fructose alters blood lipids (Sugar Task Force, 1986). Yet this conclusion is not accepted by a sizable portion of the scientific community. Dissent from the "sugar hypothesis" began early. A typical example (Bossetti et al., 1984) is a study of eight healthy volunteers receiving one third of their carbohydrate as either sucrose or fructose. No beneficial effects of fructose were found as compared to sucrose (Bossetti et al., 1984). Such a possible beneficial effect was suggested earlier (Brunzwell, 1978; Olefsky and Crapo, 1980), but the authors used regular food (and regular meals with sweetened drinks) with no starch control. Apart from arguments about the possible equivalence of sucrose and fructose, the effect of sucrose in humans is also challenged on various grounds. Size of meals, amount of carbohydrate fed, selection of subjects, and other food components besides carbohydrate are all factors cited to confound the data. Even the Framingham Study (Gordon et al., 1977) is cited by both sides to prove/disprove the possible role of sugar in heart disease. The final word on the subject seemed to have been the FDA report that concluded that sucrose consumption is not an "independent" risk factor (Sugar Task Force, 1986). Since that time, the subject has receded into obscurity.

Dietary carbohydrates can have diverse effects on lipid metabolism. Carbohydrate intake influence ketogenesis in the obese during weight loss (Pasquali et al., 1987) by a noninsulin-mediated process; carbohydrate can also alter blood lipid levels by lowering lipoprotein lipase (Haito et al., 1984; Lithell et al., 1985). A fructo-oligosaccharide was shown to reduce fasting blood glucose, total serum cholesterol, and low-density lipoprotein (LDL) cholesterol in diabetic subjects (Yamashita et al., 1984) at an intake level of 8 g/day per subject. It is very likely that polyfructose exerts its effect via the production of volatile fatty acids in the lower intestine, as do some other complex carbohydrates, rather than by providing fructose.

B. Thermogenesis and Energy Utilization

If there is a more controversial area of nutrition than the role of carbohydrates in heart disease, it is the role of thermogenesis in obesity. The problems are perhaps similar—relatively small effects, cumulative effects that take a long time to develop, confounding variables that are difficult to control, a relatively large number of scientists who cannot confirm the results. It is a common claim that obese patients find it difficult to maintain a desired body weight because of a low-energy requirement. Although many scientists dismiss such claims on various grounds, there is strong evidence to indicate that a twofold difference in food intake may exist in children (Widdowson, 1947)

and adults (Edholm et al., 1970), who nonetheless manage a similar amount of physical activity. The resting metabolic rate of obese and obese/reduced human subjects was found to be one third to one half that of normal-weight patients (who report that they can eat anything and still maintain normal weight) when heat production is stimulated by the infusion of norepinephrine (noradrenaline) (Jung et al., 1979). Brown adipose tissue (BAT) had been suggested as a major site of thermogenesis by the work of Foster and Frydman (1978, 1979). The "proton conductance" or "leakage pathway" (Heaton et al., 1978; Nicholls, 1979) is generally believed to be the mechanism by which BAT generates heat. Heat production by this mechanism involves the coupling of H^+ transport from ATP production in the mitochondria (Heaton et al., 1978). The uncoupling is prevented by purine dinucleotides (such as GDP) binding to the mitochondria via a 32-kDa protein (Heaton et al., 1978). Other processes, such as Na^+ transport and futile cycles (e.g., fatty acid turnover), may also be a source of heat production. This has been confirmed in "cafeteria-fed" animals, obese mice, and animals with ventromedial lesions (Trayhurn et al., 1982). However, although some experimenters failed to show a role for the sympathetic nervous system in BAT thermogenesis (Seaton et al., 1984), other experiments do imply a role for the nervous system in the regulation of BAT (Granneman and Campbell, 1984).

It has been known for many years that the ingestion of food is followed by the production of an increased amount of heat, the so-called thermic effect of food. As a percentage of energy given, carbohydrate has been reported to produce an average of 18% dissipation of the energy intake (13.8%–26.1%) in overfed men (Bisdee and James, 1984) compared to a 10% dissipation from men overfed fat (Dallosso and James, 1984). Jequier (1986) reported a glucose-induced thermogenesis in young adult equivalent to 12%–15% of the total carbohydrate load. This total thermic effect was divided into two parts: "obligatory thermogenesis" (due to the cost of carbohydrate storage) and "faculative thermogenesis" (due to other processes such as active transport and futile cycles) (Jequier, 1986). In another study, the thermic effect of a 500-g carbohydrate load was found (as percentage of the load) to be 5.2% from a high-fat diet, 6.5% from a mixed diet, and 8.6% from a high-carbohydrate diet (Acheson et al., 1984). Dietary fat, therefore, seems to reduce the utilization of carbohydrate (Acheson et al., 1984) and its thermic effect. The thermic effect of either a mixed meal or a high-fat meal is lower in obese humans (Swaminathan et al., 1985). Other experiments suggest that glucose is used as a primary fuel for exercise in the obese and that restriction of dietary carbohydrate in these subjects will reduce endurance (Bogardus et al., 1981). The foregoing reports would predict that a high fat/carbohydrate ratio would promote increased energy retention even in healthy individuals. However, a study with healthy Trappist nuns showed no significant difference in energy consumption or retention when the carbohydrate/fat ratio was varied (Van Stratum et al., 1978).

Overfeeding is a technique used to assess population variability and the ability of specific foods (or food in general) to promote weight gain. In an experiment that overfed young men diets high in fat, greater than expected variability was found in weight and fat gain (Norgan and Durnin, 1980). In another study, where carbohydrate was overfed (Welle et al., 1984), thyroid hormone status was altered (T_3 was increased and T_4 was reduced). Such changes indicate an adaptive mechanism to waste energy in normal subjects who are overfed, especially when such subjects are overfed diets rich in carbohydrate.

Another way to study this phenomenon is by feeding a "cafeteria diet" (Sundin and Nechad, 1983; Tulp and Shields, 1984; Bailey et al., 1985; Naim et al., 1985). Such diets have the composition of diets found in a cafeteria (hence the name) and are usually very palatable and tasty. LA/N-cp fats were shown to have an impaired adaptation to cold (Tulp and Shields, 1984). Lean LA rats had an improved thermic response to cold, and no improvement to cold adaptation was noted in the obese rats (Tulp and Shields, 1984). Cafeteria diets increased food intake even in normal rats (Naim et al., 1985). Dietary fat alone is enough to increase food intake (Naim et al., 1985). In addition, glucose was shown to induce a trophic response in the size and functionality of BAT but not lipids (Sundin and Nechad, 1983). The glucose effect can be suppressed by L-adrenergic blockade (chemical sympathectomy) (Sundin and Nechad, 1983). Finally, dietary glucose (but not fructose)

(Glick et al., 1984) can produce this trophic response in rats. Some of the effects may be confounded by other variables such as the protein/carbohydrate ratio (Kanarek et al., 1987) or the heating of food (Westringel and Potter, 1984).

In summary, dietary glucose produces a thermic response and a trophic response in BAT, the latter being dependent on the sympathetic nerves. Dietary fat produces less thermic response and may even reduce or eliminate the thermic response to glucose. Dietary fructose does not produce the trophic BAT response. The thermic response is reduced by obesity. Thus, the feeding of a cafeteria diet (high in fat and fructose, well-flavored, and palatable) can be lead to weight gain. These conclusions are based on solid data and make sense in terms of what is known about physiological adaptations. Negative evidence from some laboratories (i.e., those who cannot replicate the results) is unimpressive.

C. Other Interactions

Another type of interaction occurs between dietary fat and an organism with impaired glucose utilization, such as a diabetic rat. The response to long-chain triglycerides (LCTs) vs. medium-chain triglycerides (MCTs) is altered in diabetic rats (Edens and Friedman, 1984). Although LCTs are preferred by both the normal and diabetic rat, fat preference disappears when MCTs are fed (Edens and Friedman, 1984). Dietary cholesterol induces insulin resistance in mildly diabetic male rats (Wey et al., 1985) but not in females. Substitution of ω-3 fatty acids from fish oil for ω-6 fatty acids was shown to prevent the development of insulin resistance in noninsulin-dependent diabetic rats (Storlien et al., 1987). A three-way interaction between dietary fat, dietary carbohydrate and dietary cholesterol has also been reported (Meijer and Beynen, 1988). It has been recognized that the intake of fats, cholesterol, carbohydrate in general, sucrose (fructose), and dietary fiber, the health status and age of the individual, and exercise are all important factors that interact in various ways to alter carbohydrate and lipid metabolism (Jenkins, 1984). The FDA report (Sugar Task Force, 1986) on sucrose was, therefore, wrong to deemphasize the importance of sucrose in cardiovascular disease, because in a multifactorial disease, all risk factors are interactive. Whether risk factors are independent of interactive is or lesser importance.

In some species, such as predators, carbohydrate intake is low, and this has given rise to work with reduced carbohydrate diets. As reported in a previous section, interactive effects are sometimes different than expected: A high-protein diet reduces lipogenesis in the rat even if fat intake is low (Schmid et al., 1984), whereas butyric acid can alter the glycosylation of the α-subunit of the glycoprotein hormones secreted by glucose-starved Chang human liver cells (Morrow et al., 1983). High-protein diets, on the other hand, can reduce the lipotropic effect of sucrose (taken in as extra calories in the water).

The effect of dietary lipid on carbohydrate metabolism is strongly interactive with the periodicity of eating (Leveille, 1970; Szepesi and Freeland, 1971; Romsos and Leveille, 1972). Intermittent feeding induces a cycle of rapid glycogen synthesis and depletion (Leveille, 1970; Szepesi and Freedland, 1971) and a large variation in lipogenesis (Romsos and Leveille, 1972). The source of metabolic fuel (and hence the RQ) can change drastically (Leveille, 1970) during a 24-h period when eating is permitted only during a 1- or 2-h period. Under such circumstances, the effect of dietary lipid is expected to vary from hour to hour.

Finally, carbohydrate vs. fat effects may cause alterations that are not immediately evident unless these effects are the specific object of the study. For example, the effect of dietary fat in increasing insulin resistance was commented upon in a previous section. Such an effect may be due in part to (or is exaggerated by) the tendency of dietary lipid to enlarge fat pad size by enlarging the average size of fat cells (Madeiros et al., 1984). In such context, the dietary effect of increasing insulin resistance is not a direct effect on the membrane but an indirect effect due to cell enlargement and reduced insulin sensitivity.

III. CONTROL OF HEPATIC ENZYME ACTIVITY AND LIPOGENESIS BY DIETARY FAT

Although the interaction of dietary fat and carbohydrate is very controversial (especially the effect on blood lipids), surprising consensus exists on the subject of fat and carbohydrate interaction with regard to the control of lipogenesis and lipogenic enzymes. Early work showed that the reduction equivalents needed for reduction in fat biosynthesis are supplied by the pentose phosphate shunt and malic enzyme (ME) (Kather and Brand, 1975). This section, therefore, concentrates primarily on the control of glucose-6-phosphate dehydrogenase (G6PDH) and ME.

A. Source of NADPH and Its Influence on Metabolism

Glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) and ME (EC 1.1.1.40) are the major sources (along with 6-phosphogluconate dehydrogenase [6PGDH, EC 1.1.1.44]) of the reducing power needed for hepatic fatty acid synthesis (Kather and Brand, 1975). A fourth NADP-linked dehydrogenase of the liver, isocitrate dehydrogenase, has only a minor role in the production of NADPH.

The G6PDH reaction involves the orderly addition of NADP and glucose-6-phosphate (in that order) and the sequential release of the endproducts (NADPH last) (Thompson et al., 1976). NADPH does not bind to the enzyme (Holten et al., 1976); this property was used to prepare NADP-free G6PDH (Holten et al., 1976). The enzyme cannot bind glucose-6-phosphate without NADP (Holten et al., 1976). The NADPH/NADP ratio regulates G6PDH and 6PGDH activity (Eggleston and Krebs, 1974), so the combination of enzyme induction and change in the NADPH/NADP ratio can alter enzyme activity by a factor of 30 (Holten et al., 1976). The NADPH/NADP ratio (decrease) and an increase in GSSG concentration could both activate the pentose phosphate cycle (Henderson, 1966). Paraquat can also activate the cycle (Fabregat et al., 1985). Current consensus is that any pathway or physiological effect that maintains a high NADPH/NADP ratio has an inhibitory effect on the pentose phosphate cycle and, conversely, that any pathway or reaction that reduces the ratio accelerates the pentose phosphate cycle.

B. Regulation of the Fatty Acid Synthetase Complex by Dietary Lipid

Fatty acid synthetase is a very large dimer, and each monomeric subunit catalyzes all the intervening reactions of adding one two-carbon unit to the growing fatty acid. When the fatty acid reaches the length of 16 carbons, it is cleaved off. This is an excellent example of concerted catalysis. During pregnancy, lactation, and weaning, the activity of the enzyme varies widely (Gimenez et al., 1984). Newly synthesized monomeric units may be inserted one by one (Strawser and Larrabee, 1976). The enzyme is induced by the feeding of carbohydrate [especially sucrose; Bruckdorfer et al. (1972)] and is decreased by a high-fat diet (Sabine et al., 1969). The effect of dietary fat is due to the polyunsaturated fats in the diet (Clarke et al., 1977; Flick et al., 1977), such as linoleic acid.

C. Regulation of G6PDH by Dietary Lipid

Rat liver G6PDH has been extensively studied for a number of reasons. The human gene for the enzyme is involved in a number of mutations (Szabo et al., 1984), and liver G6PDH is believed to be an important factor in the control of lipogenesis and hence blood lipids. The importance of the enzyme is underscored by the fact that its control parallels the nature of dietary and biological factors (Szepesi, 1976; Szepesi and Michaelis, 1986): Factors that tend to increase the level of liver G6PDH are believed to increase the risk of disorders in lipid metabolism, whereas factors that reduce G6PDH levels tend to reduce disorders in lipid metabolism and the health risks associated with them. Rat (Matsuda and Yugari, 1967) and mouse (Hizi and Yagil, 1974) liver enzymes have

been purified, and the amino acid sequence of the human enzyme (Persico et al., 1986; Takizawa et al., 1986), the sequence of G6PDH from fruit fly (Fouts et al., 1988), and a partial sequence of the rat liver enzyme (Jeffery et al., 1988) have been published. A cDNA clone for the rat liver enzyme was reported (Kletzien et al., 1985). This study indicated only pretranslational control (Kletzien et al., 1985). Antibody precipitation of the enzyme gave data that failed to indicate the existence of an inactive to active enzyme conversion with either G6PDH (Peavy and Hansen, 1975) or 6PGDH (Hutchison and Holten, 1978). Levels of these enzymes undergo adaptive changes with age (Wang and Mays, 1977), as do those of a number of other enzymes (Greengard and Jamdar, 1971). The possible role of Iysosomal fragility in regulating enzyme levels has been suggested (Schroeder et al., 1976).

Dimeric and tetrameric forms of G6PDH from human red blood cells were prepared and were found to show a different pattern of binding to NADP (Bonsignore et al., 1970). The existence of the many variants of human G6PDH gave rise to a vigorous effort to identify possible isozymes of rat liver G6PDH (Schmukler, 1970; Holten, 1972; Watanabe et al., 1972; Martins et al., 1985; Angel and Back, 1986). At least six bands were found in electrophoresis of the native enzyme (Holten, 1972). A glutathione-mediated conversion (Watanabe et al., 1972) of the electrophoretic bands, a preferential induction of certain isozymes (Martins et al., 1985), and a possible role of the isoenzymes in the turnover of the enzyme (Grigor, 1984) were suggested. The identity and role of these isoenzymes, however, might be a more difficult problem than was first believed. There was early evidence of enzymes that inactivate G6PDH by removing NADP plus a third enzyme that may also inactivate G6PDH *in vitro* (Bonsignore et al., 1968). A low molecular weight inactivator of G6PDH has been found in some leukemic cells (Kahn et al., 1976). And the isolation of a nascent form of G6PDH suggests processing of the enzyme (Cummings and Barker, 1986). Experiments in our laboratory indicated that G6PDH prepared from starved rats refed a high-fat diet was less stable *in vitro* than rats refed a low-fat diet (Thimaya and Szepesi, 1986). Calculations of molecular weight from electrophoretic mobility showed that most of the isoenzymes had a molecular weight between 58,000 and 62,000 (monomeric), whereas one to three bands had a molecular weight of 122,000 (dimeric) (Thimaya and Szepesi, 1986). In spite of our taking numerous precautions to repeat the experiment in exactly the same way, the number of isoenzymes we found varied from three to seven; at times bands split into closely spaced doublets, and at other times bands may have been missing altogether, although a minimum of three bands could always be found. Molecular exclusion with a Sephadex 100 column indicated that almost all of the freshly prepared enzyme was at least in the dimeric form (Thimaya and Szepesi, 1986). This would suggest that the production of monomeric units is an artifact of the electrophoretic process. This was also suggested by the fact that when the electrophoresis gel was turned 90° and current was applied, the dimeric band split into monomers (Thimaya and Szepesi, 1986). Finally, in starved rats, we found an odd-sized G6PDH band that moved more slowly than the dimer and may have been a precursor G6PDH (Thimaya and Szepesi, 1986).

The control of G6PDH has been studied in great detail. Much of the work involved the use of the starvation-refeeding regimen (Tepperman and Tepperman, 1958). Most of the early work was previously reviewed (Szepesi, 1976). Starvation-refeeding produces a profound change in the activities of NADP-linked dehydrogenases of rat liver. It was noted that G6PDH, 6PGDH, and ME seemed to vary together (Glock and McLean, 1955; Tepperman and Tepperman, 1958; Fitch and Chaikoff, 1960; Weber and MacDonald, 1961; Potter and Ono, 1962) under a wide variety of conditions; therefore, it was proposed that these changes were "coordinate" (Pette et al., 1962). It was shown, however, that G6PDH and ME respond differentially to thyroxine (Tepperman and Tepperman, 1964); ME is induced to a greater extent than G6PDH, especially in rats fed a high fat diet. In hypophysectomized rats, the starvation-refeeding episode failed to produce the expected coordinate induction; this was restored if the animals were injected with somatotropin, thyroxine, and cortisone (Tepperman and Tepperman, 1962). Later work showed that the actual hormone requirement was insulin and glucocorticoid (Williams and Berdanier, 1982), with a possible interaction between the glucocorticoid and thyroxine (Nessmith et al., 1983). Glucagon inhibits the induction (Hahn and

Kirby, 1974; Rudack and Holten, 1975). The role of thyroxine was suggested to be an antagonizing effect of dietary PUFA (Szepesi and Thimaya, 1989). The PUFA effect will be examined shortly. The induction of the enzyme was shown to be due to an increase in the rate of synthesis (rather than activation of an inactive form) in both the liver (Rudack and Holten, 1975; Winberry and Holten, 1977) and free hepatocytes (Morikawa et al., 1984). Free hepatocytes in culture were shown to have a 12-fold higher G6PDH mRNA from refed rats than free cells from fasted rats (Stumpo and Kletzien, 1984). These same studies suggested that glucocorticoid alone can elevate the level of mRNA without increased synthesis of the enzyme; hence the role of insulin is presumed to be in translation (Stumpo and Kletzien, 1984). Glucose and ethanol also increased G6PDH synthesis in free cells (Kelley and Kletzien, 1984) by increasing specific mRNA levels (Stumpo and Kletzien, 1985). In these studies, it was concluded that the role of glucocorticoid is permissive and amplifies the induction produced by insulin.

The picture that emerges from the work cited in the previous paragraph explains the fundamentals of G6PDH induction, but it may be incomplete. The question that can be raised is this: Is there another factor essential for the induction of G6PDH by the starvation-refeeding regimen? Previously published work indicates that there is (Szepesi, 1976). One possibility, the substrate induction hypothesis (Sassoon et al., 1973), proposed that carbohydrate per se was the inducer. This hypothesis was later elaborated as follows: Upon refeeding, a very large increase in liver glycogen occurs during the first day of refeeding. Figure 25.1 shows the time course of events in a typical starve-refeed experiment (figure drawn from previously published data; see Szepesi, 1976). When liver glycogen reaches a high enough level, glycogenolysis is triggered, which in turn elevates the level of hepatic glucose-6-phosphate. This in turn induces G6PDH synthesis and fat synthesis. Both the enzyme activity and total liver lipid overshoot normal levels (enzyme overshoot). As the level of hepatic lipid reaches a triggering concentration, enzyme synthesis and lipid synthesis begin to fall until normal levels of hepatic glycogen (2 days), lipid, and G6PDH are established (the latter two in about a week). To establish such a cause and effect relationship, one has to show two things: (1) that the accumulation of a large amount of liver glycogen will trigger the enzyme overshoot and (2) that the enzyme overshoot can occur only with (and subsequent to) an overshoot of the normal level of liver glycogen (such as occurs on day 1 or refeeding; see Figure 25.1). The first condition is violated

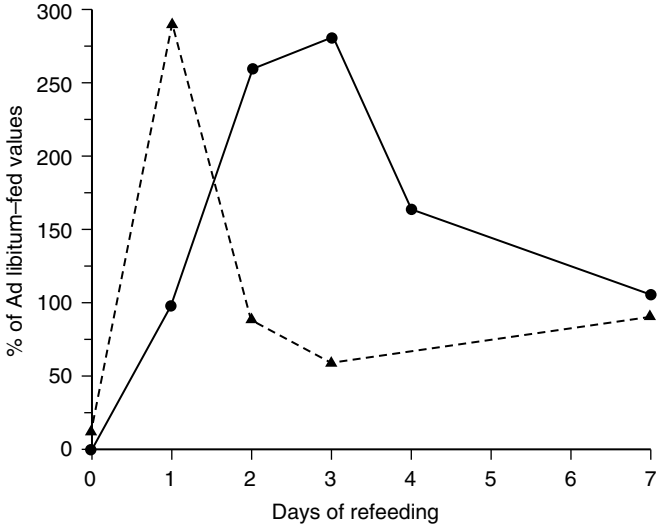

FIGURE 25.1 Time course of changes in liver glycogen and glucose-6-phosphate dehydrogenase. Rats were starved 2 days and refed a 5% corn oil diet (65% glucose + 25% casein) for the number of days indicated. (•) G6PDH; (▲) liver glycogen.

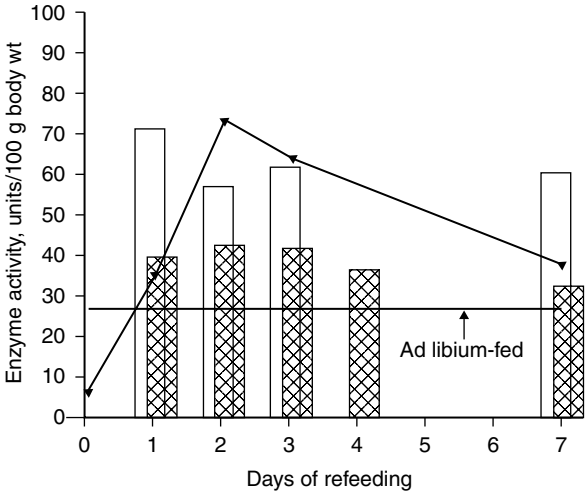

FIGURE 25.2 Requirement for starvation per se as the inducing (potentiating) stimulus for the enzyme "overshoot" of G6PDH upon refeeding an inducer diet. Three experiments are contrasted. Experiment 1 (▼): Rats were starved 2 days and refed the inducer diet (65% glucose, 25% casein, 5% corn oil) for the number of days indicated. Experiment 2 (▼): Rats were starved 2 days, refed a 90% casein, 5% corn oil diet for the number of days, then given the induced diet for 3 days. Experiment 3 (▼): Rats were given the high-protein diet for the number of days indicated (without prior starvation) and then fed the inducer diet for 3 days. The difference between Experiments 2 and 3 shows that starvation is required to potentiate the overshoot. An abnormally high level of liver glycogen was observed on the first day of feeding the inducer diet in all three experiments.

by data shown in Figure 25.2 (Szepesi and Moser, 1971). If starvation is followed by refeeding, a high-protein diet, G6PDH only returns to normal; when such rats are fed an inducer diet, the overshoot occurs even if the high-protein regimen is interposed for up to a week (Szepesi and Moser, 1971). No such overshoot occurs if rats fed ad libitum are fed a high-protein diet and then the inducer diet; yet the glycogen overshoot on day 1 of feeding the inducer diet is the same (Szepesi and Moser, 1971). Therefore, the enzyme overshoot is not a direct consequence of the glycogen accumulation. The second condition is violated by the presence of a G6PDH overshoot in starved-refed rats that are made hyperthyroid by the injection of thyroxine (Liao and Szepesi, 1980). What may happen is that the influx of extra carbohydrate during day 1 is deposited as excess glycogen, which later is converted to lipid when lipogenesis is driven by excess capacity to make NADPH. The isotope data confirm this (Chang and Johnson, 1976). Equally important is the fact that it is starvation that potentiates the overshoot, and that even if the G6PDH level is normalized (by a 90% protein diet), the overshoot occurs after the inducer diet is fed even after the interposition of the high-protein diet for as long as 7 days (Szepesi and Moser, 1971). When starved-refed rats are starved and refed a second time, the level of G6PDH climbs even higher (Szepesi et al., 1973). This extra induction has been referred to as an "induction increment" (Szepesi, 1976). It can be shown even after the G6PDH level returns to normal following the first refeeding. In fact, even if 21 days are interposed between the first and second refeeding, the induction increment is still present (Szepesi, 1976). The nature of this potentiating effect of starvation is not known, but it is not due to the sudden withholding of a single dietary component but to the drop in caloric intake (Szepesi, 1973). The "metabolic memory" of starvation can be erased by feeding a high-fat diet (Szepesi et al., 1973). In this case, if the high-fat diet is fed during the first refeeding, no overshoot occurs. And if the high-fat diet is interposed between two starvations, the rat responds to the second starvation-refeeding as if it were starved-refed for the first time (Szepesi et al., 1973) (i.e., no induction increment). Interestingly, this latter effect of a high-fat diet is inhibited by injections of 8-azaguanine, indicating that the effect of

the fat diet itself (like the enzyme induction) requires de novo RNA synthesis (Szepesi et al., 1973). The effect of dietary fat (i.e., the erasure of the metabolic memory of starvation) occurs during day 2 of refeeding (Szepesi et al., 1974b).

The role of dietary fat in controlling the activity of G6PDH and ME has long been recognized (Hill et al., 1958; Niemeyer et al., 1962; Tepperman and Tepperman, 1963, 1965; Vaughn and Winder, 1964; Leveille, 1967a,b; Novello et al., 1969; Bartley and Abraham, 1972; Century, 1972; Gozukara et al., 1972; Slayton and Szepesi, 1973; Wiley and Leveille, 1973; Szepesi et al., 1974a; Nace and Szepesi, 1975, 1976, 1983; Williams et al., 1977a,b). These studies included work with the liver (Niemeyer et al., 1962; Tepperman and Tepperman, 1963, 1965; Vaughn and Winders, 1964; Novello et al., 1969; Bartley and Abraham, 1972; Century, 1972; Gozukara et al., 1972; Slayton and Szepesi, 1973; Wiley and Leveille, 1973; Szepesi et al., 1974a; Nace and Szepesi, 1975, 1976, 1983; Williams et al., 1977a,b; Berdanier et al., 1989), adipose tissue (Leveille, 1967a,b; Wiley and Leveille, 1973), starvation-refeeding (Niemeyer et al., 1962; Tepperman and Tepperman, 1963; Vaughn and Winders, 1964; Novello et al., 1969; Bartley and Abraham, 1972; Slayton and Szepesi, 1973; Szepesi et al., 1974a; Nace and Szepesi, 1975, 1976), ad libitum feeding (Tepperman and Tepperman, 1965; Leveille, 1967a,b; Century, 1972; Gozukara et al., 1972; Wiley and Leveille, 1973), and meal feeding (Leveille, 1967a,b). In all cases, the levels of liver G6PDH and ME were reduced by the inclusion of dietary fat. Although in a number of cases the fat used was derived from natural sources, it was nonetheless clear (or at least strongly indicated) that the fats responsible for this effect were polyunsaturates (Tepperman and Tepperman, 1965; Bartley and Abraham, 1972; Century, 1972; Gozukara et al., 1972; Wiley and Leveille, 1973; Nace and Szepesi, 1975, 1976; Williams et al., 1977a,b). Our own studies indicated that (1) synthetic triglycerides containing only saturated fats were very poorly absorbed and (2) even when absorbed, they were ineffective in reducing G6PDH and ME levels (Nace and Szepesi, 1975). MCTs (C_8 and C_{10}) acted like LCTs and actually increased adipose tissue enzymes (Wiley and Leveille, 1973). Triglycerides containing single fatty acids (8:0, 10:0, 16:0, 18:0) had no lowering effect on rat liver G6PDH and ME, whereas triglycerides containing the single fatty acids 18:2 and 18:1 did lower both enzymes (Nace and Szepesi, 1977). Linoleic acid was more effective in this than oleic acid (Musch et al., 1974; Nace and Szepaesi, 1977). In starved-refed rats, trilinolein was found to decrease liver G6PDH exponentially and ME linearly (Nace et al., 1979). When these enzymes were increased by feeding a fat-free diet for 7 days, the trilinolein given was not effective in lowering liver enzymes during the first 2 days (Nace et al., 1979). Adipose tissue enzymes were slightly reduced in starved-refed rats (Nace et al., 1979). Linolenic acid and linoleic acid were equally effective in preventing an overshoot (Szepesi et al., 1989). Comparison of menhaden oil to corn oil in Sprague-Dawley and BHE rats indicates that the PUFAs of fish oils may be even more effective in lowering G6PDH and ME levels than linoleic acid, although this effect is strain dependent and is reduced in sucrose-fed rats (Berdanier et al., 1989).

It was already pointed out that starvation itself may be the potentiating stimulus for the enzyme induction that occurs during refeeding (Szepesi, 1973). As the length of starvation increases, so does the subsequent enzyme induction (Szepesi et al., 1971). Maximum enzyme overshoot can be produced if starved rats are refed a fat-free diet (Szepesi and Kamara, 1986). This would indicate that more than one effect of dietary PUFAs may be at work and that some endproduct of PUFA metabolism, or some product that contains PUFA, may be destroyed during starvation and resynthesized during refeeding (Szepesi, 1976). There have been efforts to ascertain the identity of such a possible compound. It was found that eicosa-5,8,11,14-tetraynoic acid inhibited the induction of fatty acid synthetase, G6PDH, and ME (Szepesi, 1976; Clarke and Clarke, 1983). From this it was inferred that conversion of 18:2 to 20:4 was required. However, since both linoleic and linolenic acid do equally well in reducing the starve-refeed response of G6PDH and ME (Szepesi et al., 1989), a single specific metabolic product as the effector is unlikely. In addition, some metabolic inhibitors of arahcidonic acid metabolism failed to prevent the PUFA effect (Szepesi et al., 1989).

There are also indications that dietary PUFAs may have a specific effect on G6PDH to destabilize the enzyme and perhaps even interfere with its processing (Nace, 1979). We had purified

G6PDH and ME and prepared specific rabbit antibodies against each enzyme. (In cooperation with R.H. Hansen, Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis.) Precipitation of ^{14}C-Leu-labeled enzyme from liver of rats refed high-fat or low-fat diets was carried out, and the resultant precipitates were electrophoresed with sodium dodecylsulfate (SDS); the gels were sliced into 2-mm pieces and counted. Figure 25.3 shows the labeling pattern of G6PDH obtained from several treatments. It is clear from this figure that in groups that are starved and refed a low-fat diet, most of the label is recovered in a narrow band in the gel that represents the approximately 62-kDa monomer of G6PDH, as expected. However, in groups refed the high-fat diet, a great deal of the label is found in areas outside of the monomeric G6PDH. Furthermore, the counts show up not only to the right of the monomeirc peak (which represents fragments smaller than the 62-kDa subunit) but also to the left of this peak. This would imply two things: (1) the high fat somehow destabilizes the enzyme and (2) the high-fat diet somehow interferes with the processing of a pre-G6PDH peptide larger than 62 kDa. Incubations of homogenates prepared from rat liver (obtained from identical treatments) indicated a much faster disappearance of G6PDH activity from rats fed a high-fat diet (Szepesi and Safir, 1983). Since the double-antibody precipitation technique employed when these experiments were done required 2- to 3-day incubation periods (Nace, 1979), it cannot be determined now if the presence of counts obtained outside of the monomeric subunit in the SDS gel were due to degradation *in vivo* or *in vitro*. However, there is little doubt that G6PDH obtained from rats fed the high-fat diet was less stable. This conclusion is bolstered by data shown in Figure 25.4. ME, when treated in a manner identical to the procedure used with G6PDH, did not significantly fragment, nor did it show a significant fraction of counts in fragments larger than the monomeric subunit.

More recent attempts to unravel to control of liver G6PDH have used rat hepatocyte monolayers (Salati et al., 1988) or a primary culture of mouse hepatocytes (Hillyard et al., 1988). The usefulness of such data depends on how much of the *in vivo* influences can be reproduced *in vitro*. What recommends such a procedure is the relative ease with which substrate and hormone levels can be changed independently of each other and the absence of secondary adaptations to such changes that could confound the response. The drawback is that *in vitro* simulation of the transport of lipid molecules to individual cells is chancy at best. In addition, the modifying effects of various tissues on the nature and amount of lipoproteins that may reach a particular cell (such as a liver cell) may be significant. In rat hepatocyte monolayers, inclusion of glucose caused a 500-fold increase in G6PDH activity (Salati et al., 1988). There was an additional twofold increase in the enzyme level when insulin as well as glucose was added to the medium. Dexamethasone or T_3 had no additional effect when added with insulin Linoleate added to the medium had no effect on enzyme induction when glucose, dexamethasone, or insulin was added singly. However, linoleate reduced enzyme induction (by about 30%) when both dexamethasone and insulin and glucose were added. With insulin and glucose both added, arachidonate caused an almost complete inhibition in the increase of G6PDH activity (Salati et al., 1988). The *in vitro* system appears to reproduce a number of features of G6PDH inductions (Szepesi, 1976).

The requirement for carbohydrate, the portion of the induction that cannot be blocked by dietary fat (i.e., the reaching of enzyme levels comparable to rats fed ad libitum a low-fat, high carbohydrate diet), and the partial inhibition of induction by linoleic acid (i.e., the inhibition of the enzyme overshoot) are all features that are qualitatively similar between the *in vitro* work (Salati et al., 1988) and the *in vivo* changes (Szepesi, 1976). The requirement for dietary protein was not demonstrated, and the effect of glucocorticoid and T_3 were not as important in the *in vitro* system as reported for the *in vivo* induction (Williams and Berdanier, 1982).

The report with adult mouse hepatocytes is even more puzzling (Hillyard et al., 1988). In these studies, G6PDH activity declined steadily during the experiment, and this decline was not affected by the addition of 18:1, 18:2, or 20:4. Although the authors reported an inhibition of the Δ9-desaturase activity, their conclusion was that the mechanism for long-term regulation of lipogenic enzymes may not be contained entirely in the liver (Hillyard et al., 1988). Another explanation may be that

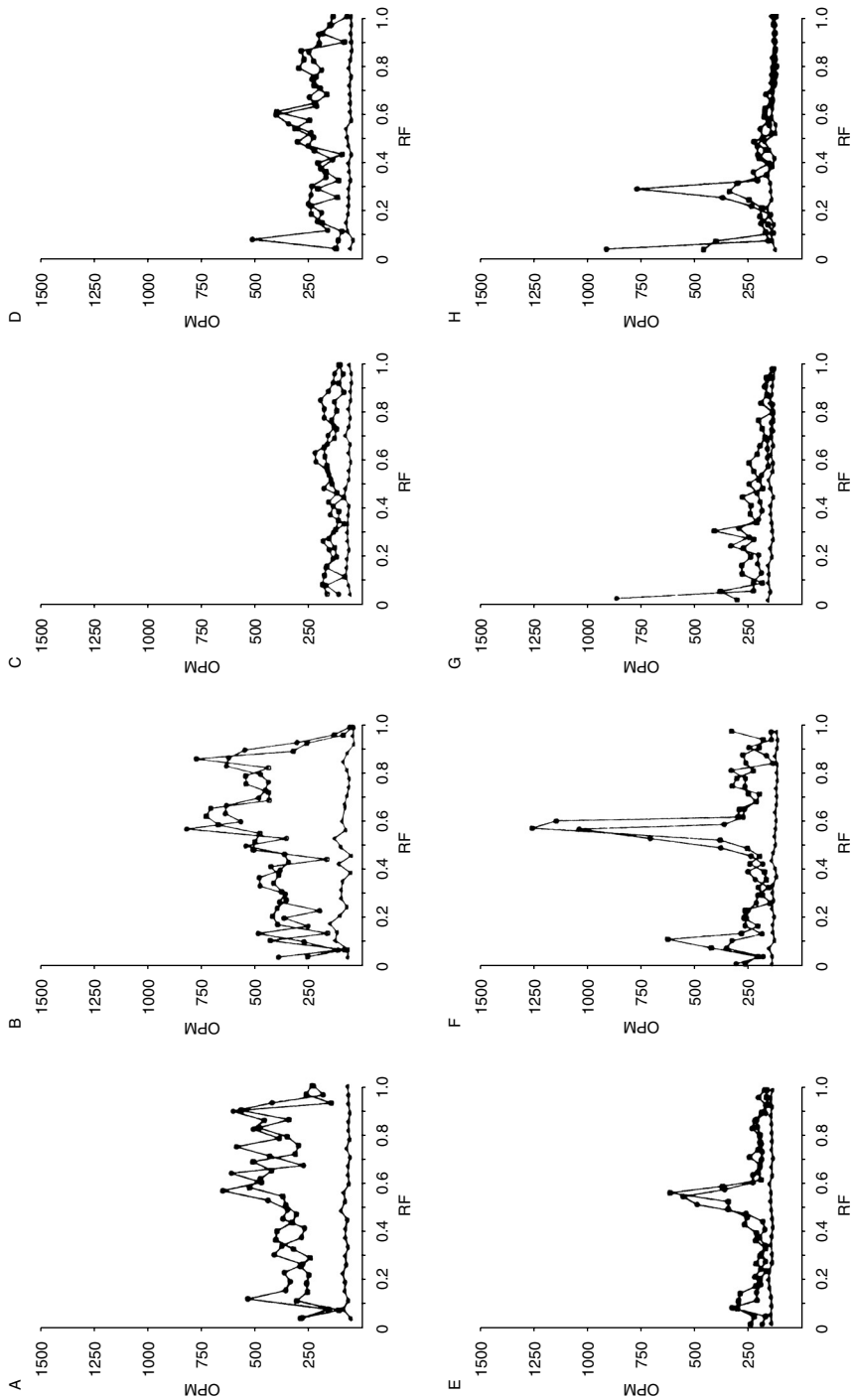

FIGURE 25.3 SDS-gel electrophoresis of rat liver glucose-6-phosphate dehydrogenase. Precipitates of rat liver homogenates with control serum (triangles) or duplicate samples using serum from immunized rabbits (squares and octagons) were treated with SDS and electrophoresed; the gel was cut into 2-mm sections and counted. The capital letters refer to treatments: A–D, starved-refed; E–H, ad libitum fed. Groups A, B, E, and F were fed the high-fat diet; C, D, G, and H, the fat-free diet. Glucagon was injected in groups A, C, E, and G.

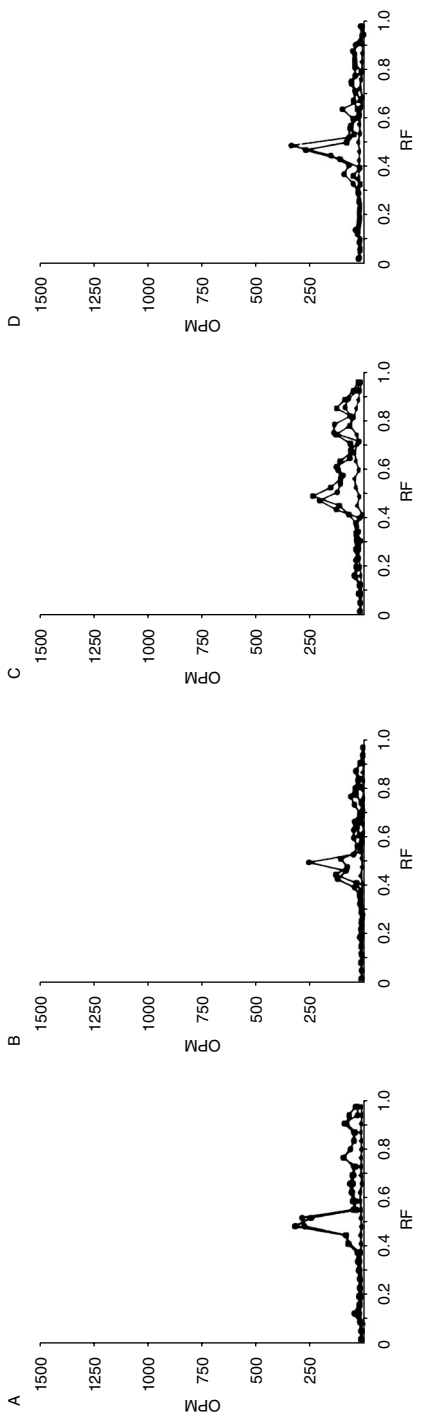

FIGURE 25.4 SDS-gel electrophoresis of rat liver malic enzyme. Precipitates of rat liver homogenates with control serum (triangles) or duplicates samples using serum from immunized rabbits (squares and octagons) were treated with SDS and electrophoresed, and the gel was cut into 2-mm sections and counted. The capital letters refer to treatments: A–D, starved-refed; E–H, ad libitum fed. Groups A, B, E, and F were fed the high-fat diet; C, D, G, and H, the fat-free diet. Glucagon was injected in groups A, C, E, and G. (From Nace, C.S. (1979). Dietary and Hormonal Regulation of Glucose-6-Phosphate Dehydrogenase and Malic Enzyme in Rat Liver and Adipose Tissue, PhD dissertation, University of Maryland, College Park, MD.)

when cultured *in vitro* the mouse liver cells lose their *in vivo* control functions or that elements necessary for the expression of the controls are missing. It is quite clear, however, that the influx of carbohydrate into the liver is a major regulator of hepatic lipogenesis and that this regulation is strongly affected by the amount and type of dietary lipid.

The control of G6PDH may respond to different stimuli in different organs. Adipose tissue G6PDH is not induced by starvation-refeeding, nor is it inhibited by dietary PUFAs (Szepesi, 1976). Uterine G6PDH may even have entirely different controls: Estrogens and NADP may be inducers (Smith and Barker, 1974), and some estrogens act as translational and posttranslational modulators (Donohue and Barker, 1983).

D. Regulation of Malic Enzyme by Dietary Lipid

As with G6PDH, there have been waves and cycles of interest related to ME. Of the *Escherichia coli* mitochondrial enzymes, only the NADP-linked enzyme has a role in lipogenesis (Murai et al., 1971). ME (NADP-linked) has been isolated not only from rat liver (Isohashi et al., 1971; Stark et al., 1975; Yeung and Carrico, 1976) but also from adrenal cortex (Sauer, 1973; Mandella and Sauer, 1975; Pfeiffer and Tchen, 1975), bovine brain (Frenkel and Cobo-Frenkel, 1973), bovine heart (Frenkel, 1971), rabbit heart (Lin and Davis, 1974), pigeon liver (Hsu and Lardy, 1967; Hsu et al., 1976), and other tissues. Other than the production of NADPH, the bacterial enzyme has a physiological role in the production of acetyl CoA (Murai et al., 1971), and it has been suggested that the rat liver enzyme may participate in glutathione-dependent dehydrogenases (via producing NADPH) (Stark et al., 1975). The NAD-dependent enzyme is involved in the oxidation of malate in both the liver and the adrenal (Sauer, 1973). The earliest studies indicated that Ca^{2+}, Mg^{2+}, or Mn^{2+} is required in the adrenal enzyme (Lin and Davis, 1974). Mg^{2+} and Mn^{2+} were also identified as cofactors for pigeon liver (Hsu, 1970; Hsu et al., 1976). Eventually, it came to be recognized that the decarboxylating-reductive function of rat liver ME is dependent on Mn^{2+}. The ability of thyroxine (T_4) and T_3 (Wise and Ball, 1964) to induce ME was recognized relatively early (Tepperman and Tepperman, 1964; Hemon, 1968). The isoenzymes of ME have not been of as much interest as that of G6PDH. However, mouse ME from the heart is mitochondrial, whereas that from the liver is cytosolic, and mutations are more likely to affect the liver enzyme than the heart enzyme (Henderson, 1966). Numerous laboratories (including our own) purified the enzyme and were able to show that (1) rat liver enzyme level changes are accompanied by changes of titratable protein (Isohashi et al., 1971; Frenkel, 1974; Le et al., 1975) and (2) the liver enzyme is immunologically different from the mitochondrial enzyme (Isohashi et al., 1971).

The control of rat liver ME is similar to the control of rat liver G6PDH with some differences (Tepperman and Tepperman, 1964; Frenkel et al., 1972). The ME level is increased by T_4 (Tepperman and Tepperman, 1964; Reed and Tarver, 1976) and by a low-protein diet (Frenkel et al., 1972) and is reduced by a high-fat diet (Tepperman and Tepperman, 1964). Premature weaning increases rat liver ME (Back et al., 1985). This increase is not suppressed by dietary fat (Angel and Back, 1985; Back et al., 1985), but glucagon inhibits the ME increase due to premature weaning. Hydrocortisone or insulin alone or together failed to invoke the adaptive increase in ME before weaning (Back et al., 1985). Diabetes (one dose streptozotocin) greatly reduces the enzyme's response to T_3, although the rats respond better when fed fructose (Kaiser et al., 1980).

Significant efforts have been expended to study the control of ME in several species using antibody precipitation and sometimes free cells. Some of the earliest of these studies were preformed using *in vivo* labeling of ME in chick liver (Silpananta and Goodridge, 1971). This study showed that the level of this enzyme was decreased by starvation and increased by refeeding and that all the changes in enzyme activity were paralleled by equal changes in antibody-precipitable protein (Silpananta and Goodridge, 1971). During starvation in a chick less than 1-week-old enzyme synthesis decreased along with the rate of ME degradation; both tendencies were reversed during refeeding (Silpananta and Goodridge, 1971). The controls of ME synthesis and degradation, therefore, impose

some limits on the magnitude of the changes. The enzyme's half-life varied with the age of the chicks: in 8- and 11-day-old chicks, $t_{1/2}$ was 55 h in the fed and 28 h in the fasted animals (Silpanata and Goodridge, 1971). Increased fatty acid synthesis upon refeeding precedes changes in ME activity by 1.5–3.0 hr (Silpanata and Goodridge, 1971). Messenger RNA is reduced by starvation and increases following refeeding in ducklings (Goldman et al., 1985). The half-life of ME-specific mRNA was 3–5 h in the fed and 1 h in the starved animals (Goldman et al., 1985). Measurement of the nuclear accumulation of mRNA showed that starvation actually reduced ME–specific mRNA transcription in a specific manner without affecting the transcription of the albumin gene (Goldman et al., 1985).

Whereas starvation-refeeding seems to increase transcription of the ME gene, one of the effects of dietary fat may operate by a different mechanism. Replacement of polyunsaturated fat with saturated fat in the diet of mice was shown to increase the amount of immunologically titratable ME without increasing the mRNA (Schwartz and Abraham, 1983). This effect of PUFA, therefore, seems to be a translational effect. The effect of a high-carbohydrate diet fed ad libitum is to elevate the level of translatable ME–specific mRNA without increased transcription by reducing mRNA degradation (Dozin et al., 1986).

IV. CONCLUSION

The interactions of dietary lipids and carbohydrates can be divided into two types: (1) those interactions that are observable on lipogenesis, the control of lipogenesis and lipogenic enzymes; and (2) the effects on blood lipids. Within this context, there is interaction with age, sex, and genetic predisposition. Animal experiments *in vivo* and *in vitro* have produced a relatively uniform conclusion, but the results of human studies are controversial. Considering the number of interactions possible, it is expected that some effects may tend to be masked by interactions and vice versa. In this light, the conclusion of the FDA report that, because dietary sucrose consumption is not an independent risk factor (Sugar Task Force, 1986). It need not be regarded as an important risk factor, is simply not valid. There is no statistical justification for the idea that only independent risk factors should be recognized.

It is true in a number of areas of nutrition that genetic predisposition is an important factor in determining the risk of various diseases that have a nutritional vector. It is therefore incumbent on those who conduct human studies to identify subpopulations in high-risk groups. The lumping together of a group of healthy human beings (such as healthy college students) for the purpose of studying the effects of dietary carbohydrates does not and cannot yield results that are useful for determining metabolic risk in other groups such as those with impaired glucose tolerance. In addition, claims that groups with already proven arterial damage (such as diabetic individuals) no longer suffer demonstrable problems from the consumption of simple sugars are disingenuous, because they ignore the basic nature of carbohydrate effects, which are long term and interactive. The purpose of this chapter is not to reignite the controversy or revisit the battleground, but to emphasize the interactive nature of the effects of dietary lipids and carbohydrate and lipid metabolism.

The nature of these interactions is becoming clear. Dietary fat in general tends to limit the production of lipid from carbohydrate, probably by affecting acetyl CoA carboxylase. Polyunsaturated fat, on the other hand, affects the synthesis of the enzymes necessary for lipogenesis, notably G6PDH and ME. I suggested years ago that the PUFA effect is partly transcriptional and partly translational (Szepesi, 1976); this has been clearly demonstrated by others. The requirement for dietary carbohydrate and protein in the starvation-refeeding experiment to induce G6PDH remains to be explained. The precise role of glucose and insulin likewise remains to be explored. As the *in vitro* methods are refined and validated, this will undoubtedly be accomplished. Although the role of PUFAs in helping to prevent arterial disease has been generally accepted (Dyerberg, 1986), the opposite role of the consumption of simple sugars remains in doubt. A new effort is necessary, therefore, to reexamine this problem. Hopefully, with recent advances in methodology, we shall get some more definitive answers.

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26 Reappraisal of the Essential Fatty Acids

Robert S. Chapkin

CONTENTS

I. INTRODUCTION

The essentiality of fat in the diet has been known since 1929 (Burr and Burr, 1929, 1930), when it was first recognized that linoleic acid (Δ9,12-octadecadienoic acid, 18:2n-6), and possibly other acids, is an essential fatty acid (EFA). Since this initial observation, there have been numerous published studies documenting the fact that certain polyunsaturated fatty acids (PUFAs) cannot be synthesized de novo from endogenous precursors and are, therefore, essential dietary elements (Alfin-Slater and Aftergood, 1968; Holman, 1971; Rivers and Frankel, 1981). Indeed, it is now recognized that there are two EFAs, 18:2n-6 and α-linolenic acid (Δ9,12,15-octadecatrienoic acid, 18:3n-3). Although the volume of literature associated with the biological significance of PUFAs in laboratory animals is immense, relatively few studies have focused on the essentiality of 18:2n-6 and 18:3n-3 with respect to humans. In fact, the critical issues related to EFA physiological functions and requirements in humans are still being investigated. The results of some of these recent investigations are discussed in this review.

II. ESSENTIAL FATTY ACIDS

A. Linoleic Acid Requirement

The physiological effects of 18:2n-6 deficiency has been very well characterized, particularly in rodent species (Burr and Burr, 1929, 1930; Alfin-Slater and Aftergood, 1968; Holman, 1971; Rivers and Frankel, 1981). The various deficiency symptoms include depressed growth, scaly dermatoses, increased permeability of skin, fatty liver, kidney damage, and impaired reproduction (Burr and Burr, 1929, 1930; Alfin-Slater and Aftergood, 1968; Holman, 1971; Rivers and Frankel, 1981) (refer to Table 26.1). The classical biochemical method of establishing EFA deficiency is to calculate the ratio of the "Mead acid," Δ5,8,11-eicosatrienoic acid (20:3n-9), to arachidonic acid (Δ5,8,11,14-eicosatetraenoic acid, 20:4n-6) (Holman, 1960). Although the validity of this index has

TABLE 26.1
Symptoms of Linoleic Acid Deficiency in Mammals

Diminished growth	Alopecia
Scaly dermatoses	Fatty liver
Inflamed epidermis	Kidney degeneration
Diminished skin pigmentation	Impaired reproduction and sterility
Increased transepidermal water loss	Increased basal metabolic rate
Increased water consumption	Impaired protein utilization
Impaired wound healing	Electrocardiographic aberrations
Increased susceptibility to infection	Increased fragility and permeability of cellular membranes
Caudal necrosis	
Loss of muscle tone	

Source: Adapted from Holman, R.T. (1971). *Prog. Chem. Fats Other Lipids 9*: 275–348; Holman, R.T. (1977). *Adv. Exp. Med. 88*: 515–534; Yamanaka, W.K., et al. (1981). *Prog. Lipid Res. 19*: 187–215; Ziboh, V.A., and Chapkin, R.S. (1988). *Prog. Lipid Res. 27*: 81–105.

been questioned (Horrobin and Cunnane, 1981; Yamanaka et al., 1981), ratios above 0.2 and 0.4 are considered the upper limit of normalcy in the rat (Alfin-Slater and Aftergood, 1968) and human (Holman et al., 1979), respectively.

Ingestion of approximately 1%–2% of daily calories as 18:2n-6 is widely accepted as the amount needed to meet the EFA requirement of rodent species and humans (Holman, 1977). However, it is important to note that the 1%–2% figure is only an approximation of 18:2n-6 dietary requirement for humans. Although many attempts have been made to determine the human 18:2n-6 requirement, most studies have met with a plethora of problems regarding the considerable recycling of PUFAs (Sprecher et al., 1995; Sheaff et al., 1996) and the physiological criteria to use for normality. For example, reports that dietary oleic acid (18:1n-9) spares 18:2n-6 oxidation, and that 16:2n-6 can be elongated to 18:2n-6, indicates that the 18:2n-6 requirement may have been overestimated by feeding diets deficient in all unsaturated fatty acids (Cunnane, 1996). In addition, the quantity and type of dietary PUFA (Pawlosky et al., 2003) as well as body composition, that is, adiposity (Villalpando et al., 2001), can alter EFA metabolism in humans.

Human infants and children are generally thought to require 1%–2% of total dietary energy as 18:2n-6 to prevent EFA deficiency (Rivers and Frankel, 1981). However, it has been argued that the minimum requirement has been set "far too high" for infants, and should be changed to less than 0.5% of calories (Cuthbertson, 1976; Naismith et al., 1978). This value is consistent with the findings of Hansen et al. (1963) who demonstrated that growing infants developed deficiency symptoms only when receiving less than 0.1% of their calories as 18:2n-6. The scientific response to these data remains mixed primarily because it is believed that children require more 18:2n-6 as a percentage of total daily calories than adults because growth increases the demand for cell membrane constituents (Holman et al., 1979). In a study on premature infants (Farrell et al., 1988), the average amount of 18:2n-6 required to achieve normal fatty acid nutrition was 1.19 g/kg/day or approximately 10% of total caloric intake. The prodigious requirement for 18:2n-6 was attributed to the premature infants' small amount of adipose tissue and the great need for energy production from fat (Farrell et al., 1988). There is growing evidence that additional long-chain n-6 PUFAs may be conditionally essential for preterm and possibly full-term infants. This is based on the limited efficiency of conversion of 18:2n-6 to 20:4n-6 and 18:3n-3 to 22:6n-3 (Carlson et al., 1993; Demmelmair et al., 1995). Carlson and coworkers (Carlson et al., 1993; Carlson, 2001) have hypothesized that dietary 20:4n-6 could improve first year growth of preterm infants. It is uncertain, however, whether growth is a biomarker that accurately reflects "optimal nutrition" (Lapillonne and Carlson, 2001). It is also

unclear whether 20:4n-6 has a significant effect on infant development (Fleith and Clandinin, 2005; Wright et al., 2006).

With respect to factors affecting EFA requirement, a higher requirement for 18:2n-6 during pregnancy (4.5% of calories) has been suggested (Mendy et al., 1986). In addition, an FAO/WHO expert committee has advised increased intakes of 18:2n-6 (5%–7% of calories) during lactation (Mendy et al., 1986). Other examples of physiological conditions that may alter the 18:2n-6 requirement are protein malnutrition (Hill and Holman, 1980; Holman et al., 1981), genetic disorders that affect the relative activity of unsaturated fatty acid metabolic pathways (Holman, 1977; Dyck et al., 1981; Hernell et al., 1982), intestinal malabsorption (Gourley et al., 1982), cystic fibrosis (van Egmond et al., 1996), anorexia nervosa (Holman et al., 1995), and n-3 fatty acid intake (Bourre et al., 1989a; Lands, 1989). Not surprisingly, EFA abnormalities may be the single most important nutritional deficiency in patients with gastrointestinal disorders (Siguel and Lerman, 1996; Jeppesen et al., 1997). Since the metabolic pathways for the biosynthesis of long-chain PUFAs consist of a series of desaturation and elongation enzymatic steps (Brenner, 1981), dietary 18:3n-6 and 20:4n-6 may have superior biopotency with respect to 18:2n-6 (Rivers and Frankel, 1981).

Attempts to determine the 18:2n-6 requirement of isolated mammalian cells in culture have created an interesting paradox. Although the phenotype of some cell lines is altered under EFA-deficient conditions (Garner et al., 1995; Lerner et al., 1995; Stoll and Spector, 1995), many studies (Bailey, 1980; Spector et al., 1981; Rosenthal, 1987; Grammatikos et al., 1994) indicate that most "normal" human cells do not require 18:2n-6 or any PUFAs for growth or metabolism. The reason for this is unknown.

Current adequate intakes for infants, children, and adults range from 4.4 to 17 g/day of n-6 PUFA (Institute of Medicine, 2005). This corresponds to approximately 2 energy percent as 18:2n-6. Modest adjustments are made for pregnancy and lactation. It is generally agreed that there may be a tolerable upper intake level of 18:2n-6, however, insufficient data exist at present to set a precise value for the upper limit.

B. Linolenic Acid Requirement

The essentiality of 18:3n-3 in higher animals and humans has been debated for many years. Although, similar to 18:2n-6, it cannot be synthesized *in vivo* (Brenner, 1981), initial attempts to elicit 18:3n-3 deficiency in mammals produced equivocal results (Tinoco et al., 1979; Tinoco, 1982). During the early 1970s (Fiennes et al., 1973; Lamptey and Walker, 1976) and again in the 1980s, cogent evidence in support of the role of 18:3n-3 in the brain and retina was presented (Holman et al., 1982; Rivers, 1984; Yamamoto et al., 1987, 1988; Bourre et al., 1989b; Neuringer et al., 1994). Estimates for the 18:3n-3 requirement of humans have been listed at 0.4% (Bjerve et al., 1989), 0.54% (Holman et al., 1982), and 0.2%–0.3% of calories (Bjerve et al., 1987a,b). In addition, numerous recommendations that the diet provide an adequate amount of n-3 fatty acids and a PUFA n-6/n-3 ratio of 4:1–10:1 (Neuringer et al., 1994), 6:1 (Bourre et al., 1989a), 4:1 (Simopoulos, 1989a), and 2:1 (Okuyama et al., 1997), particularly during infancy, pregnancy, and lactation, have been made. The current estimate of the PUFA n-6/n-3 ratio in the Western diet is 10–11:1 (Simopoulos, 1996) and 4–5:1 in the Japanese diet (Okuyama et al., 1997) (refer to Table 26.2).

The very limited ability of 18:3n-3 to substitute for 18:2n-6 (or its metabolic derivatives) and to reverse classical EFA deficiency signs, that is, growth, reproduction, or dermal symptoms (Mohrhauer and Holman, 1963; Houtsmuller, 1975; Tinoco, 1982; Bivins et al., 1983; Crawford, 1992), is a major reason why the essentiality of 18:3n-3 has remained controversial (Rivers, 1984; Koletzko, 1987) and why the requirement remains ill defined. However, substantial evidence now indicates that 18:3n-3 and its metabolic fatty acid derivatives (Δ5,8,11,14,17-eicosapentaenoic acid, 20:5n-3 and Δ4,7,10,13,16,19-docosahexaenoic acid, 22:6n-3) have their own specialized and distinct functions in the retina and central nervous system (Wheeler et al., 1975; Bivins et al., 1983; Hoffman et al., 1993; Neuringer et al., 1994; Ikemoto et al., 2001; Lauritzen et al., 2001). Indeed, precisely

TABLE 26.2
Recommendations for Dietary PUFA Intake

(For *normal healthy adults* free of genetic disorders, medical disorders, etc.)
Polyunsaturated fatty acids
18:2n-6 = 12–17 g/day (4%–5% of calories, perhaps as low as 2%)
18:3n-3 = 1.1–1.6 g/day (0.7% of calories)
20:5n-3 (EPA) plus 22:6n-3 (DHA) = 0.5–0.85 g/day (0.27% of calories)
Total PUFA = 18 g/day (6%–7% of calories)
(n-6/n-3 ratio) = (4/1 or perhaps as low as 2 or below), *note:* there is no scientific evidence to support a recommendation of 5–10
(n-3 as 18:3/n-3 as EPA plus DHA ratio) = (4/1)
*Estimated Current Japanese vs. U.S. Fatty Acid Intake (as energy percentage)

	Japan	United States
n-6 PUFA	6.4	7.1
n-3 PUFA	1.6	0.8
n-6/n-3	3.9	8.3
n-6, % of total	26	19

Source: Adapted from Simopoulos, A.P. (1989a). Executive summary, in *Dietary ω3 and ω6 Fatty Acids. Biological Effects and Nutritional Essentiality* (C. Galli and A.P. Simopoulos, eds.), Plenum Press, New York, pp. 391–404; Anonymous (1995). *Nutr. Rev. 53*: 202–205; Okuyama, H., et al. (1997). *Prog. Lipid Res. 35*: 409–457; Institute of Medicine of the National Academies (2005) *Dietary Reference Intakes*, National Academy of Sciences, Washington, DC.

because long-chain fatty acids are required for the development and function of the brain and retina (Ikemoto et al., 2001; Lauritzen et al., 2001), there is a consensus that bottle-fed healthy term infants should be supplemented with long-chain n-3 PUFA (Bouwstra et al., 2003; Birch et al., 2005). This may in part be related to the neuroprotective properties of 18:3n-3 and its metabolic product, 22:6n-3 (Lauritzen et al., 2000; Akbar et al., 2005). In addition, because preterm infants do not receive the third trimester intrauterine supply of 22:6n-3 and full-term infants are at risk of 22:6n-3 deficiency since some formulas are devoid of this fatty acid, 22:6n-3 is considered provisionally essential for infant nutrition (Hoffman et al., 1993; Agostoni et al., 1995; Uauy et al., 1996). Therefore, infant foods should contain a level of n-3 fatty acids (ratio of 10:1 n-6:n-3 fatty acids, 20:4n-6 at ~0.5% and 22:6n-3 at 0.1%–1.5% of energy) similar to human milk (Anonymous, 1995). Historically, U.S. infant formulas contained >4% of energy as 18:2n-6 and 0.75% of energy as 18:3n-3 (up to a (16:1) n-6:n-3 ratio), and none contained 20:4n-6 or 22:6n-3 (Innis et al., 1996; Ratnayake et al., 1997). In February 2002, infant formulas with added 20:4n-6 and 22:6n-3 for infants born to term became commercially available in the United States and Canada. Additional concerns related to the nutritional adequacy of infant formulas have focused on the presence of *trans* geometrical fatty acid isomers in powdered and liquid infant formulas (Ratnayake et al., 1997). Therefore, infants should be fed breast milk if at all possible (Anonymous, 1995). Further studies are required to determine whether *trans* acids adversely affect fetal and infant n-6 and n-3 fatty acid metabolism, neurodevelopment, and growth (Carlson et al., 1997). The potential for an excessive or imbalanced intake of long-chain PUFA exists with the increasing fortification of n-6 and n-3 fatty acids into infant foods other than formulas (Koo, 2003).

Dietary estimates to prevent n-3 PUFA depletion of liver and brain phospholipids are 800–1100 mg/day of 18:3n-3 and 300–400 mg/day of 20:5n-3 and 22:6n-3 combined (Simopoulos, 1989b). It has been estimated that 1–2 g/day of 18:3n-3 is consumed by the U.S. population, which provides approximately 75%–85% of the 400–500 mg/day of long-chain n-3 fatty acids that are required by

adults (Emken et al., 1994; Kris-Etherton et al., 2000). Current adequate intakes for infants, children, and adults range from 0.5 to 1.6 g/day of n-3 PUFA (Institute of Medicine, 2005). This corresponds to approximately 0.7 energy percent as 18:3n-3. Modest adjustments are made for pregnancy and lactation. It is recommended that up to 10% of the total n-6 and n-3 PUFA requirements can come from long-chain PUFA. It is highly likely that a minimum intake level of 20:5n-3 and 22:6n-3 (500 mg/day) will be adopted in the near future (ISSFAL, 2004).

III. METABOLIC FATES OF LINOLEIC AND LINOLENIC ACIDS

Once ingested, 18:2n-6 and 18:3n-3 can be desaturated and elongated, primarily in the liver, in most mammals including humans (DeGomez Dumm and Brenner, 1975; Brenner, 1981; Biagi et al., 1990) in a manner such that the methylene-interrupted pattern of unsaturated double bonds is maintained (Brenner, 1981). The microsomal enzymatic reactions do not permit crossover between fatty acid metabolites from the 18:2n-6 and 18:3n-3 sequences (Brenner, 1981; Sprecher, 1981, 2000). It is also likely that considerable recycling of fatty acids occurs between peroxisomes and microsomes (Sprecher et al., 1995). This implies that individuals with peroxisomal disorders could suffer from defects in long-chain PUFA metabolism (Martinez, 1990). The desaturation and elongation steps are influenced by numerous nutritional and hormonal factors, possibly involving obesity and lifestyle variables (Brenner, 1981; Brenner et al., 1981; Naughton, 1981; Cho et al., 1999a,b; Warensjo et al., 2006). A competitive interaction between 18:2n-6 and 18:3n-3 exists, such that n-3 PUFA suppress the metabolism of n-6 PUFA and n-6 PUFA suppress the metabolism of n-3 PUFA less strongly (Rahm and Holman, 1964; Brenner and Peluffo, 1969). It is generally believed that the capacity to convert 18:2n-6 and 18:3n-3 into 20- and 22-carbon fatty acids in humans is very limited (Nichaman et al., 1967; Stone et al., 1979; Dyerberg, 1986; Mark and Saunders, 1994; Burdge et al., 2002a,b). Interestingly, in an investigation using deuterated 18:2n-6 (Emken et al., 1987), desaturation/elongation products were not detected in plasma lipids. The authors concluded that 18:2-$^{2}H_4$ to 20:4-$^{2}H_4$ conversion is extremely low in normal subjects. In a separate series of studies using mixtures of triglycerides containing deuterium-labeled fatty acids, Emken et al. (1988, 1990) demonstrated the conversion of 18:3n-3 to 20:5n-3 and 22:6n-3, but were again unable to detect conversion of 18:2n-6 to 20:4n-6. The exclusive conversion of 18:3n-3, but not 18:2n-6, in these studies is most intriguing. It is possible that the 18:2n-6 conversion products were sequestered in tissues following ingestion and, therefore, could not be released into the plasma lipid pool, which was subsequently analyzed. In comparative studies, Siguel and Maclure (1987), using derivative precursor ratios, and El Boustani et al. (1989), using 20:3n-$^{2}H_4$, have demonstrated that desaturase activity operates in humans and that the desaturation/elongation enzymes have a preference for n-3 vs. n-6 fatty acids. The conversion (Siguel and Maclure, 1987) of 18:3n-3 suggests that vegetarians can accumulate 20:5n-3 and 22:6n-3 and need not alter their diets to include fish products (which are rich in 20:5n-3 and 22:6n-3). Recently, a number of human studies (Pawlosky et al., 2001; Burdge et al., 2002a,b; Hussein et al., 2005) have demonstrated that 22:6n-3 synthesis from 18:3n-3 is generally low. However, these tracer kinetic studies are complicated, and overall, the regulation of 22:6n-3 studies remains an enigma. Currently, there is consensus that 22:6n-3 can be formed from 18:3n-3 *in vivo*, albeit at a low rate, but cannot be readily increased by dietary 18:3n-3. In addition, dietary 20:5n-3 is well utilized in the biosynthesis of 22:6n-3. With respect to the influence of dietary 20:4n-6 on metabolism of 20:3n-6, although the conversion of 20:3n-6 to 20:4n-6 (Δ5 desaturase activity) is low in humans, it can be enhanced by feeding 20:4n-6 (Emken et al., 1997).

The recent cloning and expression of Δ6,Δ5 desaturases (Cho et al., 1999a,b) and the long-chain PUFA elongases (Leonard et al., 2002, 2004) provide direct evidence of the position-specific enzymes responsible for the interconversion of 18:2n-6 and 18:3n-3 to 20-carbon fatty acids. Structure–function studies of mammalian desaturases indicate that combinations of multiple transcription

factors, for example, sterol regulatory binding protein-1c (SREBP-1c), peroxisome proliferator-activated receptor-α (PPAR-α), carbohydrate response element binding protein (ChREBP), and liver X receptor response element (LXR), regulate 20:4n-6, 20:5n-3, and 22:6n-3 levels in tissues (Tang et al., 2003; Nakamura and Nara, 2004; Li et al., 2005).

Many epidemiological and clinical studies have demonstrated that long-chain n-3 PUFAs are bioactive food components, and attenuate diseases with an immune-mediated inflammatory component such as heart disease, arthritis, and certain forms of cancer. Since new studies are raising serious concerns about the long-term safety of many popular pain-relief anti-inflammatory drugs, it is important to recognize that there are dietary alternatives to these pharmaceutical agents. At present, sources of n-3 fatty acids include vegetable and nut oils, which can contain up to 50% 18:3n-3, and marine oils and single cell oils derived from the fermentation of microalgae, which contain 20:5n-3 and/or 22:6n-3 in varying amounts. However, there are limitations to each of these sources. Yields from global fisheries have been reported to be stagnant and there is concern regarding the levels of methyl mercury in a number of species (Ursin, 2003). Aquaculture requires fish meal and fish oil, and microalgae is currently not being produced in sufficient quantities for wide scale impact. As already noted, with respect to vegetable oils, the bioconversion of 18:3n-3 to long-chain n-3 PUFA, which is necessary for the therapeutic and preventive benefits, is extremely inefficient. In contrast, it has been recently shown that stearidonic acid (Δ6,9,12,15-octadecatrienoic acid, 18:4n-3), a metabolic intermediate between 18:3n-3 and 20:5n-3, is efficiently converted in humans to 20:5n-3 (James et al., 2003). Therefore, the use of 18:4n-3-containing land-based plant oils could become a valuable tool for increasing tissue concentrations. Along these lines, in an attempt to generate n-3 PUFA-enriched foods, Kang and coworkers (Kang, 2005; Lai et al., 2006) have successfully cloned n-3 fatty acid desaturase into pigs in an attempt to produce high tissue levels of n-3 fatty acids from n-6 analogs. This genetic approach may very well prove to be an effective and sustainable way to produce n-3 PUFA-enriched bioactive foods.

IV. PHYSIOLOGICAL FUNCTIONS

The broad array of deficiency symptoms suggests that EFAs play a prominent physiological role in different organs. For example, 18:2n-6 and 18:3n-3 and their desaturation/elongation products (20:4n-6, 20:5n-3, and 22:6n-3) were historically recognized in the homoviscous control of the membrane lipid bilayers of most cells (Burr and Burr, 1930; Bernsohn and Spitz, 1974; Mead, 1984). The identification of 18:2n-6 as a constituent of epidermal sphingolipids is an example of the regulation of membrane barrier function by an EFA (Hansen et al., 1986).

The molecular mechanisms of cellular uptake and intracellular translocation of EFAs have been extensively studied. In general, this is a multistep process that involves various membrane-associated proteins utilizing both passive and carrier-mediated transmembrane translocation (Glatz and vand der Vusse, 1996; Hamilton et al., 2002; Daleke, 2003). Upon incorporation into structural lipids, long-chain PUFAs can modify membrane fluidity, membrane thickness, and alter specific interactions with membrane proteins (Carrillo-Tripp and Feller, 2005). Of relevance to membrane biology, it has been demonstrated that dietary PUFAs are capable of altering reversible fatty acylation of membrane-anchored proteins in platelets (Laposata and Muszbek, 1996). Complimentary studies have shown that both n-3 and n-6 PUFAs are capable of inhibiting fatty acylation, plasma membrane localization, and intracellular signaling in T-cells (Webb et al., 2000; Liang et al., 2001). These data suggest that dietary PUFAs may alter the hydrophobic contribution of lipid groups responsible for membrane localization of lipidated proteins. This represents a putative mechanism by which dietary PUFAs can alter intracellular signal transduction pathways.

Our view of biological membranes has evolved dramatically over the past 5 years (Helms and Zurzolo, 2004; Ma et al., 2004a,b). Within the cell membranes, there are specific detergent-resistant domains in which key signal transduction proteins are localized. These regions are classified as "lipid rafts" (Xavier et al., 1998; Viola et al., 1999). Rafts are composed mostly of cholesterol and

sphingolipids and therefore do not integrate well into the fluid phospholipid bilayers causing them to form microdomains that influence protein–protein interactions (Hancock, 2006). The emerging role of n-3 PUFAs in maintaining the fluid microenvironment of membranes was originally supported by studies showing an altered electroretinogram, decreased visual activity, and impaired learning ability in animals deprived of 18:3n-3 (Menon and Dhopeshwarkar, 1982; Neuringer et al., 1994). Deprivation of 18:3n-3 and its major fatty acid metabolite, 22:6n-3, eventually results in decreased brain and retina 22:6n-3 membrane content (Hoffman et al., 1993; Uauy et al., 1996). It is generally believed that 22:6n-3 is synthesized from 18:3n-3 by the liver (Emken et al., 1988, 1990), secreted into the bloodstream in lipoprotein form and taken up by the brain and retina, possibly by a 22:6n-3 receptor (Scott and Bazan, 1989).

22:6n-3 is the most unsaturated membrane fatty acid commonly found in biological systems and can be obtained by dietary consumption of cold-water fatty fish. Long-chain n-3 PUFAs present in dietary fish oil, such as 20:5n-3 and 22:6n-3, affect diverse physiological processes including cognitive functions, visual acuity, eicosanoid signaling, and whole body glucose and lipid metabolism (Jump, 2002), thereby providing significant protection against a variety of apparently unrelated human diseases (Kris-Etherton et al., 2002; Hu et al., 2003; Stulnig, 2003; Terry et al., 2003; Lupton and Chapkin, 2004). 22:6n-3 is rapidly incorporated into cells, primarily into membrane phospholipids at the *sn*-2 position (Anderson and Sperling, 1971), and in general, the cellular level is readily influenced by diet (Katan et al., 1997; Vidgren et al., 1997). The presence of 22:6n-3 in membrane phospholipids imparts unique physicochemical properties to cellular membranes and 22:6n-3-induced alterations in membrane structure and function have been proposed to underlie its pleiotropic salutary effects (Ma et al., 2004a,b; Seo et al., 2006; Stillwell et al., 2006).

The EFAs are also the precursors for prostaglandins, hydroxy fatty acids, sulfidopeptide-leukotrienes, and lipoxins, collectively referred to as eicosanoids (Lands, 1979; Samuelsson et al., 1991). These oxygenated metabolites are formed rapidly on stimulus and, acting as autacoids, exert a profound influence on many cellular reactions. A flurry of interest focusing on the dietary modulation of eicosanoid formation in humans has surfaced (von Schacky et al., 1985; Knapp et al., 1986; Kinsella et al., 1990; Zhou and Nilsson, 2001). For example, diets rich in select n-3 and n-6 PUFAs are capable of influencing *in vivo* levels of 13-hydroxyoctadecadienoic acid (13-HODE) (Miller et al., 1990). This is significant because 13-HODE, a 15-lipoxygenase-1 metabolite of 18:2n-6, is capable of inhibiting diacylglycerol-activated protein kinase C (PKC) activity, which may have relevance in modulating epidermal hyperproliferation (Cho and Ziboh, 1994). Curiously, 13-HODE may play a promoting role in the development of prostate (Kelavkar et al., 2004) and a protective role in colorectal cancer (Zuo et al., 2006). In addition, increased 20:5n-3/20:4n-6 ratios can inhibit cyclooxygenase I and thus thromboxane formation. Cyclooxygenase II-mediated signaling is also modulated via the reduction of PGE_2 and the increase in PGE_3 (Smith, 2005). This is significant because recent evidence indicates that PGE_2 induces angiogenesis at the earliest stage of tumor development (Wang and DuBois, 2004), while PGE_3 is antiproliferative/chemoprotective (Bagga et al., 2003; Yang et al., 2004). Results from several laboratories indicate that a number of cell types are capable of generating novel oxygenase products from n-3 PUFA (Lukiw et al., 2005; Serhan, 2005). These findings have implications for neurogenerative diseases (Calon et al., 2004) and for the resolution of inflammatory processes (Serhan and Savill, 2005).

Dietary PUFA manipulations are highly significant because many diseases are associated with an overproduction of eicosanoids from 20:4n-6 (derived from 18:2n-6), and the formation of n-6 eicosanoids can be antagonized by dietary n-3 PUFA (Lands, 1989; Kinsella et al., 1990; Kremer et al., 1995). In humans who ingest 0.2–0.5 g of 20:4n-6 and 10–20 g of 18:2n-6 per day on the typical Western diet, the formation of 20:4n-6 from 18:2n-6 exceeds the dietary supply of 20:4n-6 (Zhou and Nilsson, 2001). As a point of reference, 20:4n-6 intakes are in the range of 100–200 mg/day (Innis and Elias, 2003). In contrast, obligate carnivores do not produce 20:4n-6 by desaturation–elongation and depend on the supply of 20:4n-6 in the diet. Recent studies on interactions between n-3 and n-6 PUFAs indicate that cycles of deacylation/reacylation of 20:4n-6 and 22:6n-3 operate

independently from each other (Contreras and Rapoport, 2002; Marszalek et al., 2005; Murphy et al., 2005). Interestingly, the 20:4n-6 pool(s) for prostaglandins in humans is not quickly influenced by dietary 18:2n-6 and 20:4n-6 because of a large pool(s) size of 20:4n-6 and a low conversion of 18:2n-6 to 20:4n-6, whereas the n-3 PUFA pool(s), which is considerably smaller, is immediately influenced by n-3 PUFA supplementation (Hamazaki et al., 1989). The relationship between dietary 18:3n-3 and its respective long-chain metabolites was also examined in humans by Mantzioris et al. (1995), who demonstrated that increasing dietary 18:3n-3 levels will elevate tissue 20:5n-3 levels in a predictable manner whereas no relationship was observed between dietary 18:2n-6 and its metabolite, 20:4n-6. There is, however, growing evidence to indicate that although the average intake of 20:4n-6 in the diet (~100 mg/day) is approximately 100 times lower than 18:2n-6 (Mantzioris et al., 1995), its consumption may have physiological relevance (Mann et al., 1995). Since dietary 20:4n-6 bypasses some of the normal regulatory rate-limiting enzymatic ($\Delta 6$ and $\Delta 5$ desaturase) steps that control membrane 20:4n-6 content, it can abrogate the salutary effects of n-3 PUFAs (Li et al., 1994; German et al., 1995; Whelan and McEntee, 2004).

Additional insight into the mechanisms by which dietary PUFAs modulate gene expression was provided by the identification of several nuclear receptors (e.g., PPARs), which are activated at micromolar concentrations by a variety of fatty acids (Kliewer et al., 1997). This class of nuclear receptor binds n-3 and n-6 PUFAs with equal affinity and lacks fatty acid class (n-3 vs. n-6) specificity (Kliewer et al., 1997; Xu et al., 1999; Fan et al., 2003). Therefore, the unique effects of n-3 PUFA are likely not mediated via PPARs. Other molecular pathways that do not require PPARs are also involved in the PUFA-mediated regulation of gene expression (Ren et al., 1997; Fan et al., 2003; Jump et al., 2005). With regard to alternative pathways of intracellular signaling, Brown and Goldstein (1997) have documented how cells sense the level of a membrane embedded lipid, and how this information is transmitted to the nucleus to regulate gene transcription. Data are emerging from studies which demonstrate that families of membrane-bound transcription factors (e.g., SREBPs) coordinate the synthesis of the two major constituents of membranes, fatty acids and cholesterol (Anderson, 2003; Jump et al., 2005; Botolin et al., 2006). These data offer insight into mechanisms by which dietary PUFAs can modify risk of disease development.

V. CONCLUSION

It is evident that large gaps exist in our present understanding of EFA requirements and 18:3n-3 functions in humans. Specific issues related to n-6/n-3 PUFA balancing, quantitative dose-response to dietary n-3 PUFA and mechanisms involved in defining the essentiality of n-3 PUFA in growth, development, and disease resistance require further investigation. In addition, because North Americans consume greater than ten times the amount of n-6 PUFA required to meet minimal requirements (Lands, 1989; Simopoulos, 1989a), the significance of the question "when does enough (18:2n-6) become more than enough" (Lands, 1979) must now be evaluated. The excesses in n-6 PUFA (18:2n-6 and 20:4n-6) consumption are creating an overall imbalance in long-chain fatty acid metabolism. This issue is of particular importance in view of the relative n-3 PUFA deficiency syndrome seen in Japan, where an increase in the n-6/n-3 ratio from 2.8 to 4 during the past 40 years is associated with the rising incidence of cancer of the colon, breast, prostate, and pancreas (Okuyama et al., 1997). In addition, provocative data related to cognitive function in the elderly also reinforces the concept that an elevated dietary n-6/n-3 PUFA ratio is creating a health risk (Moriguchi and Salem, 2003; Calon et al., 2005; Lukiw et al., 2005; McCann and Ames, 2005). Kalmijn et al. (1997) have demonstrated that in men, aged 69–89 years, high 18:2n-6 intake was positively associated with cognitive impairment. In contrast, fish consumption (containing 20:5n-3 and 22:6n-3) was inversely associated with cognitive impairment, and no protective effect of any dietary antioxidants was observed. Although the complexity of this issue precludes any conclusions, it can be argued that more attention must be paid to promoting the consumption of n-3 PUFA-rich foods while reducing the intake of n-6 PUFA (Burchfiel et al., 1996; Connor, 1997; Daviglus et al., 1997; Okuyama et al., 1997).

In conclusion, effective public intervention to encourage the consumption of foods with an appropriate n-6/n-3 PUFA ratio is needed. As well, new food and agriculture policies are needed in order to expand federal efforts to disseminate relevant nutrition information. It is also important to recognize the inaccuracies of the dietary instruments used in epidemiological studies and the databases used to translate foods into nutrients. Finally, future efforts should concentrate on defining single nucleotide polymorphisms that influence EFA metabolism. Several loci are providing proof-of-principle for the potential application of genetics and diet (nutrigenomics) in the context of personalized nutritional recommendations for chronic disease prevention (Corella and Ordovas, 2005).

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27 Fatty Acids and Membrane Function

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CONTENTS

I. INTRODUCTION

Within and around all living cells are membranes. These structures have been studied intensely over the past 75 years as scientists have become increasingly aware of their importance in metabolic regulation as well as in the regulation of the cell's life cycle. Membranes serve as biological boundaries to the various cell compartments and to the cell itself. If these boundaries altered, cell function is affected. Every organelle within each cell, and each cell type has this boundary. Some of the functions of these membranes are unique to the particular organelle or cell under study while other functions may be common to all cells or all similar organelles. In general, the plasma membrane (the membrane surrounding the cell) serves to exclude certain toxic ions and molecules from the cell. The plasma membranes also accumulate cell nutrients and serves in energy and signal transduction. To a limited extent in the mammal, the plasma membrane has a role of cell locomotion and cell division. This chapter focuses on the lipid components of membranes and discusses how changes in these lipids can affect the functions of membranes and hence the function of the cell. Indeed, the metabolism of the whole body may be affected if the membrane lipids are drastically altered.

II. MEMBRANE LIPID COMPOSITION AND STRUCTURE

A. Fluid Mosaic Model

Biological membranes contain a large number of lipids, proteins, lipid–protein complexes, glycolipids, and glycoproteins. The arrangement of these many compounds within the membrane structure has been studied extensively.

In the early twentieth century, Gortner and Grendel (1925) studied the composition and function of the lipids in the erythrocyte membrane. These lipids were primarily phospholipids.

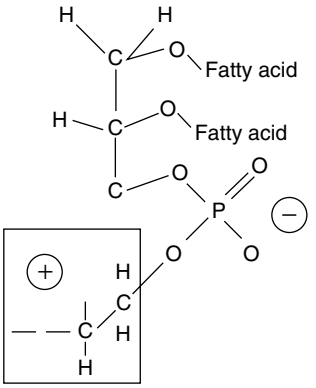

FIGURE 27.1 Structure of the major phospholipids. Head group can be ethanolamine, serine, glycerol, choline, or inositol.

Phospholipids can be thought of as substituted triacylglycerols. Attached to the glycerol backbone (Figure 27.1) are two fatty acids at carbons 1 and 2, respectively, and a charged moiety connected to carbon 3 via a phosphate group. Ethanolamine, inositol, choline, serine, or another glycerol can also be connected. Hence, one can find phosphatidylethanolamine, phosphatidylcholine (also known as lecithin), phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol in the plasma membrane. In the mitochondrial membrane cardiolipin can be found. Cardiolipin has two phosphatidylglycerol groups (diphosphatidylglycerol) attached to the glycerol backbone. This structure has some unique properties as will be described in Section III. Cardiolipin serves as the signature for the mitochondrial membrane. Biochemists separating the various membranes for study use this signature to determine what membrane fraction they have. Cholesterol and cholesterol esters can also be found in the lipid portion of the plasma membrane. There is less cholesterol in the mitochondrial membrane than in the plasma membrane.

Gortner and Grendel extracted the lipids from the erythrocyte membrane and found that they covered more surface area than they had expected considering their calculations of the cell surface area of the erythrocyte. They concluded that these lipids must exist as a bilayer rather than as a monolayer around the cell contents. A decade later, Danielli and Dawson (1935) proposed that this lipid bilayer wore a protein coat. Then, in 1972, Singer and Nicholson, in their now classic paper, proposed that biological membranes exist as lipid bilayers and have a number of different proteins distributed throughout them. Some of these proteins might extend entirely through the layer, whereas others might rest on either aspect of the layer or be partially drawn into the layer. Singer and Nicholson thus proposed that biological membranes exist as fluid mosaics with regional differences in composition, structure, and function.

The reason the membrane exists as a lipid bilayer has to do with the amphipathic character of the phospholipids. They have both polar (the phosphorylated substituent at carbon 3) and nonpolar (the fatty acids) regions. The polar region is hydrophilic and is positioned such that it is in contact with the aqueous media around and within the cells. The nonpolar or fatty acid region is hydrophobic and is oriented toward the center of the bilayer so that it is protected from contact with the contents of cell and the fluids that surround it.

The ability of these amphipathic compounds to self-assemble into a bilayer can be demonstrated *in vitro*. Lipid vesicles can be made by the addition of these phospholipids to water. A lipid bilayer will form just as described above. This feature of the phospholipids is consistent with one of the many roles a membrane serves: that of a permeability barrier for the cell and cell compartments. The lipid bilayer forms the matrix into which specific proteins are placed. Each of the individual phospholipids and the cholesterol provide specific regional characteristics that satisfy the insertion

requirements of each of the many membrane proteins. The lipid bilayer serves as a seal around these membrane proteins and thus prevents nonspecific leakage. The lipids also serve to maintain the proteins in their most appropriate functional conformations. The polar position of the phospholipids satisfies the requirements for the electrostatic charge that is needed for the surface associations of specific cell surface proteins. All of the characteristics are needed and are critical to normal cell function. For example, an intact permeability barrier to sodium, potassium, calcium, and hydrogen ions is needed so that electrochemical gradients, which in turn drive other membrane transport process, are maintained.

The phospholipids are distributed asymmetrically depending on the cell type and the membrane in question. In the erythrocyte, for example, phosphatidylcholine is distributed such that 76% of it is located in the outer aspect of the membrane while the remainder can be found in the inner aspect. Asymmetric distribution of membrane components applies not only to the phospholipids as just mentioned but also to proteins and other membrane components. The glycolipids, for example, are almost exclusively found on the outer aspect with their carbohydrate moiety extending beyond the outer aspect. There they can participate in the cell recognition process.

Cell membranes usually work best when their lipids are in the liquid crystal state. This means that there are regional differences in the physical state of the lipid. Some portions may be fairly fluid, whereas other regions may be fairly solid. The localized difference in physical state or fluidity has to do with the chain length and saturation of the fatty acids attached at carbons 1 and 2 of the phospholipid; it is this portion of the molecule that extends into the center of the bilayer. Membranes whose phospholipid fatty acids are saturated are less fluid than those membranes containing polyunsaturated fatty acids in their phospholipids. Even within a membrane there can be regional differences in fluidity due to the nature of the fatty acids in the phospholipids of that region and in the amount of cholesterol. Although there can be local regions which differ in fluidity, the overall fluidity of the plasma membrane is usually constant in normal individuals despite drastic dietary change. This has been shown in human erythrocytes (Popp-Snijders et al., 1986), rat brush-border membranes (Stenson et al., 1989; Wahnon et al., 1992), and myocardial plasma membranes (Abeywardena et al., 1984). Differences in fluidity are not only due to the ratio of saturated fatty acids to unsaturated fatty acids but also due to the ratio of cholesterol to fatty acids (Naito, 1978a,b; Shinitzky, 1984). This ratio varies according to the location of the membrane within the cell and the tissue or cell type. Plasma membranes, for example, contain more cholesterol than do mitochondrial membranes. Hence, dietary fatty acid variation could have a greater influence on mitochondrial membrane fluidity than on plasma membrane fluidity. Indeed such has been shown (McMurchie and Raison, 1979; McMurchie et al., 1983a,b,c; Kim and Berdanier, 1988, 1998). When diets differing in the degree of unsaturation, that is, hydrogenated coconut oil vs. fish oil, mitochondrial membrane fluidity as well as the activity of certain mitochondrial membrane-embedded enzymes and transporters is affected. Clouet et al. (1995) reported that altering the outer mitochondrial membrane fatty acid content through diet affected carnitine palmitoyltransferase I, but had no effect on monoamine oxidase, glutamine dehydrogenase, and acylester hydrolase activities. Carnitine palmitoyltransferase I activity was reduced in outer membranes when rats were fed an arachidonic acid-enriched diet, whereas this membrane-bound enzyme was less sensitive to malonyl coenzymes A (CoA) inhibition when rats were fed a fish oil-enriched diet. Enzymes of the respiratory chain, particularly those in complexes III and IV, were less active in hepatic mitochondria isolated from rats fed highly saturated fats, for example, hydrogenated coconut oil, than in hepatic mitochondria isolated from rats fed polyunsaturated fat diets (McMurchie and Raison, 1979; Blomstrand and Svenson, 1983; McMurchie et al., 1983a,b; Deaver et al., 1986; Stillwell et al., 1997; Kim and Berdanier, 1998). Furthermore, in instances where there are small genetically determined differences in one or more of the proteins involved in mitochondrial oxidative phosphorylation, dietary effects on mitochondrial fluidity and function are more pronounced (Deaver et al., 1986; Kim and Berdanier, 1998; Mathews et al., 1999).

Microsomal membranes likewise have been examined. Through manipulating the cholesterol content and the fatty acid composition of the dietary lipid, the microsomal fatty acid profile as well as the Δ5 desaturase activity was affected. Rats fed a beef tallow diet had less desaturase activity than rats fed a linseed oil diet but more activity than rats fed a fish oil diet. When these diets were enriched with cholesterol, desaturase activity was reduced but the dietary fat effect on membrane lipid was unchanged (Garg et al., 1988). These observations suggest that the cells of these rats could adjust microsomal membrane fluidity not only through adjusting the ratio of saturated to unsaturated fatty acids but also through adjusting the ratio of membrane cholesterol to unsaturated fatty acids. Such adjustments have not been reported to occur in the mitochondrial membranes or the nuclear membranes. In these organelles, fluidity apparently is solely responsive to the ratio of saturated to unsaturated fatty acids.

B. Phospholipid Composition

As mentioned earlier, there are three major classes of lipids in membranes: glycolipids, cholesterol, and phospholipids. The glycolipids have a role in the cell surface-associated antigens, whereas the cholesterol serves to regulate fluidity. The phospholipids have fatty acids attached at carbons 1 and 2. It is usual to find a saturated fatty acid attached at carbon 1 and an unsaturated fatty acid at carbon 2. In addition, phosphatidylethanolamine and phosphatidylserine usually have fatty acids that are more unsaturated than phosphatidylinositol and phosphatidylcholine. Shown in Table 27.1 are some observations of the fatty acid composition of phospholipids extracted from normal human erythrocytes (van Deenen and de Gier, 1974) and rat hepatic mitochondrial membranes (Sire et al., 1986). Note the diversity in fatty acids, both in amount and kind, in these two types of membranes. Note too that the plasma membrane of the erythrocyte has no cardiolipin, whereas the rat mitochondrial membrane contains very little phosphatidylserine. Less than 10% of the membrane phospholipid is phosphatidylinositol. The fatty acid pattern shown in Table 27.1 for the erythrocyte membrane phospholipids can be altered. Similarly, the fatty acid composition as well as phospholipid composition of the B and T cells of the immune system can be influenced by the fatty acid

TABLE 27.1
Fatty Acids in Different Membrane Phospholipids

	PC		PE		PS[a]	CL[b]
Fatty Acid (mol. %)	**RBC**	**RLM**	**RBC**	**RLM**	**RBC**	**RLM**
16:0	31.2	21.0	12.9	14.5	2.7	7.9
18:0	11.8	21.3	11.5	27.1	37.5	6.0
18:1	18.9	6.6	18.1	5.2	8.1	11.2
18:2	22.8	12.8	7.1	6.3	3.1	63.9
20:3	1.9	1.0	1.5	0.4	2.6	4.5
20:4	6.7	30.9	23.7	31.1	24.2	3.2

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanoamine; PS, phosphatidylserine; CL, cardiolipin; Pl, phosphatidylinositol; RBC, red blood cell (erythrocyte); RLM, rat liver mitochondria.

[a] Plasma membrane contains phosphatidylserine but not cardiolipin.
[b] Mitochondrial membrane contains cardiolipin but not phosphatidylserine.

Source: From van DeenanDeenen, L.L.M., and de Gier, J. (1974). Lipids of the red cell membrane, in *The Red Blood Cell*, D. Surgenor, Ed., Academic Press, New York, pp. 147–160; Sire, O., et al. (1986). *Biochim. Biophys. Acta* 860: 75–83.

composition of the diet (Tiwari et al., 1987). Other cell types have also been studied, and again dietary fat composition affects the fatty acid array of the phospholipid. Mention should be made here of the role that fatty acids as well as the phosphatidylinositol play in the regulation of metabolism that occurs through the inositol cascade and through eicosanoid production. The arachidonic acid that is often attached to carbon 2 of the phospholipid can be released via the activity of phospholipase B. Diet can influence the amount of arachidonic acid present in the plasma membrane, and this arachidonic acid can then serve as a precursor for the eicosanoids, particularly those of the prostaglandin 2 (PG_2) series (see Chapters 29 and 31). These eicosanoids act as localized hormones to stimulate one or more metabolic processes such as uterine contraction ($PGF_2\alpha$). In this instance, arachidonic acid serves not only in the maintenance of membrane fluidity and function but also as a substrate for the synthesis of a number of metabolic regulators.

In the second instance, the phospholipid, phosphatidylinositol, serves as substrate for phospholipase C that stimulates the release of phosphatidylinositol and diacylglycerol (DAG) from the membrane. Phosphatidylinositol (PIP_2) is further phosphorylated to PIP_3 and PIP_4. Both PIP_3 and PIP_4 and DAG are second messengers for a number of hormone-mediated cellular processes and thus can be included in the list of lipids that are metabolic controllers. Fatty acids and eicosanoids are covered in detail in Chapter 29 and signal transduction is discussed in Chapter 31.

C. Disease Effects on Membrane Lipids

Holman (1986) reviewed the literature on the effects of essential fatty acids and disease on the fatty acid content of membrane fatty acids. Goldberg and Riordan (1986) reviewed the literature on the role of membranes in the pathophysiology of a number of genetic diseases as well as contagious and degenerative diseases. Although these authors focused primarily on the abnormalities of the proteins in the membrane lipid bilayer, they indicated a role for the lipid in some of these disorders. For example, muscular dystrophy and multiple sclerosis are characterized by changes in the lipid structure of the membrane. In the former, the change consists of an increase in the amount of lysophosphatidylcholine and cardiolipin (Kalofoutis et al., 1977). In multiple sclerosis, there is a degeneration of the myelin of both central and peripheral nerves. The myelin is 75% lipid and 25% protein. Although the disease could be attributed to a specific abnormality in either component, there are reports that the protein contains about 27% fatty acid (palmitate and oleate) in a covalent linkage (Wood et al., 1971) and that multiple sclerosis is associated with a derangement in this association (Boggs and Moscarello, 1978) and a reduction in the amount of phosphatidylserine (Winterfield and Debuch, 1977). Another example is diabetes. Cardiolipin depletion in the myocardial tissues occurs in diabetes (Han et al., 2005). Diabetes is also characterized by excessive utilization of fatty acids as metabolic substrates as well as by diminished glucose transport into cells and mitochondrial dysfunction. Some of these dysfunctions could be due to the effects of the disease on the cardiolipin content of the inner mitochondrial membrane.

There have been many reports of secondary rather than primary differences in the lipid portion of the membrane. In these situations, it is the specific membrane protein(s) that somehow become abnormal. Other diseases—for example, heart arrhythmias coupled with sudden death, renal disease, ethanol intoxication, spur cell anemia, and diabetes—likewise result in secondary effects on the membrane lipids. These changes can, however, make the disease state worse by compromising the functionality of the membrane and its role in metabolic regulation. Alvaro et al. (1982) reported that the erythrocyte membrane fatty acids change in humans with liver disease (cirrhosis). In cirrhotic subjects, the membranes contain less phosphatidylethanolamine and more phosphatidylcholine than membranes from normal subjects. Although the membrane cholesterol content remained unchanged, the ratio of cholesterol to phospholipid increased. The fatty acid content of the phospholipid remained unchanged. Finally, cardiolipin, the mitochondrial membrane phospholipid, has an atypical fatty acid profile. The other phospholipids can have a number of different fatty acids attached to the glycerol backbone at positions 1 and 2 and thus be quite diverse in

this respect. Although there could be enormous combinational diversity among cardiolipin species in normal individuals there is not. Using sophisticated analytical tools, Schlame et al. (2005) reported that cardiolipin contained only one or two types of fatty acids and this generated a high degree of structural uniformity and molecular symmetry. The exception was found in patients with Barth's syndrome. Barth's syndrome is characterized by a loss in activity of acyltransferase. With this loss there is a wide diversity of fatty acids found in the cardiolipins of mitochondrial membranes from these patients. Since cardiolipin plays such an important role in mitochondrial physiology and cell viability (Hatch, 2004; Webster et al., 2005), these changes explain some of the features of this syndrome (Zhong and Greenberg, 2005). Additional concerns with respect to fatty acids and disease states are discussed in Chapters 39–53.

D. Diet Effects on Membrane Fatty Acids

Diet can alter the fatty acid content of the membrane phospholipid fatty acids both directly and indirectly (Valentine and Valentine, 2004; Hulbert et al., 2005; Leaf et al., 2005; Cury-Boaventura et al., 2006). Listed in Table 27.2 are some examples of reports in the literature of how the dietary fatty acids can affect the kinds and mounts of fatty acids in the membranes. Although this list is incomplete with respect to many published works in the literature, one can readily realize that the kind of dietary lipid and the lipid in the diet can affect the membrane phospholipid fatty acids. This is especially apparent from those fatty acids deemed essential because of inadequate synthesis (18:2, 20:4). Most animals can synthesize arachidonic acid (20:4) from linoleate (18:2) by desaturation and chain elongation (Sprecher, 1981; Holman, 1986). Felines cannot do this (MacDonald et al., 1984a,b); for this reason, felines must have both the 18:2 and 20:4 fatty acids in their diets. As detailed in Chapter 25 in this volume, palmitate, stearate, and oleate are the main fatty acids

TABLE 27.2
Effects of Dietary Fats on Selected Membrane Fatty Acids[a]

	Fatty Acids (mol. %)							
Diet	**16:0**	**18:0**	**18:1**	**18:2**	**20:4**	**22:6**	**Tissue**	**Reference**
12% Sunflower oil	7.8	25.1	6.3	18.4	19.6	13.2	RHM	McMurchie et al. (1983)
12% Sheep fat	9.1	23.0	9.7	13.7	16.6	16.7	RHM	
12% Sunflower oil	12.5	24.1	7.5	13.8	30.0	7.1	RLM	
12% Sheep fat	14.0	24.3	11.5	11.2	20.4	10.8	RLM	
15% Corn oil	10.9	26.1	5.6	18.5	22.8	6.3	RHM	Royce and Holmes (1984)
15% Hydrogenated coconut oil	10.1	18.3	8.3[b]	15.0	21.9	9.1	RHM	
15% Lard	11.9	28.4	7.5	9.8	26.4	5.3	RHM	
15% Sunflower oil	11.4	11.1	12.9	22.3	9.5	4.7		
4% Lard	11.3	11.1	36.5	4.0	6.1	3.2	Mouse tumor plasma membrane	Solomonson et al. (1976)
Fat-free diet	16.4	7.0	23.5	4.1	10.5	—	RLM	Stancliff et al. (1969)
10% Hydrogenated coconut oil	19.6	19.3	24.7	0.3	7.32	7.7	Rat brain	Anding and Hwang (1986)

Abbreviations: RHM, rat heart mitochondria; RLM, rat liver mitochondria.

[a] Only the major fatty acids are listed here. Membrane phospholipids also contain a variety of other fatty acids in small amounts.

[b] Significant amounts of *trans* 18:1 fatty acid were also found.

synthesized de novo. Monounsaturated as well as polyunsaturated fatty acids can be synthesized from the saturated fatty acids via the activity of the microsomal desaturases. The dietary fat effect on desaturase activity is influenced by the genotype of the consumer. Park et al. (1997) studied two different strains of mice-fed diets containing 4% or 20% coconut or corn oil. The BALB/cByJ mice had significantly less desaturase activity and mRNA than normal C57BL/6J mice. Mice fed the 20% fat (corn oil) diet had less desaturase activity than those fed the 4% fat or the hydrogenated coconut oil diets. The desaturase enzymes (as well as the enzymes for fatty synthesis—acetyl CoA carboxylase, fatty acid synthase complex, for example) can be altered by the nature of the diet and by hormonal status. Note in Table 27.2 that rats fed a high-carbohydrate, fat-free diet have significant quantities of 16:0 and 18:1 fatty acids. Rats fed high-carbohydrate, low-fat diets where the carbohydrate is either sucrose of starch will evidence differences in their fatty acid profiles. Those fed sucrose will have more saturated fatty acids in their membrane phospholipids than those fed starch (Wander and Berdanier, 1985). The large amount of carbohydrate in the diets of these rats stimulated the activity of the enzymes for both de novo synthesis and fatty acid desaturation. This activation was probably due in part of the stimulatory effect of the diet on insulin release. Insulin is a potent stimulant of the desaturase and lipogenic enzymes. In addition, certain of the fatty acids (18:2, 20:4) are involved in the regulation of transcription of the mRNA for the lipogenic enzymes (see Chapter 29). Stearic acid (18:0) serves in a similar fashion for the Δ5 desaturase.

Note in Table 27.2 that within the same dietary treatment, membrane phospholipid fatty acid differs depending on the cell type from which the membrane was extracted. McMurchie et al. (1983a) studied mitochondrial membranes from the heart and liver and found significant differences in the fatty acid profiles between these tissues in rats fed the same diet as well as the aforementioned differences in the profiles of these mitochondria from rats fed different fats. Although it might appear from Table 27.2 that only a few tissues have been studied, this is not the case. The literature is vast. Many cell types have been studied. What can be concluded from Table 27.2 is that the type of dietary fat (degree of saturation), the amount of fat in the diet, and the tissue and organelle examined all contribute to what may be observed with respect to the fatty acid profile of the membrane phospholipids.

E. Hormones and Their Effects on Membrane Lipids

As mentioned above, the hormone insulin can affect the fatty acid profile of the membrane phospholipids through its effect on glucose conversion to fatty acids and through its effect on the desaturases. Other hormones can influence the profile as well. Examples of this influence are shown in Table 27.3. Daily injections of the synthetic glucocorticoid, dexamethasone, resulted in an increase in the mol.% of linoleic acid (18:2) and a decrease in arachidonic acid (20:4). Thyroidectomy resulted in a small increase in rat liver mitochondrial levels of 18:2 and 20:4. Hypophysectomy, which causes a decrease in growth hormone levels, resulted in an increase in 18:2 and a decrease in 20:4 in hepatic mitochondria. Studies on the influence of all the many hormones that affect fatty acid synthesis, phospholipid synthesis, and membrane phospholipid fatty acid levels are not as readily available as are reports on the dietary fat effects on these parameters. However, they are of interest, because these hormones may also have a large influence on the function of the protein components that are embedded in the various membranes. These hormones may act directly on the synthesis and activation of these proteins or indirectly through their effects on the membrane lipids that surround the proteins. Some of these hormones affect desaturase activity and thereby affect the fatty acid profile of the membrane. As mentioned, this could affect the activity of the membrane-embedded enzymes (Hoch et al., 1980, 1981; Withers and Hulbert, 1987). For example, microsomal cytochrome b_5 activity is dependent on the lipid environment. This is, it must be surrounded by a very fluid lipid, and this lipid must have a number of unsaturated fatty acids in it (Hoch et al., 1980). Just as thyroidectomy results in a decrease in membrane phospholipid fatty acid unsaturation, hyperthyroidism has the reverse effect (Mak et al., 1983; Berdanier, 1988a; Raederstorff et al., 1991). The thyroid hormones

TABLE 27.3
Hormonal Effects on Fatty Acids Profiles of Selected Tissues

	Fatty Acids (mol. %)							
Treatment	**16:0**	**18:0**	**18:1**	**18:2**	**20:4**	**22:6**	**Tissue[b]**	**Reference**
1 mg GC/day	14.6	22.7	6.6	28.5	19.0	3.5	Liver	Huang et al. (1986)
Control	17.0	20.3	7.6	15.1	27.4	1.5	Liver	
Thyroidectomy	12.5	21	8.6	19.5	19.0	8.1	RLM	Withers and Hulbert (1987)
Control	13.0	20	9.0	17.4	15.7	7.0	RLM	Hoch et al. (1981)
Hypophysectomy	27.1	21.5	13.5	17.0	14.5	2.0	RLM	Clejan and Maddaiah (1986)
Control	27.4	22	12.2	12.1	17.4	2.8	RLM	
Diabetes[a]	24.5	23.3	7.0	22.4	15.2	4.2	RLM	Labonia and Stoppani (1988)
Control	16.9	23.2	9.3	22.7	22.2	3.1	RLM	

Abbreviations: GC, synthetic glucocorticoid, dexamethasona; RLM, rat liver mitochondria.

[a] Streptozotocin-induced diabetes.

also affect fatty acid elongation by inducing an increase in the activity of the microsomal fatty acid elongation system, whereas they have little effect on mitochondrial elongation (Landriscina et al., 1976). Thyroxine also affects the synthesis of phospholipids. Pasquini et al. (1980) reported that the incorporation of labeled choline into brain and liver phosphatidylcholine was less in thyrotoxic rats than in normal rats. In turn, the type of dietary fat affects triiodothyronine (T_3) production (Takeuchi et al., 1995; Kim and Berdanier, 1998). Rats fed a lard diet had lower serum T_3 levels and Na^+, K^+-ATPase activity in liver and muscle than did rats fed a safflower oil diet (Takeuchi et al., 1995). Diabetes-prone BHE/cdb rats fed a hydrogenated coconut oil diet had higher levels of thyroxine and T_3 than rats fed a corn oil or menhaden oil diet. Sprague-Dawley rats fed these same diets had no difference in T_3 levels, and the difference in thyroxine was significant only between the coconut oil and corn oil groups not between the corn and menhaden oil groups (Kim and Berdanier, 1998). In this same study, there were similar strain differences in the effects of these fats on mitochondrial fluidity and function.

Nuclear T_3 receptor number (but not binding affinity) on the other hand is affected by the type of dietary fat (Knopp et al., 1992). Rats fed a saturated fat diet had decreased T_3 receptor binding in the nucleus. Hypothyroidism is characterized by reduced phosphate transport (Paradies and Ruggiero, 1990; Paradies et al., 1991) as well as by a reduction in mitochondrial respiratory activity and ATP synthesis efficiency (Hoch, 1968; Hulbert et al., 1976; Sugiyama et al., 1991). Essential fatty acid deficiency mimics many of these same effects both with respect to mitochondrial fatty acid composition, fluidity, and function (Levin et al., 1957; Hayashida and Portman, 1963; Ito and Johnson, 1964; Stancliff et al., 1969; Williams et al., 1972; Divakaran and Venkataraman, 1977; Rafael et al., 1984).

F. Age Effects on Membrane Lipids

As animals age, their hormonal status changes, as does the lipid component of their membranes (Pepe, 2005a,b). With age, there is a decrease in growth hormone production, an increase followed by a decrease in the hormones for reproduction, and, as the animal develops, larger fat stores. There may develop peripheral resistance of the action of insulin, and insulin levels may rise as a result of increased fat cell size. As mentioned in the preceding section, these hormones can affect the lipid portion of the membranes within and around the cells and hence affect how these cells regulate their metabolism. Yechiel and Barenholz (1985) studied aging myocytes isolated from newborn rats. As these cells aged *in vitro*, sphingomyelin and cholesterol levels increased by 100% and 50%, respectively, whereas phosphatidylcholine fell by 15%–20%. There were no changes in total

phospholipids. These myocytes were grown in vitro in a defined medium without the influences of the many factors that, in the aging whole body, can affect membrane lipids. Hegner (1980) studied rats as they aged. He found that with age, the degree of unsaturation of the plasma membrane fatty acids decreased and the cholesterol level rose. He also reported that with age, there was an increase in the number of superoxide radicals and proposed that this increase led to the degradation of the membrane lipids, which in turn might explain the age-related changes in membrane function. Others (Wahnon et al., 1989; Yen et al., 1989) have reported that membranes from aging animals are less fluid and have reduced transport capacities. Yen et al. (1989) reported that, as human's age, there is a decline in hepatic mitochondrial respiratory rate and a decrease in the respiratory rate and a decrease in the respiratory ratio and the ADP/O ratio. Wei and Kao (1996) have also shown that free radicals rise with age as does the number of mitochondrial DNA mutations. This might explain the age-related decline in mitochondrial respiratory function as reported to occur in aging rats as well as humans. Since mitochondrial membranes are rich in unsaturated fatty acids, free radicals could readily form and damage mitochondrial DNA which in turn would result in malfunctioning respiratory proteins (Chen et al., 1972; Murfitt and Sanadi, 1978; Berdanier and McNamara, 1980; Kim and Berdanier, 1988). In addition, there are reports of an age-related decrease in membrane fatty acid unsaturation coupled with a decrease in membrane fluidity and a decrease in the exchange of ATP for ADP across the mitochondrial membrane (Nohl and Kramer, 1980), a decrease in ATP synthesis (Clandinin and Innis, 1983), and an amelioration of these age-related decreased mitochondrial function by restricted feeding (caloric intake reduced by 50% over the lifetime of the animals) (Weindrach et al., 1980).

III. MEMBRANE FUNCTION

In the previous section, the importance of diet, age, and hormonal status was described in terms of their influence on the composition of the membrane lipids. Although not emphasized, these compositional differences have important effects on metabolic regulation. This regulation consists of the control of the flux of nutrients, substrates, and/or products into, out of, and between compartments of the cell.

The terms metabolic regulation implies that metabolic flux is controlled such that there is an interdigitation of the pathways within cellular compartments, between compartments, between cells or tissues, and between tissues within the body and that this is closely regulated. The mechanisms that control metabolism at all of these levels are various. Both genetic and dietary factors may influence these mechanisms, and individuals within a population group may vary in the activity of given reactions or pathways. Hence, we have genotypes for obesity and/or diabetes whose controls of lipid metabolism and/or glucose metabolism are aberrant regardless of diet, and we have other genotypes whose phenotypic expression of their genotype is inhibited or enhanced by the composition of the diet they consume. Still other genotypes will express normal phenotypic features of tight control of energy balance, fat synthesis, fat storage, glucose use, glycogen synthesis, and so forth. In normal individuals, metabolic control mechanisms include redox state, calcium ion flux, phosphorylation state, substrate and product levels, enzyme amount and activity, concentrations of cofactors, coenzymes, hormones, second messengers such as cAMP, and probably a whole host of other factors as yet not identified or described. Some of these are listed in Table 27.4.

Although the cellular membranes serve as geographical boundaries of the cell and its compartments, these structures have metabolic control properties as well. They are the "gatekeepers" of the cells and their compartments. They regulate the influx and efflux of nutrients, substrates, hormones, and metabolic products produced or used by the cell or compartment in the course of its metabolic activity. For example, the mitochondrial membrane through its transport of two- and four-carbon intermediates and though its exchange of ADP of ATP regulates the activity of the respiratory chain and ATP synthesis. If too little ADP enters the mitochondria because of decreased ADP transport across the mitochondrial membrane, respiratory chain activity will decrease, less ATP will be

TABLE 27.4
Dietary Effects on Membrane-Embedded Enzyme or Transporter or Receptor Function

Diet Treatment	Effect	Comment	Reference
Coconut vs. safflower oil (20% w/w diet)	↑ Δ5 Desaturase activity	↑ mRNA for stearoyl CoA	Dang et al. (1989); Park et al. (1997)
Coconut vs. soybean oil (15% w/w)	↑ Alkaline phosphatase activity	No effect on enterocyte plasma membrane fluidity	Wahnon et al. (1992); Stenson et al. (1989)
Mackeral vs. soybean oil (15% w/w)	↓ Alkaline phosphate activity	No effect on enterocyte plasma membrane fluidity	Wahnon et al. (1992); Stenson et al. (1989)
Fish vs. vegetable oil in a fat mixture (50% w/w diet)	↓ Sensitivity of carnitine palmitoyl transferase I to inhibition	Increase in hepatic outer mitochondrial membrane fluidity	Clouet et al. (1995)
n-6 vs. n-3 deficiency (60% fat w diet)	↓ Secretagogue effect of prolactin on mammary cells	Plasma membrane fatty acids reflected dietary fatty acids	Ollivier-Bousquet et al. (1993)
Lard vs. safflower oil	↓ Hepatic and muscle Na^+K^+ATPase activity No difference in lipoprotein lipase activity ↓ Serum T_3 levels	Rats were fatter when fed the lard diet	Takeuchi et al. (1995)
Coconut vs. corn oil (5%–6% w/w) or a fat-free diet	↑ Activity of soluble and microsomal phosphatidate phosphatase ↑ α-Glycerol-P shuttle ↓ Coupling of mitochondrial respiration to ATP synthesis	↑ Mitochondrial membrane fluidity ↓ 18:2 and 20:4 content of membrane lipid	Deaver et al. (1986); McMurchie et al. (1983a–c); Stillwell et al. (1997); McMurchie and Raison (1979); Kim and Berdanier (1998)
Sheep vs. safflower oil	No change in ion transporting ATPases	No change in plasma membrane fluidity; membrane fatty acid profile reflects diet fat	Abeywardena et al. (1984)

synthesized, and there may be a decrease in other mitochondrial reactions that are either driven by ADP influx or dependent on ATP availability. Through its export of citrate from the matrix of the mitochondria, it regulates the availability of citrate to the cytosol for cleavage into oxaloacetate and acetyl CoA, the beginning of fatty acid synthesis. If more citrate is exported from the mitochondria than can be split to oxaloacetate and acetyl CoA, this citrate will feed back onto the phosphofructokinase reaction and glycolysis will be inhibited. Thus, the activity of the mitochondrial membrane tricarboxylate transporter has a role in the control of cytosolic metabolism. Other transporters such as the dicarboxylate transporter or the adenine nucleotide translocase have similar responsibilities vis-á-vis the control of cytosolic and mitochondrial metabolic activity. Receptors embedded in the plasma membrane have a similar function; that is, they control the entry of nutrients or hormones into the cell. Further, the plasma membrane hormone receptor may bind a given hormone, and, with binding, elicit a cascade of reaction characteristic of the hormone effect without permitting the entry of the hormone itself into the cystosolic compartment. An example here is the hormone insulin. Insulin binds to its receptor and in so doing elicits the cascade of events that include the transport and metabolism of glucose by the cell. The insulin, bound to the receptor site, is inactivated and is brought into the cell by pinocytosis for further degradation. Other hormones, notably the nonprotein steroids and the low-molecular weight hormones such as epinephrine and thyroxine, pass through the plasma membrane and attach to receptors in the cytosol and/or nuclear membrane or on the endoplasmic reticulum. Once attached to their respective biding sites, they also elicit a metabolic response.

These examples illustrate the importance of the cellular and intracellular membranes in the regulation of metabolism. They also illustrate the fact that the gatekeeping property of the membrane is vested in the structure and function of the various transporters and receptors or binding proteins embedded in the membrane. Whereas the genetic heritage of an individual determines the amino acid sequence of the proteins and hence their function, this function can be modified by the lipid milieu in which they exist. An example of a diet–genetic interaction with respect to membranes is seen in the function of the mitochondrial $F_1 \cdot F_0$ ATPase. This enzyme complex consists of two parts. The base, the F_0 is embedded in the inner mitochondrial membrane while the stem and head projects out into the mitochondrial matrix. Both parts must be mobile. They rotate around the stem as the F_0 captures the energy generated by the respiratory chain and transmits it to the head which traps it in the high-energy bond of the ATP. Rotation of the F_0 proton is hindered when the inner mitochondrial membrane contains more then the usual amounts of saturated fatty acids. This occurs when the animal is fed hydrogenated coconut oil or an essential fatty acid-deficient diet. Indeed, essential fatty acid deficiency is characterized by a reduction in ATP synthesis. In the diabetes-prone BHE/cdb rat (a rat with a mutation in the mitochondrial ATPase 6 gene), ATP synthesis efficiency is reduced. When this rat is fed a highly saturated fat, the combined effects of the diet and the genetic defect are additive: that is, there is a considerable loss in ATP synthesis efficiency (Deaver et al., 1986; Kim and Berdanier, 1998). Thus, diet as in a fatty acid deficiency, or genetics, or an interaction of both these factors can result in mitochondrial malfunction. Fatty acid deficiency affects other cell types as well. Ollivier-Bousquet et al. (1993) showed that a deficiency of n-6 fatty acids but not n-3 fatty acids inhibited the secretagogue effect of prolactin on lactating rat mammary epithelial cells. This is due to inadequate supplies of arachidonic acid in the mammary cell plasma membrane. Arachidonic acid is an essential component of the signal cascade that is elicited when the hormone prolactin is bound to its cognate receptor. This cascade involves the conversion of arachidonic acid to the E series of eicosanoids. Diet, hormonal state, and genetics in turn control the lipid milieu in terms of the kinds and amounts of the different lipids that are synthesized within the cell and incorporated into the membrane.

In the membranes are a variety of closely packed proteins and lipids. The membrane-bound proteins have extensive hydrophobic regions and usually require lipids for the maintenance of their activity. Adenylate cyclase (Engelhard et al., 1978), Cytochrome b_5 (Holloway et al., 1982), and cytochrome c oxidase (Robinson, 1982) have all been shown to have phospholipid affinities. Cytochrome c oxidase (E.C.1.9.3.1) from mitochondria has tightly bound aldehyde lipid that cannot be removed without destroying its activity as the enzyme that transfers electrons to molecular oxygen in the final step of respiration (McMillen et al., 1986). Marinetti and Cattieu (1982) found a number of membrane proteins that have tightly bound fatty acids as part of their structures. These fatty acids are covalently bound to their proteins as a post-translational event and act of direct, insert, and anchor the proteins in the cell membranes. Alkaline phosphatase in the enterocyte is an example of one such enzyme. This enzyme increases in activity as the membrane increases in eicosapentaenoic acid (20:5) without any change in membrane fluidity (Stenson et al., 1989; Wahnon et al., 1992). Alkaline phosphatase is an integral enzyme known to be intimately associated with the hydrophobic core of the intestinal microvillus plasma membrane. Other lipids are bound differently to membrane proteins. Some are acylated acids with fatty acids during their passage from their site of synthesis on the rough endoplasmic reticulum to the membrane, whereas others acquire their lipid component during their placement in the membrane. β-Hydroxybutyrate dehydrogenase (E.C.1.1.1.30), for example, requires the choline head of phosphatidylcholine for its activity (Isaacson et al., 1979). If hepatocytes are caused to increase their synthesis of phosphatidylmethylethanolamine, which substitutes for phosphatidylcholine in the membrane, β-hydroxybutyrate dehydrogenase activity is reduced (Clancy et al., 1983).

On the other hand, low membrane levels of phosphatidylethanolamine are related to low levels of calcium pump activity in the muscle and high levels of calcium pump activity in reconstituted membrane systems (Hui et al., 1981; Hidalgo et al., 1982; Navarro et al., 1984). The difference

between the activity of the calcium pump in the reconstituted system and in the muscle preparation probably has to do with the conformation of the phospholipid in the two preparations. In the reconstituted system, the phosphatidylethanolamine assumes a form that allows for a lipid bilayer to surround the pump and stimulate it. If in a monolayer (or prevented from surrounding the pump to hold it in its active form), then the pump is less active (Yeagle, 1989).

Cholesterol also modulates the ion pumps in the plasma membrane. Two of these, the Na^+,K^+-ATPase and the Ca-ATPase, have been studied (Yeagle, 1989). The Na^+,K^+-ATPase is responsible for pumping sodium out of the cell and potassium into the cell against their respective gradients. The ratio of Na^+ to K^+ is 3:2, so the pump is electrogenic. Thus, this enzyme has a central role in metabolic regulation. High levels of membrane cholesterol are associated with low levels of Na^+,K^+-ATPase activity (Kimelberg and Paphadjopoulus, 1974; Giraud et al., 1981; Yeagle, 1983). Decreased plasma membrane fluidity, due, not only to increase cholesterol content but also to an increase in the amount of saturated fatty acids in the phospholipids, also results in decreased Na^+,K^+-ATPase activity (Solomonson et al., 1976; Storch and Schachter, 1984). Adenine nucleotide exchange via adenine nucleotide translocase is also affected by the mitochondrial membrane lipid. It is less active in less fluid membranes (Mak et al., 1983). Mg^{2+} ATPase is 18 times as active in the presence of lysolecithin and to a smaller extent in the presence of cardiolipin, phosphatidylinositol, and phosphatidylethanolamine as in the presence of lecithin or in a lipid-free environment (Swanljung et al., 1973). If ATP synthesis is less due to less active Mg^{2+} ATPase, and if ADP influx and ATP efflux from the mitochondria are decreased, respiration (which is dependent on ADP influx) will also decrease. Cytosolic reactions that are affected by phosphorylation state (ATP:ADP) will be decreased or increased depending on the reaction in question. Lipogenesis is favored by an increase in the phosphorylation state, whereas gluconeogenesis is favored by a decrease.

Microsomal HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis, is similarly affected (Wulfert et al., 1981), as are the plasma receptor proteins for insulin, glucagon (Liepnieks et al., 1983), prolactin (Knazek and Liu, 1979), epidermal growth factor (Shoyab and Todaro, 1981), and the enkephalins (Deber and Behnam, 1984).

The hepatic mitochondrial membrane fatty acid composition in relation to the activities of the enzymes embedded in this membrane has been particularly well studied. This tissue is very responsive to dietary changes and has received considerable attention, because the mitochondria manifest a number of functional changes as part of the animal's response to a diet deficient in essential fatty acids. The uncoupling of mitochondrial respiration to ATP synthesis has been reported, as has the increased activity of the α-glycerol phosphate shuttle (Levin et al., 1957; Divakaran and Venkataraman, 1977; Deaver et al., 1986). Increased respiratory chain activity and increased succinate dehydrogenase activity in rats fed an essential fatty acid–deficient diet has also been reported (Hayashida and Portman, 1963; Ito and Johnson, 1964; Williams et al., 1972; Rafael et al., 1984; Royce and Holmes, 1984). When these findings are coupled with the reports on the effects of diet, particularly the effects of low-fat or no-fat diets, on the membrane phospholipid fatty acid composition and function, one can readily appreciate the importance of these membranes in the regulation of metabolism throughout the body. One can also appreciate the fact that Mother Nature has designed a number of systems that, under normal conditions of diet, exercise, growth, and health, ensure the maintenance of the most appropriate fluidity in each and every cell membrane. Only those conditions that cause severe perturbations in metabolism will affect cell membrane fluidity. If highly saturated fats are produced de novo, the cell increases the activity of the microsomal desaturases; the ratio of unsaturated fatty acids to saturated fatty acids to cholesterol is thus carefully controlled. On top of this is the maintenance and positioning of amounts of the different phospholipids. There is an asymmetrical distribution of these phospholipids (Krebs et al., 1979) just as there is an asymmetrical distribution of the membrane proteins. As mentioned, certain of these proteins require certain phospholipids for their activity. Fortunately, these molecules are arranged according to need. This asymmetry may be due in part of the action of the different phopholipases (Fujii and Tamura, 1979) although these lipases may be found on either side of a given membrane, activation may not be

equal of the two sides, thus leaving some phospholipids degraded, whereas others are left in place. Also, certain of the phospholipids may be protected by the proteins that are closely associated with them. The membrane phospholipids do turn over rapidly, and they exchange their fatty acids rapidly with their neighbors and with the fatty acids present in the media surrounding them. Thus, there is ample opportunity to maintain a steady state with respect to membrane lipid composition and function.

There is one further consideration where membrane lipid has a profound effect on cell function and that is the role of lipid particularly cardiolipin in programmed cell death, apoptosis. This is the process whereby the body regulates cell number (cell turnover). Cell turnover has two parts: cell replacement and cell death. Cell death is either a concerted, all at once, event (necrosis) or a programmed, gradual process. Necrotic cell death is preceded by cell enlargement and a swelling of all the organelles within the cell. The DNA disintegrates and its nucleotides are degraded. In contrast, programmed cell death or apoptosis involves a shrinkage of the cell and enzyme catalyzed DNA fragmentation. This fragmentation can be detected as a laddering when the DNA is extracted from the cell and separated by electrophoresis. Apoptosis is viewed as a defense mechanism to remove unwanted and potentially dangerous cells such as virally infected cells or tumor cells. The process involves the mitochondria as the "central executioner." The mitochondria produce reactive oxygen (free radicals) as well as other materials that participate in apoptosis. Alterations in mitochondrial function have been observed to occur prior to any other feature of apoptosis. A decrease in mitochondrial membrane potential that in turn affects membrane permiability is an early event. The increase in permiability is followed by a release of cytochrome c. Cytochrome c acts as a cardiolipin oxygenase required for the release of proapoptoctic factors (Kagan et al., 2005; Orrenius and Zhivotovsky, 2005). In turn, cytochrome c regulates the caspases, which are cysteine proteases. These caspaces stimulate the proteolysis of key cell proteins in various parts of the cell. Altogether the fragmentation of the DNA and the destruction of cell proteins result in the death of the cell via a very orderly process.

Apoptosis is the mechanism used in the thymus to eliminate thymocytes that are self-reactive. By doing so the development of autoimmune disease is suppressed. Figure 27.2 illustrates the process of apoptosis and Table 27.5 lists some of the factors that influence apoptosis.

Many cells can be stimulated to become apoptotic. The p53 protein, for example, can induce apoptosis as one of its modes of protecting the body against tumor cells. The p53 protein is a DNA-binding protein. Mutations in tumor cells have been found that inactivate p53. The result of such mutation is that tumor cells grow and multiply. The gene for p53 likewise can mutate and this mutation has been associated with tumor development and growth. Apparently, this gene has several "hot spots" (likely places for mutation to occur), which explains its role in carcinogenesis and its

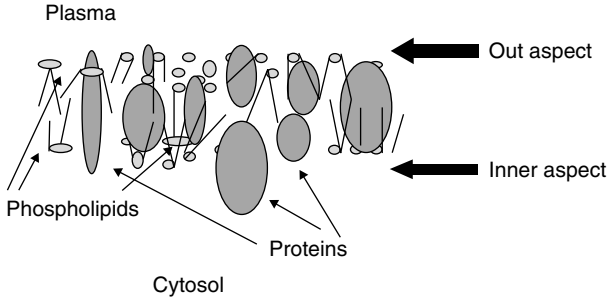

FIGURE 27.2 Cartoon representation of the membrane structure showing that some proteins extend through the lipid bilayer while others can be found on either aspect of the membrane. The phospholipids are shown as having their fatty acids (the straight lines) extending into the center of the bilayer while the phosphatidyl component is on either aspect of the membrane.

TABLE 27.5
Factors That Influence Apoptosis

Factor	Effect
Age	↑
Apaf-1 protein	↑
p38 protein	↑
p53 protein	↑
Bcl-2 protein	↓
Bcl-x_3 protein	↑
Bcl-x_L protein	↓
Bax protein	↑
Bak protein	↑
WAF-1	↑
ced 3 and 4	↑
Glucocorticoids	↑
ced 9	↓
High zinc	↓
Low zinc	↑
Low-retinoic acid	↓
High-retinoic acid	↑
Interleukin 1β	↑
Leptin	↑
TNFα	↑
Insulin	↓
Low manganese	↑
Insulin-like growth factor (IGF_1)	↓
Low-SOD activity	↑
Peroxidized lipid	↑

loss in normal function. The bcl-2 protein, a membrane bound cytoplasmic protein, is another player in this regulation of apoptosis. It is a member of the family of proteins called protooncogenes. Its normal function is to protect valuable cells against apoptosis. It is down regulated when cells are stimulated to die. Inappropriately, high bcl-2 protein levels can provoke cell overgrowth. The cell regulates bcl-2 protein so as to maintain a normal homeostatic state. Bcl-2 and its homologue, Bcl-x_L, blocks apoptosis. Both Bcl-2 and Bcl-x_L can heterodimerize with Bax or Bcl-x_s and when Bcl-2 or Bcl-x_L is over expressed an enhancement of oxidative stress mutagenesis can be observed. This occurs because these proteins suppress the apototic process allowing more exposure of the cell to DNA damage by mutagens. As an example, if there is a deficiency of respiratory complex I as happens in several of the mitochondrial genetic diseases, this deficiency primes BAX-dependent apoptosis through mitochondrial oxidative damage (Perier et al., 2005).

Nutrients play a role in apoptosis. Intakes of highly peroxidized lipids or lipids that are easily peroxidized, that is, fish oils, can stimulate apoptosis. High levels of zinc (500 μm) have been found to block apoptosis in cultured thymocytes. The zinc blocks the action of glucocorticoids in stimulating apoptosis. Zinc also interferes with tumor necrosis factor (TNF) induced apoptosis as well as heat-induced death. In contrast, low levels of zinc (0.3–200 μm) have the reverse effect. That is, at low levels of zinc, apoptosis could be stimulated by TNF, glucocorticoid and heat exposure. TNF induces apoptosis through an alteration of membrane lipid (Sandra et al., 2005). Manganese, another essential nutrient, also is involved as a cofactor for superoxide dismutase (SOD). In instances where SOD is less active than normal, apoptosis is stimulated coincident with an increase in C2-ceramide,

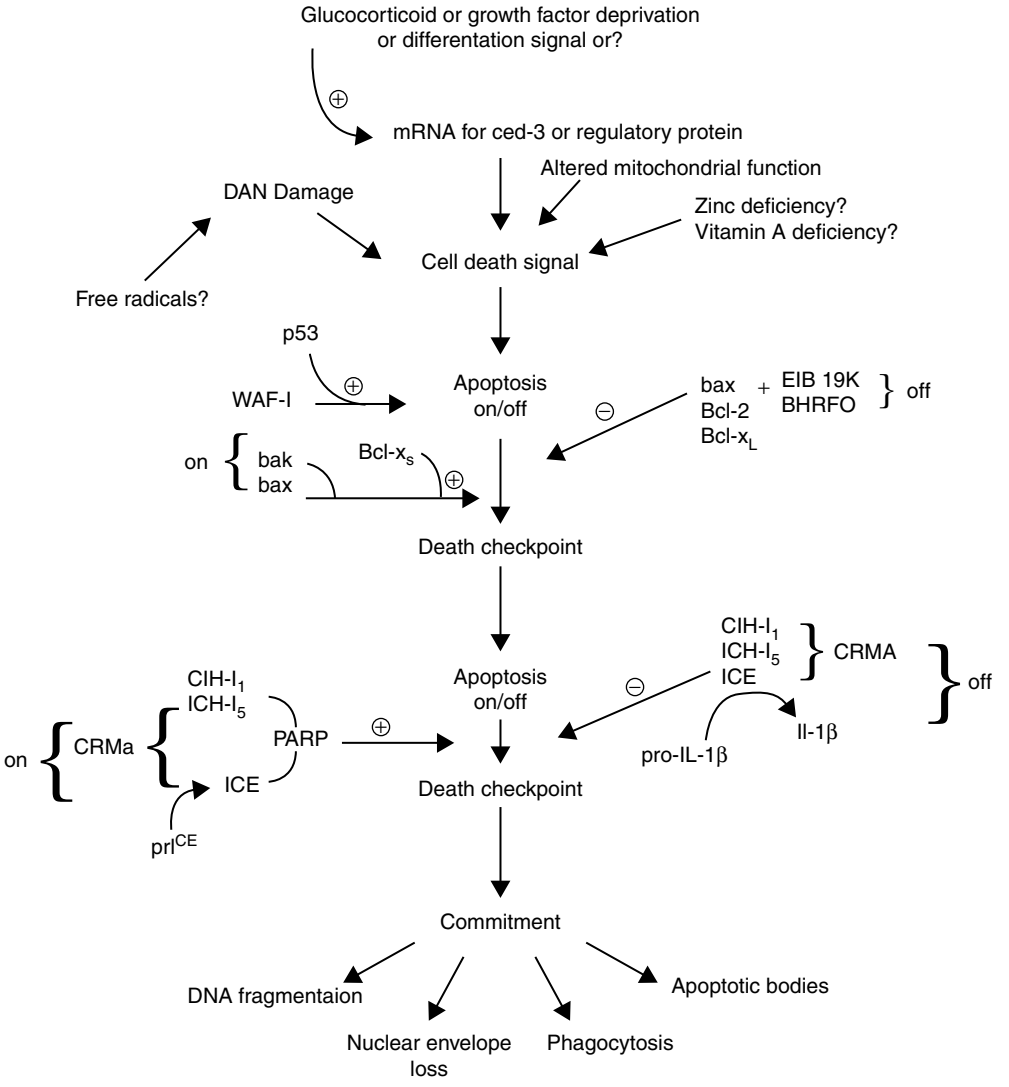

FIGURE 27.3 Roles of fatty acids in gene expression and cell signaling.

TNFα, and hydrogen peroxide. Ceramide displaces cholesterol from lipid rafts and decreases the association of the cholesterol-binding protein caveolin-1 (Yu etal., 2005). Ceramide is a positively charged molecule that induces mitochondrial permeabilization (Novgorodov et al., 2005). Ceramide induces nonapoptotic death as well (Kim et al., 2005). Peroxides form from unsaturated fatty acids as well as certain of the amino acids. There may be a link therefore between the fatty acid intake of the individual and apoptosis of certain cell types. In one study of genetically obese diabetic rats, an increase in fatty acid intake was associated with an increase in β cell apoptosis.

Retinoic acid (and dietary vitamin A) deficiency stimulates apoptosis. The well known feature of vitamin A deficiency (suppressed immune function) suggests that vitamin A and its metabolite, retinoic acid, serve to influence the sequence of events leading to cell death. This link is made because it is known that T cell apoptosis is an important defense mechanism and T cells are part of the immune system. Vitamin A is also an antiproliferative agent so its effect on abnormal cell growth is two pronged. One, it stimulates apoptosis and two, it suppresses proliferation.

Genetic regulation of apoptosis involves not only the transcription factors mentioned above but also specific genes. Already described are the p53 and bcl-2 DNA-binding proteins that (like zinc, retinoic acid, and fatty acids) affect gene expression. Add to this list the mitogen activated protein kinases (p42/44) Erk1 and Erk2 and mitochondrial p38 mitogen-activated protein kinase (Kong et al., 2005). When phosphorylated these proteins suppress apoptosis in brown adipocytes. Apoptosis is also suppressed by certain of the cytokines (IL6, IL3, and interferon γ) and stimulated by others (leptin). Lastly, the gene Nedd 2 encodes a protein similar to the nematode cell death gene ced-3 and the mammalian interleukin 1β converting enzyme. Overexpression of this gene induces apoptosis.

With the understanding of apoptosis comes the understanding of how and why the body changes in its composition as it ages from conception to death. The young animal has little fat while the older one has gained fat sometimes at the expense of body protein. Clearly, the body is continually being remodeled and quite clearly this remodeling is the result of a combination of many factors including the apoptotic process.

IV. CONCLUSION

This chapter presents an overview of the many factors that affect the fatty acid composition of the membrane phospholipids. These membrane lipids under normal circumstances are carefully balanced so as to maintain optimal function of the membrane as an important determinant of metabolic function of the cell. Not included in this chapter is a summary of the signaling properties of some of the complex lipids, eicosanoid formation, or the roles of specific fatty acids in gene expression (Figure 27.3). This information is covered in the chapters that follow.

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28 Dietary Fatty Acids and Eicosanoids

Joo Y. Lee and Daniel H. Hwang

CONTENTS

I. INTRODUCTION

One of the important functions of dietary fat is to provide essential fatty acids (EFAs). However, the requirement for EFAs as linoleic acid (18:2n-6) is only 1%–2% of the total caloric intake. Thus, EFA deficiency seldom occurs in the general population consuming ordinary diets. In spite of this, much greater amounts of intake for dietary polyunsaturated fatty acids (PUFAs) are generally recommended. This recommendation is primarily based on the cholesterol-lowering action of dietary PUFAs, and this, in part, resulted in changes in trends in the consumption of dietary fat in the United States (Stephen and Wald, 1990).

Dietary PUFAs has been generally used as an entity without differentiating the type of PUFA. Epidemiological, clinical, and biochemical studies conducted during the past 20 years suggest that dietary n-3 PUFA is beneficial in reducing certain risks of chronic diseases. Revelation of the difference existing between n-6 and n-3 fatty acids in metabolic pathways leading to the formation of eicosanoids and their precursor acids suggests that not only the level of PUFAs but also the types of PUFAs need to be defined for recommendation of dietary PUFAs in dietary guidelines in the future.

In this chapter, discussion is focused on how different dietary fatty acids affect the capacity of tissues to synthesize eicosanoids with diverse physiological actions and their nutritional implications.

II. DIETARY PUFAs AS A SOURCE OF ESSENTIAL FATTY ACIDS

In 1929, Burr and Burr first reported the essentiality of dietary fat (Burr and Burr, 1929). Later, it became clear that the essentiality of dietary fat is due to the requirement of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), which cannot be synthesized by mammals. The requirement for linoleic acid is known to be only 1%–2% of the total daily caloric intake (Holman, 1970). This requirement is based on the amounts of linoleic acid that can clear the dermal lesion resulting from EFA deficiency or the amounts that normalize the triene/tetraene ratio (Mohrhauer and Holman, 1963). Ordinary American diets can provide linoleic acid at amounts much higher than required. Thus, EFA deficiency (based on overt symptoms and an increased triene/tetraene ratio) rarely occurs in the general population. However, it should be noted that the amount of EFAs required to prevent the deficiency symptoms may not represent the amount needed for optimal functioning of the body.

The major PUFAs in our diets are the n-6 fatty acids including linoleic acid, which is the predominant PUFA in most edible vegetable oils consumed in the United States. The major vegetable oils consumed in the United States are soybean, corn, and cottonseed oils, which comprise more than 82% of the total fats and oils used in foods (ISEO, 1988). More than 50% of the total fatty acids of these vegetable oils are linoleic acid.

Although a wide range of symptoms was ascribed to the deficiency of EFAs, their biological functions were unknown until early 1964, when Van et al. (1964) and Bergstroem et al. (1964) demonstrated that prostaglandins (PGs) are synthesized from PUFAs derived from dietary EFAs in animal tissues. It is now generally recognized that EFAs have both a structural role in maintaining the integrity of cell membranes and a functional role in serving as precursors of eicosanoids.

III. DIETARY ESSENTIAL FATTY ACIDS AS PRECURSORS OF EICOSANOIDS

Long-chain PUFAs, which are the precursors of eicosanoids, can be generated by the desaturation and elongation of dietary fatty acids in tissues, as shown in Figure 28.1.

Oleic acid (18:1n-9), which is the most abundant fatty acid in our food system, can be converted to 20:3n-9. This fatty acid cannot serve as a substrate for cyclooxygenase (COX). Therefore, no PGs is synthesized from 20:3n-9. However, 20:3n-9 is a substrate for 5-lipoxygenase (5-LOX) from rat RBL-1 cells leading to the formation of leukotriene A_3 (LTA_3) (Stenson et al., 1984).

There is an indication that 20:3n-7 derived from palmitoleic acid can be converted to PGs (Lands et al., 1977). However, 20:3n-7 does not accumulate in animal tissues in significant amounts. It has been shown that the 5- LOX in RBL-1 cells requires double bonds at C-5 and C-8 for catalysis to occur. This structural requirement suggests that 20:3n-7 is not a substrate for 5-LOX (Jakschik et al., 1980).

Linoleic acid (18:2n-6) can be desaturated and elongated to arachidonic acid (20:4n-6) in animal tissues. Arachidonic acid is the predominant 20-carbon PUFA present in most tissues of terrestrial animals. Thus, eicosanoids synthesized in animal tissues are mostly derived from arachidonic acid. The metabolic map for the arachidonic acid cascade is shown in Figure 28.2. Arachidonic acid is a precursor of "2-series" PGs and thromboxanes and 4-series leukotrienes. The biosynthesis and physiological actions of various eicosanoids have been reviewed extensively (Nicosia and Patrono, 1989; Samuelsson, 1983; Samuelsson et al., 1987; Smith et al., 1991).

α-Linolenic acid (18:3n-3) can be converted to longer-chain n-3 PUFAs by the same desaturases used for n-6 or n-9 fatty acids. The extent to which α-linolenic acid is converted to longer-chain PUFAs is not known. Dyerberg et al. (1980) suggested that α-linolenic acid is not converted to 20:5n-3 in humans on the basis of their observations that insignificant amounts of 20:5n-3 in plasma lipids were found in human subjects who consumed linseed oil. Similarly, Adam et al. (1986) showed

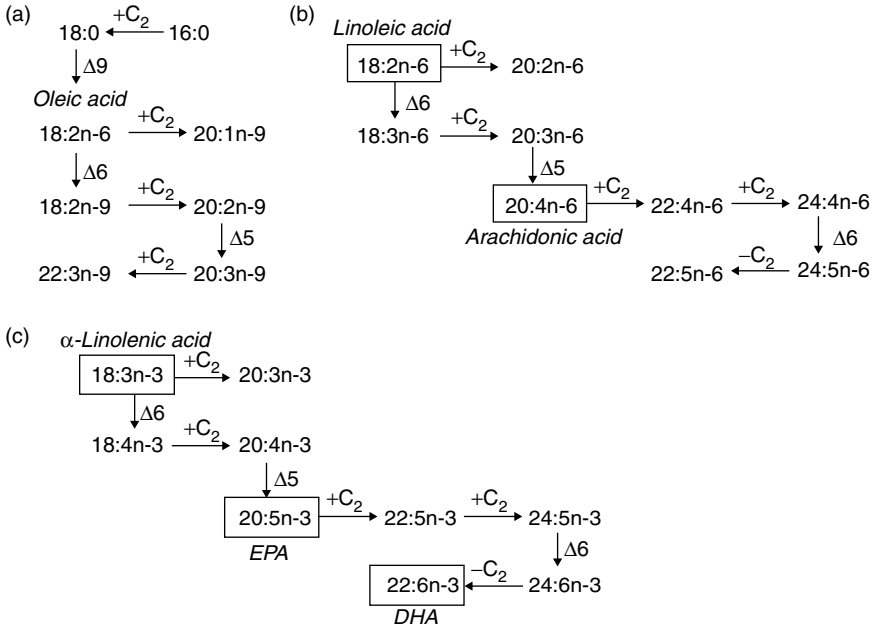

FIGURE 28.1 Conversion of dietary fatty acids to longer-chain polyunsaturated fatty acids and precursor acids for eicosanoids. The first number denotes the number of carbon atoms, the number after the colon denotes the number of double bonds, and the number after n denotes the position of the last double bond from the methyl end of fatty acids. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

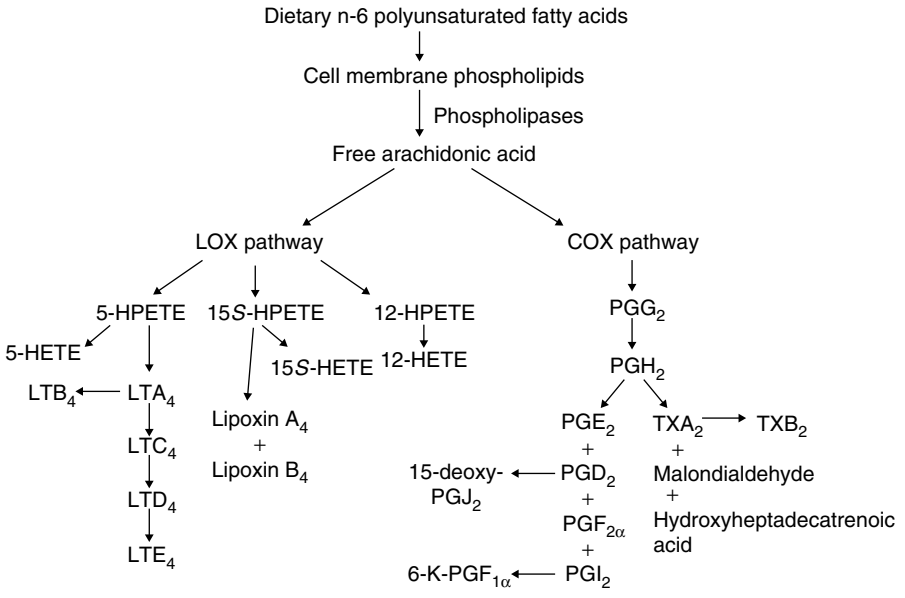

FIGURE 28.2 Eicosanoid formation from arachidonic acid via the cyclooxygenase and lipoxygenase pathways. LOX, lipoxygenase; COX-2, cyclooxygenase; LTA_4, leukotriene A_4; TXA_2, thromboxane A_2; PGH_2, prostaglandin H_2; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid.

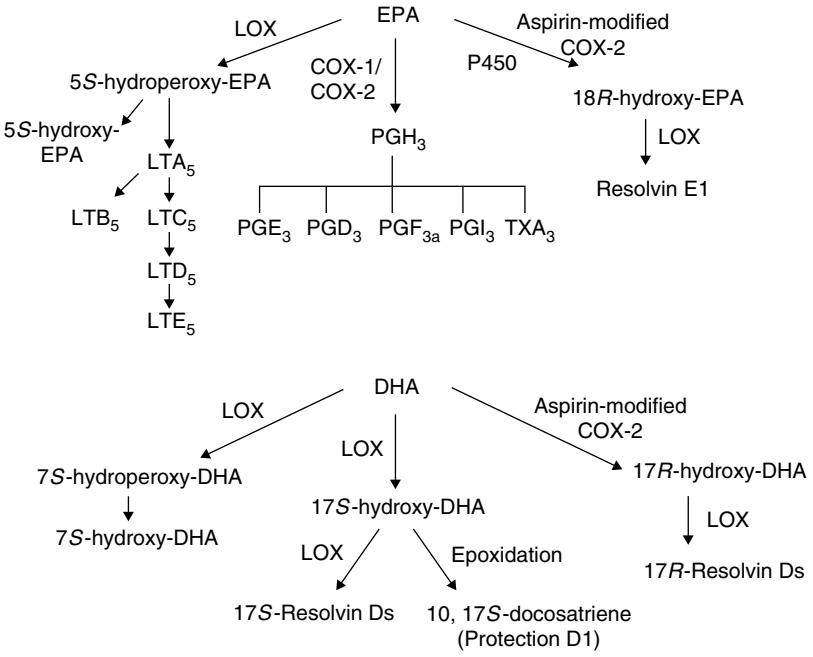

FIGURE 28.3 The biosynthesis of prostanoids, resolvins, and protectins from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The metabolism of EPA by cyclooxygenase (COX) leads to the formation of the "3-series" of prostanoids. The acetylated COX-2 by aspirin produces 18*R*-hydroxy-EPA in endothelial cells that are sequentially metabolized to Resolvin E1 by LOX in neutrophils.

that ingestion of α-linolenic acid did not affect levels of 18:3n-6 or 20:4n-6 in plasma and platelet phospholipids in human volunteers. However, levels of PGE_2 in urine samples were reduced by dietary α-linolenic acid in a dose-dependent fashion. Thus, the possibility that dietary α-linolenic acid can be desaturated and elongated to longer-chain n-3 fatty acids in other tissues cannot be ruled out on the basis of fatty acid composition of plasma and platelet lipids alone. Furthermore, the results of these studies were derived from relatively short-term feeding trials (less than 2 weeks).

Eicosapentaenoic acid (EPA, 20:5n-3) can be metabolized by both COX and 5-LOX leading to the formation of "3-series" PGs, thromboxane A_3 (TXA_3), and "5-series" leukotrienes (Figure 28.3) (Dyerberg et al., 1978; Fischer and Weber, 1983; Gryglewski et al., 1979; Needleman et al., 1979). EPA is a poorer substrate for COX than arachidonic acid (Culp et al., 1979). Thus, it acts as a competitive inhibitor. Since EPA is a poor substrate, tissues of animals fed fish oil diets synthesized *in vitro* very small amounts of triene PGs and TXA_3. However, it has been shown that urine samples of human subjects consuming fish oil contain greater amounts of metabolites of triene PGs than those of diene PGs derived from arachidonic acid (Fischer and Weber, 1983; FitzGerald et al., 1984). The reason for the discrepancy between the *in vivo* and *in vitro* results has not been resolved. Overall, these studies appear to have established the cause and effect relationships among dietary fatty acids, eicosanoid formation, their cellular responses, and perhaps certain chronic diseases. Aspirin-treated COX-2 converts EPA to 18*R*-hydroxy-EPA (18-HEPE) in vascular endothelial cells, which is further metabolized to resolvin E1 (5*S*,12*R*,18*R*-trihydroxy-6Z,8E,10E,14Z,16E-EPA) by 5-LOX in neutrophils (Serhan et al., 2000). EPA was shown to be a preferred substrate for 5-LOX as compared to arachidonic acid (Ochi et al., 1983). Thus, increasing dietary n-3 PUFAs suppressed LTB_4 formation and increased formation of LTB_5 in human neutrophils.

Docosahexaenoic acid (DHA, 22:6n-3) can be converted to 7*S*- or 17*S*-hydroperoxy-DHA by LOX. 17*S*-hydroxyDHA is further metabolized to 17*S*-Resolvin D by LOX or converted to 10,

TABLE 28.1
Physiological Actions of Eicosanoids

Eicosanoid	Effect
PGE_1	Inhibits platelet aggregation
PGE_2	Vasodilation; increases cAMP levels; decreases gastric acid secretion; suppresses immune response; luteotropic action
PGI_2	Relaxes smooth muscle; vasodilation; inhibits platelet aggregation; raises cAMP levels
TXA_2	Contracts smooth muscle; causes platelet aggregation; bronchoconstriction
PGD_2	Inhibits platelet aggregation; raises cAMP levels; causes peripheral vasodilation
LTB_4	Neutrophil and eosinophil chemotaxis; leakage in micro circulation; raises cAMP levels; causes neutrophil aggregation
LTC_4–LTD_4	Contracts smooth muscle; constricts peripheral airways; leakage in microcirculation; decreases cAMP levels
12-HETE–12-HPETE	Neutrophil chemotaxis; stimulates glucose-induced insulin secretion
15-HETE	Inhibits 5- and 12-lipoxygenases
Lipoxin A	Superoxide anion generation; chemotaxis; activates protein cell activity
Lipoxin B	Inhibits NK cell activity
Resolvins	Block PMN transmigration and infiltration; inhibit microglial cell cytokine expression
Protectins	Block PMN recruitment and activation; inhibit TNF-α secretion

Abbreviations: PG, prostaglandin; TX, thromboxane; LT, leukotrine; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; NK, natural killer.

17*S*-docosatriene called neuroprotectin D1 through epoxidation process (Hong et al., 2003). DHA is a poor substrate for COX. However, DHA can be converted to 17*R*-hydroxyDHA by aspirin-treated COX-2 and subsequently, to 17*R*-resolvin D series by 5-LOX (Serhan et al., 2002).

IV. PHYSIOLOGICAL ACTIONS OF EICOSANOIDS

The eicosanoids exert diverse actions on the cardiovascular, reproductive, respiratory, renal, endocrine, skin, nervous, and immune systems (Table 28.1). Many of the actions are due to their capacity to change vascular tone and cAMP concentrations in the tissues. The kinds of eicosanoids synthesized vary with the type of tissue. As an example, thromboxane A_2 (TXA_2), which is a potent platelet-aggregating agent, is mainly synthesized in platelets (Hamberg et al., 1975), whereas prostaglandin I_2 (PGI_2), which is a potent inhibitor of platelet aggregation, is formed by endothelial cells (Bunting et al., 1976). Eicosanoids are synthesized when and where cells are stimulated. Eicosanoids are not stored in cells, and they are rapidly metabolized. Thus, the effects of eicosanoids are locally expressed.

A. Prostaglandins and Thromboxanes

Prostanoids, PGs, and TXA_2 are produced by the action of COX, or PGHS (prostaglandin G/H synthase). The series 2 PGs and thromboxanes derived from arachidonic acid generally promote inflammation with potent chemotactic activity, serve as vasoconstrictors, and stimulate platelet aggregation. In contrast, the "3-series" PGs and thromboxanes from EPA act as vasodilators and antiaggregators. Prostanoids act as autacoids by activating specific G-protein coupled receptors (GPCRs). Prostacyclin, the IP; $PGF_{2\alpha}$, the FP; TXA_2, the TP; PGE_2, EP1-4; PGD_2, DP1, and DP2; EP2, EP4, IP, and DP1 are the relaxant receptors that increase cAMP levels, whereas EP1, FP, and TP are the contractile receptors that increase intracellular calcium levels. EP3 increases intracellular calcium, but decreases cAMP level.

TXA_2 is a potent vasoconstrictor and platelet agonist. TXA_2 is produced by platelet COX-1 and macrophage COX-2. The protective effect of aspirin on cardiovascular system is considered to be attributed to the inhibition of platelet COX-1. PGI_2 is a potent vasodilator and inhibitor of platelet aggregation. Increase of PGI_2 production suppresses the proliferation and migration of vascular smooth muscle cells in response to injury. PGD_2 is the major product released from mast cells during allergic responses and asthma. PGE_2 is involved in a variety of biological processes including cell growth and proliferation, contraction and dilation of smooth muscle, reproduction, and inflammation. There are four PGE_2 receptors, EP1–4. The differential expression of four receptors and PGE_2 level dictate the outcome of PGE_2 action in the specific cell type. EP4 activation leads to platelet inhibition at low-PGE_2 concentration while high concentration of PGE_2 activates EP3 resulting in platelet aggregation. $PGF_{2\alpha}$ induces smooth muscle contraction. The activation of FP is known to lead to vaso- and bronchoconstriction and cell proliferation. TXA_3 is much less potent than TXA_2 in inducing platelet aggregation (Needleman et al., 1979). However, PGI_3 is as effective as PGI_2 in inhibiting platelet aggregation. For this reason, dietary EPA is implicated to reduce the risk of thrombosis.

B. Leukotrienes

4-Series leukotrienes are generated from arachidonic acid by 5-LOX in leukocytes. Leukotriene A_4 (LTA_4) is an unstable intermediate that is metabolized to leukotriene B_4 (LTB_4). LTB_4 increases the adhesion of leukocytes and the recruitment of $CD8^+$ cytotoxic T lymphocytes at sites of inflammation. LTA_4 is also converted to LTC_4, LTD_4, and LTE_4 that are slow-reacting substances of anaphylaxis and promote endothelial cell permeability and airway smooth-muscle constriction during anaphylactic reactions. LTB_4 activates BLT_1 and BLT_2 while LTC_4, LTD_4, and LTE_4 activate $CysLT_1$ or $CysLT_2$ to exert proinflammatory activities (Funk, 2005).

LTB_5 that is generated from EPA is less potent than LTB_4 in chemotactic and aggregating activities for human neutrophils (Lee et al., 1984, 1985). However, the efficacy of 5-series leukotrienes compared with that of 4-series leukotrienes on other physiological responses is not known.

C. Lipoxins

5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (LXA_4) and its positional isomer 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid (LXB_4) are the major lipoxins found in mammals. The 15-epi-lipoxins are endogenous 15*R*-enantiomers of LXA_4 and LXB_4 and also called as the aspirin-triggered lipoxins. Lipoxins can be generated from arachidonic acid through three different enzymatic pathways. First, arachidonic acid is converted to LTA_4 by 5-LOX in polymorphonuclear leukocytes (PMNs). LTA_4 is subsequently metabolized to LXA_4 and LXB_4 by platelet 12-LOX. Second, arachidonic acid is also a substrate of 15-LOX to generate 15*S*-HPETE that is subsequently converted to 5,6-epoxytetraene by 5-LOX in PMNs. Then LXA_4 and LXB_4 are generated by epoxide hydrolases in leukocytes. Third, 15-epi-LXA_4 and 15-epi-LXB_4 can be generated by 5-LOX in leukocytes from 15*R*-HETE that is converted from arachidonic acid by aspirin-acetylated COX-2. Lipoxins are known to be expressed during the resolution phase of inflammation. Lipoxins are anti-inflammatory mediators that inhibit leukocyte migration, NF-κB activation, and the expression of chemokines, cytokines, and adhesion molecules (Gilroy et al., 2004). Lipoxin A_4 (LXA_4) activates two receptors, a G-protein-coupled receptor, LXAR (or FPRL1, formylpeptide receptor-like 1), and a nuclear receptor, AhR (Devchand et al., 2003; Maddox et al., 1997; Schaldach et al., 1999). LXA_4 decreases LTB_4- and fMLP-induced Ca^{2+} mobilization in PMNs resulting in the suppression of neutrophil chemotaxis, adhesion, and superoxide generation. LXA_4 stimulates clearance and phagocytosis of apoptotic PMNs, which is associated with increased production of TGF-β1 and decreased release of IL-8 and monocyte chemotactic protein-1 (MCP-1). A recent report has shown that the stimulation of denderitic cells with lipoxins resulted in the expression of suppressor of cytokine signaling (SOCS)-2 (Machado et al., 2006).

D. Resolvins

Whereas arachidonic acid is metabolized to epi-lipoxins by consecutive action of aspirin-aceylated COX-2 and LOX, resolvins can be generated from n-3 PUFAs, DHA, and EPA by COX-2 and LOX. Aspirin-acetylated COX-2 in vascular endothelial cells and LOX in leukocytes synthesize 18*R*-Resolvin E1 from EPA and 17*R*-Resolvin D series from DHA, respectively. 17*S*-Resolvin D series and protectin D1 are generated from DHA by LOX (Schwab and Serhan, 2006). The resolvins and protectins are endogenously found during the resolution phase of inflammation. The resolvins and protectins showed potent anti-inflammatory activities through the inhibitory effects on the leukocytes activation and the proinflammatory mediator synthesis (Serhan and Savill, 2005). Resolvin E1 showed the protective effect on inflammatory responses in periodontitis and 2,4, 6-trinitrobenzene sulfonic acid-induced colitis by decreasing PMN recruitment (Arita et al., 2005b; Hasturk et al., 2006). Resolvin E1 acts on a specific GPCR, ChemR23 to downregulate NF-κB activation (Arita et al., 2005a). Resolvin D series inhibits infiltration of leukocytes and IL-1β production in microglial cells (Hong et al., 2003). Both 17*S*-resolvin Ds and 17*R*-resolvin Ds have similar activity in inhibiting PMN infiltration. Protectin D1, 10,17*S*-docosatriene, possess potent neuroprotective activity by inhibiting NF-κB activation, COX-2 expression, and PMN infiltration in cultured neuronal cells and ischemic stroke animal model (Marcheselli et al., 2003).

Excessive and/or imbalanced synthesis of eicosanoids has been implicated in various pathological conditions, including thrombosis, inflammation, asthma, ulcers, and kidney disease. Specific inhibitors of enzymes in COX and LOX pathways and receptor antagonists for specific eicosanoids have been tried and used as therapeutic agents for many of the disorders mentioned above. Accumulated evidence suggests that the amounts and types of eicosanoids synthesized in tissues can also be modulated by manipulating dietary fatty acids. Thus, it may be possible that risk factors of certain chronic diseases can be reduced by modulating eicosanoid biosynthesis through changes in the composition of dietary fatty acids.

V. REGULATION OF EICOSANOID BIOSYNTHESIS

There are many factors that can regulate eicosanoid biosynthesis in tissues. First, substrate availability is an important limiting factor in the biosynthesis of eicosanoids. The immediate substrate for eicosanoid biosynthesis is free arachidonic acid (Lands and Samuelsson, 1968). Arachidonic acid is incorporated into tissue phospholipids. Cellular concentration of free arachidonic acid is extremely low. Thus, arachidonic acid has to be released by the action of phospholipases to initiate the synthesis of eicosanoids.

Second, pharmacological agents, which can inhibit activities of phospholipases or key enzymes in the arachidomic acid cascade, suppress the formation of eicosanoids. Corticosteroid and mepacrine, which inhibit phospholipase A_2, can inhibit release of the immediate precursor acids of eicosanoids (Flower et al., 1989; Hirata, 1989). Thus, they can suppress the formation of both COX- and LOX-derived eicosanoids. Many nonsteroidal anti-inflammatory drugs inhibit COX, resulting in preferential suppression of the formation of eicosanoids derived from the COX pathway (Nelson, 1989). COX requires peroxides, particularly lipid hydroperoxides, as activators (Lands et al., 1984). Thus, agents that affect peroxide levels would modulate the COX activity. Phenolic antioxidants are known to inhibit PG synthesis as a result of reduced lipid peroxide levels. Glutathione peroxidase also suppresses PG biosynthesis by removing lipid hydroperoxides (Lands et al., 1977).

The studies (Raz et al., 1988; Wu et al., 1988) indicate that the synthesis of COX enzyme itself is another important factor affecting PG biosynthesis. COX is irreversibly inactivated by the substrate (Hemler and Lands, 1980). This may be due to the oxidizing equivalent released during the reduction of PGG_2 to PGH_2 by PGH synthase, an enzyme that possesses both COX and peroxidase activity (Gale and Egan, 1984). If purified enzyme is incubated with the substrate, oxygen consumption ceases before the substrate is depleted. The reaction can be started again only if the fresh enzyme

is added. This suggests that the synthesis of new enzyme would be required to maintain the biosynthesis of PGs in tissues.

VI. MODULATION OF EICOSANOID BIOSYNTHESIS BY DIFFERENT DIETARY FATTY ACIDS

The immediate precursor acid of eicosanoids is nonesterified free fatty acid (Lands and Samuelsson, 1968). Since most PUFAs are esterified mainly in the *sn*-2 position of phospholipids, the amounts of precursor acid released from phospholipids can limit the rate and amounts of eicosanoid synthesis. The amounts and types of precursor acid released from phospholipids depend on the composition of fatty acids in tissue phospholipids, which in turn is influenced by the composition of dietary fatty acids.

There are two steps in which dietary fatty acids can modulate eicosanoid biosynthesis from arachidonic acid. The first step is in the desaturation and elongation. There is a competitive inhibition among linoleic acid and linolenic acid families for the conversion of the fatty acids to longer-chain PUFAs. The observation that n-3 PUFAs can decrease the conversion of linoleic acid to arachidonic acid was first made by Machlin (1962) and subsequently by Mohrhauer and Holman (1963).

Although these observations were made even before the discovery that PGs are synthesized from 20:3n-6 and arachidonic acid derived from EFAs, they provided a predictable basis for the modulation of eicosanoid biosynthesis by modifying dietary fatty acid composition. The far-reaching nutritional significance of this is that modification of the composition of dietary fatty acids can be used to modulate the biosynthesis of eicosanoids and consequent physiological responses. Furthermore, it can be used as a new strategy to prevent and/or ameliorate certain chronic diseases for which modulation of eicosanoid biosynthesis is desirable.

The second step in which dietary fatty acids can modulate the biosynthesis of eicosanoids is at the formation of endoperoxide intermediate. The n-3 fatty acids competitively inhibit the oxygenation of arachidonic acid by COX (Lands et al., 1973). The competitive inhibition between n-3 and n-6 PUFAs for desaturases and COX suggests that increasing n-3 PUFAs in diets would reduce arachidonic acid levels in tissue lipids and, consequently, would decrease the formation of eicosanoids derived from arachidonic acid. It has been suggested that the bleeding tendency and low incidence of coronary heart disease among native Greenland Eskimos, whose diets consist mainly of cold water marine animals, is attributed to enhanced levels of n-3 PUFAs in their blood lipids (Dyerberg and Bang, 1979; Dyerberg et al., 1978).

It has been shown that α-linolenic acid can suppress arachidonic acid levels in tissue lipids and eicosanoid biosynthesis from arachidonic acid, although it is less effective than longer-chain n-3 PUFAs, which are abundant in marine lipids (Hwang et al., 1988). α-Linolenic acid is not a substrate for COX or 5-LOX; thus, no thromboxanes or leukotrienes can be formed from it.

In addition to competitive inhibitory effects, another mechanism through which PUFAs reduce eicosanoid biosynthesis has been recently proposed. The expression of COX-2, which is a rate-limiting enzyme to generate PGs from arachidonic acid released from plasma membrane, is upregulated in response to proinflammatory stimuli. The activation of Toll-like receptors in response to microbial infection or endogenous agonists leads to enhanced expression of inflammatory gene products including COX-2 and cytokines (Beutler, 2004; Lee et al., 2001, 2003). Various PUFAs (n-6 and n-3) suppress the expression of COX-2 induced by bacterial components mediated through the inhibition of Toll-like receptor activation (Lee et al., 2001, 2003). n-3 PUFAs (EPA and DHA) showed the highest potency in inhibiting the activation of Toll-like receptors 2 and 4 resulting in the reduced PGE_2 synthesis (Lee et al., 2003, 2004). This provides a novel mechanism as to how dietary PUFAs regulate the level of eicosanoids at inflammatory sites.

If inclusion of n-3 PUFAs in diets is desirable to suppress eicosanoid synthesis, an important practical question would be how much of them should be added into the diet. It has been shown that when rats were fed graded amounts of n-3 fatty acids (trilinolein and fish oil) in the presence of a

constant amount of linoleic acid, the levels of arachidonic acid in tissue lipids and the formation of eicosanoids derived from arachidonic acid were suppressed in a dose-dependent fashion (Hwang et al., 1988). However, if rats were fed graded amounts of the n-3 fatty acids with constant ratios of n-3/n-6 fatty acids (0.62 for linolenate and 0.3 for menhaden oil groups) by concomitant increases in n-6 fatty acid, then there was no dose response within groups fed different levels of the same dietary fat at a constant n-3/n-6 ratio (Boudreau et al., 1991). These results indicate that the ratio of n-3/n-6 fatty acids in the diet, rather than the absolute amount of n-3 fatty acids, is the determining factor in inhibiting eicosanoid biosynthesis from arachidonic acid. Thus, in assessing desirable levels of dietary n-3 fatty acids, not only the absolute amounts of n-3 fatty acids but also the amounts of dietary n-6 fatty acids may have to be adjusted.

However, the results from human studies demonstrated that absolute amounts of fish oil, and not the relative amounts of fish and vegetable oil (ratios of n-3 and n-6 PUFAs), determined the magnitude of the reduction of arachidonic acid and increase in eicosapentaenoic in phospholipids of plasma and platelets (Hwang et al., 1997). The suppression of plasma triglycerides by fish oil was also not affected by varying amounts of dietary n-6 PUFAs (Hwang et al., 1997).

VII. INCORPORATION OF n-3 FATTY ACIDS INTO FOOD SYSTEMS

If incorporation of n-3 fatty acids into processed foods is desired in the future, plant oils containing α-linolenic acid as a major PUFA would have certain advantages over fish oil. α-Linolenic acid is abundant in certain plant seed oils, and it is the major PUFA in chloroplast lipids, as shown in Table 28.2. In spite of the suggested beneficial effect of n-3 PUFAs in reducing certain risks of coronary heart disease, there would be a limitation for the direct incorporation of fish oil into food systems owing to its undesirable flavor and the greater susceptibility of EPA and DHA to autoxidation compared to α-linolenic acid.

Fish oil or plant seed oils containing α-linolenic acid can be added as supplements to feeds for poultry or aquacultured fish to increase the n-3/n-6 fatty acid ratios. Feeding linseed oil or menhaden oil at the 1.0%, 2.5%, or 5.0% level to broiler chickens resulted in a significant increase in n-3 PUFAs and a decrease in n-6 PUFAs, with a consequent increase in the n-3/n-6 fatty acid ratio in thigh muscle lipids compared to the control group, which was fed a corn oil diet (Chanmugam et al., 1992). The increase in the n-3/n-6 fatty acid ratio by linseed oil was much greater than by menhaden oil.

It has been demonstrated that the lipids from pond-reared prawn and catfish have considerably lower amounts of n-3 fatty acids compared with lipids of their wild counterparts (Chanmugam et al., 1986). This difference is due to the diet used for commercial aquaculture, which contains soybean meal in which n-6 PUFAs predominate, whereas marine animals consume diets derived from plankton, which are rich in n-3 fatty acids. The content of n-3 fatty acid in aquacultured fish

TABLE 28.2
Fatty Acid Composition of Total Lipids from Selected Vegetables and Nuts (wt. %)

Fatty Acid	Frozen Spinach	Green Bell Pepper	Green Cabbage	Broccoli	Romaine Lettuce	Walnut	Linseed Oil
16:0	16.22	23.79	16.42	18.90	12.75	2.05	7.33
16:1n-7	3.82	0.09	3.66	2.72	3.76	8.45	—
18:0	0.99	9.54	2.87	2.70	1.27	1.81	3.48
18:1n-9	3.41	2.36	5.81	5.14	1.01	15.74	24.15
18:2n-6	13.97	47.91	25.24	18.13	13.51	55.80	16.97
18:3n-3	61.57	16.31	45.99	52.37	67.70	16.14	48.28
n-3/n-6 ratio	4.41	0.34	1.81	2.89	5.01	0.29	2.84

can also be increased by supplementing their feeds with fish oil or plant oil containing a high level of α-linolenic acid.

VIII. DIETARY γ-LINOLENIC ACID (18:3n-6)

Certain plant oils such as primrose seed and borage seed oil contain considerable amounts of γ-linolenic acid. Approximately 8% of the total fatty acids of primrose oil is 18:3n-6. Some studies suggested that primrose oil suppresses acute and chronic inflammation in rats (Kunkel et al., 1988; Tate et al., 1988). It was speculated that this anti-inflammatory effect of primrose oil is due to accumulation of dihomo-γ-linolenic acid (20:3n-6) derived from γ-linolenic acid, and dihomo-γ-linolenic acid competes with arachidonic acid for COX, resulting in suppressing the formation of diene PGs derived from arachidonic acid. Furthermore, dihomo-γ-linolenic acid cannot be converted to leukotrienes. However, dihomo-γ-linolenic acid does not normally accumulate in animal tissues in significant amounts. Dietary γ-linolenic acid would be converted mostly to arachidonic acid. The ratio of dihomo-γ-linolenic acid (20:3n-6) to arachidonic acid (20:4n-6) is normally less than 1:30 in rat tissue lipids. In fact, one of the studies (Tate et al., 1988) suggesting the beneficial effect of primrose oil on acute inflammation showed that arachidonic acid levels in serum lipids of rats fed primrose oil were much higher than those of rats fed chow or a safflower oil diet. Some studies (Kernoff et al., 1977) also suggested that the oral administration of dihomo-γ-linolenic acid is beneficial in the treatment of thromboembolic diseases. This suggestion may be based on the fact that, unlike PGE_2, PGE_1 derived from dihomo-γ-linolenic acid inhibits platelet aggregation. However, since dihomo-γ-linolenic acid can be converted to TXA_1 but cannot be converted to prostacyclin, the beneficial effects of long-term administration of dihomo-γ-linolenic acid are questionable. Furthermore, dihomo-γ-linolenic acid can be readily converted to arachidonic acid in tissues.

IX. DIETARY *TRANS* FATTY ACIDS

Geometrical and positional isomers of octadecenoate (18:1) comprise the major portion of fatty acid isomers present in partially hydrogenated vegetable oil. Various positional isomers of *trans*-octadecenoate can be desaturated *in vitro* by Δ9, Δ6, or Δ5 desaturases (Mahfouz et al., 1980). Some of them may be further elongated to longer-chain PUFAs (Kameda et al., 1980). However, the presence of 20-carbon PUFAs derived from *trans*-octadecenoate in significant amounts in tissue lipids has not been reported. Possible effects of geometrical and positional isomers of octadecenoate and longer-chain PUFAs derived from the isomers on eicosanoid formation have not been reported.

Three different kinds of geometrical isomers of linoleic acid are present in hydrogenated vegetable oil: 9-*trans*-, 12-*trans*-; 9-*trans*; 12-*cis*; and 9-*cis*, 12-*trans*-linoleic acid. 9-*cis*, 12-*trans*-linoleic acid is present in most vegetable shortenings in much greater quantities than 9-*trans*, 12-*trans*-linoleic acid (Lanza and Slovar, 1981). Unlike 9-*trans*, 12-*trans*-linoleic acid, 9-*cis*, 12-*trans*-linoleic acid can be converted to arachidonic acid containing a *trans* double bond (Privett et al., 1967). It was shown that *trans* isomers of arachidonic acid inhibited the formation of PGs from all-*cis*-20:4n-6 (Nugteren, 1970). Some *trans* isomers (9-*trans*, 12-*cis* and 9-*trans*, 12-*trans*) of linoleate inhibit the conversion of *cis*-linoleate to arachidonic acid (Privett and Blank, 1964; Privett et al., 1977). It was demonstrated that dietary *trans* (9-*trans*, 12-*trans*)-linoleate at levels equal to or greater than that of *cis*-linoleate suppresses arachidonic acid levels in tissue lipids and the formation of eicosanoid by platelets in rats (Hwang and Kinsella, 1979; Hwang et al., 1982). However, the level of 9-*trans*, 12-*trans*-linoleate does not exceed 0.5% of total fatty acids of partially hydrogenated vegetable oils (Lanza and Slovar, 1981). Such a level does not appear to be enough to suppress the formation of eicosanoids derived from arachidonic acid.

X. CONCLUSION

The level of intake of dietary PUFA recommended by the American Heart Association is based on the lowering effect of dietary PUFA on the blood cholesterol level, which is one of the important risk factors of coronary heart disease, the leading cause of death in the United States. Dietary PUFA has been used as an entity without differentiating the kind of PUFA. There are two types of dietary PUFA: n-3 and n-6 fatty acids. Linoleic acid (18:2n-6) is the predominant PUFA in the major vegetable oils consumed in the United States. Thus, linoleic acid is the major dietary PUFA in the diet. Among n-3 PUFA, α-linolenic acid (18:3n-3) is abundant in certain plant seed oils and is the major PUFA in chloroplast lipids. Longer-chain n-3 PUFAs such as EPA (20:5n-3) and DHA (22:6n-3) are abundant in marine lipids.

Twenty-carbon PUFAs derived from EFA are precursors of eicosanoids with diverse pathophysiological actions in the cardiovascular system and inflammatory processes. It has been implied that excessive and/or imbalanced synthesis of eicosanoids in tissues can lead to the development of certain pathological conditions. It is now well documented that the amounts and types of eiocosanoids synthesized in tissues can be modulated by manipulating the composition of dietary fatty acids. Epidemiological, clinical, and biochemical studies performed during the past two decades suggest that replacing dietary n-6 PUFA with n-3 PUFA to some extent is beneficial in reducing risks of certain chronic diseases. It has been revealed that dietary n-3 PUFA suppresses tissue levels of arachidonic acid, the biosynthesis of eicosanoids derived from arachidonic acid and the expression of inflammatory gene products including COX-2. Furthermore, eicosanoids derived from EPA possess a different potency with respect to various cellular responses as compared with those derived from arachidonic acid. This suggests that biosynthesis of eicosanoids and their cellular responses can be modulated by modifying the relative amounts of n-3 and n-6 PUFA in the diet. For this reason, types of PUFA may have to be defined in recommendations of dietary PUFA levels in the future dietary guidelines.

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29 Polyunsaturated Fatty Acids and Regulation of Gene Expression

Harini Sampath and James M. Ntambi

CONTENTS

I. INTRODUCTION

It has now long been understood that lipids not only serve as important stores of energy, but also function as fine modulators of cellular signaling and metabolism. Fatty acids and especially polyunsaturated fatty acids (PUFAs) of the n-3 and n-6 series have been implicated in the modulation of various biochemical pathways. PUFAs decrease plasma lipid levels (Rambjor et al., 1996; Harris et al., 1997), improve the immune response (Hwang, 2000; Ntambi et al., 2002a), and increase insulin sensitivity (Storlien et al., 1998; Suresh and Das, 2003). The n-3 PUFAs are also preventive in several chronic diseases including coronary heart disease and stroke (Siscovick et al., 2000; von Schacky, 2000; Skerrett and Hennekens, 2003), inflammatory bowel disease (Kremer, 1996; Beluzzi et al., 2003), lung disease (Schwartz, 2000) as well as certain kinds of cancer such as breast, prostate, and colorectal cancers (Rose, 1997; de Deckere, 1999). Although the beneficial effects of n-3 PUFAs are clear, there is also some concern about possible detrimental effects of n-6 PUFAs due to their known roles in inflammatory and aggregatory pathways. Thus, better understanding of the mechanisms by which these fatty acids affect cellular metabolism will be invaluable in establishing a valid dietary n-3/n-6 fatty acid ratio for optimal health (Sampath and Ntambi, 2004).

Once fatty acids are taken up into the cell, they are rapidly converted to fatty acyl CoA thioesters (Figure 29.1) by one of six characterized acyl CoA synthetases (Coleman et al., 2000). These fatty acyl CoAs can then be shunted toward incorporation into complex lipids such as triglycerides (TG) and phospholipids, thereby decreasing the availability of fatty acyl CoAs within the cell (Figure 29.1). Alternately, they can be elongated/desaturated or oxidized through various pathways to give rise to second messengers such as prostaglandins and leukotrienes (Figure 29.1). Furthermore, the generation of fatty acyl CoAs is an energy requiring process that increases the cellular AMP/ATP ratio. This leads to secondary effects such as activation of AMP activated protein kinase (AMPK), which in turn can lead to changes in cellular and nuclear signaling (Kawaguchi et al., 2002). Thus, the

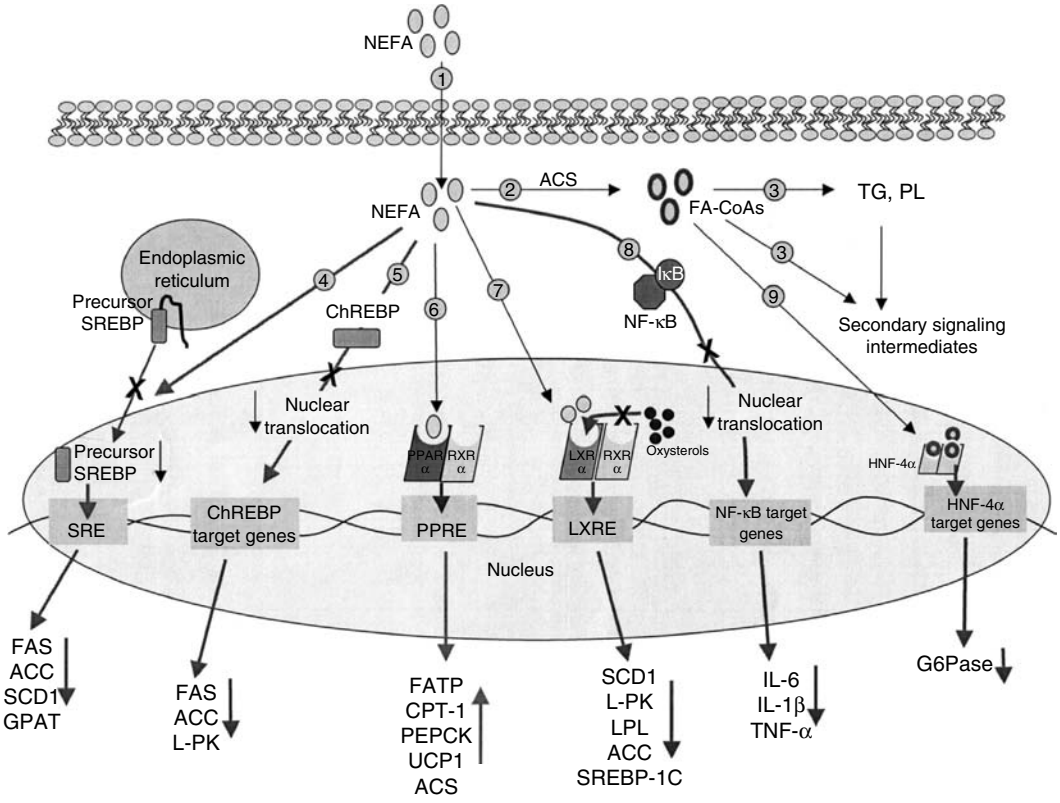

FIGURE 29.1 Once nonesterified fatty acids (NEFA) enter the cell (1), they can be rapidly converted to fatty acyl CoAs (2) by acyl CoA synthetases (ACS). Fatty acyl CoAs can then be esterified to complex lipids or give rise to secondary signaling intermediates (3). Alternately, NEFA or fatty acyl CoAs themselves can affect cellular signaling. Polyunsaturated fatty acids (PUFAs) can reduce lipogenic gene expression by decreasing the maturation of SREBP (4) as well as by reducing its expression and mRNA stability. PUFAs can also decrease the half-life and activity of ChREBP (5). On the other hand, PUFAs can serve as endogenous ligands for PPARa (6), thereby increasing oxidative gene expression. PUFAs can compete with oxysterols for binding to LXR and can also LXR binding to LXREs (7). PUFAs are also thought to inhibit NF-κB nuclear translocation (8). Fatty acyl CoAs can modulate the activity of HNF-4α (9). *Abbreviations:* ACC, acetyl CoA carboxylase; ChREBP, carbohydrate responsive element binding protein; CPT-1, carnitine palmitoyl transferase-1; FAS, fatty acid synthase; FATP, fatty acid transport protein; GPAT, glycerol phosphate acyl transferase; HNF-4α, hepatocyte nuclear factor-4α; IκB, inhibitor κB; IL, interleukin; L-PK, L-type pyruvate kinase; LPL, lipoprotein lipase; LXR, liver X receptor; NF-κB, nuclear factor κB; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RXR, retinoid X receptor; SCD1, stearoyl CoA desaturase-1; SRE, sterol response element; SREBP, sterol regulatory element binding protein; TNF, tumor necrosis factor; UCP1, uncoupling protein-1.

cellular and nuclear effects of fatty acids can be modulated not only by the site-specific metabolism and availability of the fatty acids, but also from the production of secondary signaling intermediates and activation of alternate pathways (Sampath and Ntambi, 2005).

II. SITE-SPECIFIC GENE REGULATION BY PUFAs

The ability of fatty acids to affect gene expression has been best characterized in the case of PUFAs. PUFAs regulate gene expression not only in liver and primary hepatocytes but also in many other

cell types including adipose tissue (Mater et al., 1998), small intestine (Niot et al., 1997), pancreas (Brun et al., 1997), the immune system (Tebbey and Buttke, 1993), and the neonatal mouse brain (DeWille and Farmer, 1993). In rodents, PUFA-rich diets repress various hepatic lipogenic genes including stearoyl CoA desaturase-1 (SCD1), acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and glucose-6-phosphate dehydrogenase (G6PDH) in the liver (Salati and Clarke, 1986; Clarke et al., 1990; Tebbey et al., 1994; Sessler et al., 1996; Cho et al., 1999; Jump et al., 1999a; Ntambi et al., 2002b; Wahle et al., 2003). In adipose tissue, PUFAs inhibit genes such as FAS in retroperitoneal fat but not in subcutaneous fat (Raclot et al., 1997), suggesting site-specific differences in fatty acid availability (Sessler and Ntambi, 1998). In addition, while hepatic genes respond to both n-3 and n-6 PUFAs in a similar manner, adipose genes are most responsive to n-3 PUFAs (Raclot et al., 1997), except for the SCD1 gene that is similarly regulated by both n-3 and n-6 fatty acids in adipose tissue (Sessler and Ntambi, 1998). Another difference between hepatic and adipose repression of lipogenic genes by PUFAs is the fact that in liver, PUFAs act directly to inhibit gene expression, while in adipose tissue, this effect may be mediated by eicosanoid intermediates (Mater et al., 1998; Jump et al., 1999).

Apart from repressing the expression of genes encoding enzymes involved in lipid synthesis, there is recent evidence that PUFAs also repress transcription of the leptin gene (Reseland et al., 2001). Leptin is an adipose-derived hormone that regulates appetite, body weight, and adiposity. Increased plasma leptin levels have been correlated with increased adiposity and obesity in humans, while weight loss results in decreased plasma leptin levels. High-fat diets increase plasma leptin levels in humans, but substitution of PUFAs for saturated fat in the diet causes a decrease in plasma leptin levels without effecting changes in body mass (Reseland et al., 2001). The effect on leptin gene expression is accompanied by decreased levels of transactivating factors such as peroxisome proliferator activated receptor-γ (PPAR-γ) and sterol regulatory element binding protein-1 (SREBP-1), discussed further below.

Conversely, PUFAs are involved in upregulation of genes of fatty acid oxidation and adipocyte differentiation. In the liver, PUFAs induce the expression of various genes including acyl CoA synthetase (Duplus et al., 2000), acyl CoA oxidase (Berthou et al., 1995), liver fatty acid binding protein (Meunier-Durmort et al., 1996), carnitine palmitoyl transferase-1 (Chatelain et al., 1996), and cytochrome P450 A1 (Duplus et al., 2000). In adipose tissue, both saturated as well as unsaturated long-chain fatty acids upregulate adipogenic genes (Duplus et al., 2000) in a direct manner (Grimaldi et al., 1992). For example, the gene encoding phosphoenolpyruvate carboxykinase (PEPCK), which in adipose tissue plays a role in TG synthesis, is upregulated in response to PUFAs; however, the same effect is not observed in the liver, where PEPCK serves as a gluconeogenic enzyme (Forest et al., 1997).

III. NUCLEAR RECEPTORS AND TRANSCRIPTION FACTORS

It was initially thought that fatty acids affect cellular metabolism solely through indirect mechanisms such as changing membrane phospholipid concentrations or producing signaling intermediates such as the eicosanoids. However, the actions of fatty acids on gene regulation occur within hours of feeding animals diets rich in PUFAs (Jump et al., 1993, 1994; Kim et al., 1999), suggesting a more direct mode of action. The discovery by Gottlicher et al. (1992) of nuclear receptors capable of binding fatty acids to modulate gene expression established a direct role for fatty acids at the nuclear level.

Nuclear receptors are found in metazoan organisms and contain two major domains (Beato, 1991). The ligand binding domain interacts with a ligand in order to induce binding of the DNA-binding domain to specific DNA sequences on target genes. These nuclear receptor specific DNA sequences are termed nuclear receptor response elements. Binding of the nuclear receptor to this sequence affects transcription of the target gene. DNA response elements can be direct repeat sequences such as AGGTCA-*Nx*-AGGTCA, where *N* can be any nucleotide and *x* refers to the number of residues

between the two direct repeats. Thus, a direct repeat sequence with a single nucleotide spacer is denoted as a DR1 element. Nuclear response elements can also exist as inverted repeats (IR) such as AGGTCA-*Nx*-ACTGGA or everted repeats (ER) such as ACTGGA-*Nx*-AGGTCA. Nuclear receptors that do not have a known ligand are termed orphan nuclear receptors (Forman and Evans, 1995). Unlike classical hormone receptors such as the thyroid hormone receptor or the retinoic acid receptor, some nuclear receptors do not bind a single compound with great specificity but rather a broad range of compounds, such as fatty acids and eicosanoids, with varying sensitivities. It has therefore been suggested that these nuclear receptors be classified as metabolic sensors rather than as classical nuclear receptors (Desvergne et al., 2006).

Transcription factors are proteins that bind to selected DNA sequences in the promoter or enhancer regions of target genes, often in conjunction with other proteins and in response to changes in cellular signaling. This binding leads to changes in the transcription of the target gene.

Our current understanding of the mechanisms whereby PUFAs affect gene expression is far from complete; however, several possible mechanisms for PUFA action have been well characterized. These include interactions of PUFAs with nuclear receptors such as peroxisome proliferators-activated receptors (PPARs), liver X receptors (LXRs), and hepatocyte nuclear factor-4α (HNF-4α), as well as regulation of the transcription factor, sterol regulatory element binding protein (SREBP), and carbohydrate response element binding protein (ChREBP).

IV. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

PPARs were the first nuclear receptors to be identified as possible mediators of fatty acid regulation of gene expression. Isseman and Green (1990) first characterized these nuclear receptors as transcription factors that belong to the superfamily of steroid nuclear receptors and include three different isoforms, α, β/δ, and γ. These three isoforms of PPARs are encoded by separate genes. PPARα is mainly expressed in brown adipose tissue and liver, as well as in the heart, skeletal muscle, kidneys, and enterocytes and is a key regulator of energy balance. PPARα regulates fatty acid uptake and oxidation in rodent liver and is activated by an array of fatty acids, eicosanoids as well as the hypolipidemic fibrate class of drugs (Desvergne et al., 2006). The prooxidative phenotype associated with PUFA feeding seems to be mainly regulated by the PPARα isoform and is reviewed further below. PPARγ is mainly expressed in adipose tissue and is shown to be a key regulator of adipocyte differentiation, energy storage and energy dissipation in white and brown adipose tissues. PPARγ is also expressed in monocytes/macrophages, endothelial cells, and vascular smooth muscle cells where its activation is associated with decreased monocyte expression and decreased production of proinflammatory cytokines (Ricote et al., 2004). PPARγ activation also results in improved insulin sensitivity, making this transcription factor the target of the thiazolidinedione class of drugs used in the clinical management of type II diabetes (Desvergne et al., 2006). Similar to PPARα, PPARγ binds fatty acids and eicosanoids as endogenous ligands. PPAR β/δ, which remains as the least characterized of the three isoforms, is ubiquitously expressed and binds fatty acids and eicosanoids without much specificity (Michalik et al., 2003).

Upon ligand activation, PPARs dimerize with the orphan nuclear receptor, RXR, and bind to DR1 elements termed peroxisome proliferator response elements (PPREs) (Desvergne and Wahli, 1999). Gottlicher et al. (1992) used a chimeric receptor of the putative PPAR ligand binding domain fused to the glucocorticoid receptor to show that linolenic and arachidonic acids activated the chimeric receptor, while cholesterol and DHEA did not. This led to PUFAs being established as the endogenous ligands of PPARs (Figure 29.1). It has since been shown that binding of PUFAs to PPARα causes rapid upregulation of target genes such as acyl CoA oxidase and CYP4A2 that are involved in peroxisomal β-oxidation (Berthou et al., 1995; Jump et al., 1999b). *In vitro* studies suggest that PPARs are capable of binding many different fatty acids. However, *in vivo*, PUFAs differentially regulate gene expression in various tissues and at different developmental stages possibly due to

differences in rates of fatty acid elongation, desaturation, and oxidation that result in alterations in fatty acid availability. The physiological significance of PUFA activation of PPARα has been established through the development of the PPARα-null mouse. Bing et al. (1997) showed that supplementation of the diet with fish oil, which is enriched in PUFAs, resulted in upregulation of genes of β-oxidation in wild-type mice. This effect was not observed in PPARα-null mice. However, fish oil feeding also resulted in repression of lipogenic genes such as S14 and FAS not only in wild-type animals, but also in PPARα knockout animals. This suggested that while the induction of oxidative genes by PUFAs occurs through a PPARα-dependent mechanism, the PUFA-induced repression of lipogenic genes is unlikely to involve PPARα (Bing et al., 1997). Overall, PUFA activation of PPARα results in increased expression of fatty acid oxidation genes (Figure 29.1), causing a decrease in hepatic and plasma TG.

V. STEROL REGULATORY ELEMENT BINDING PROTEINS

SREBPs were first identified due to their ability to regulate cholesterolgenesis by binding to specific sterol response elements (SREs) on target genes in response to fluctuations in cellular sterol concentrations. SREBPs belong to the family of helix–loop–helix family of transactivators (Osborne, 2000). Thus far, three SREBP isoforms have been identified that include SREBPs-1a and -1c and SREBP-2. SREBP-1c is the predominant isoform in rodent and human liver and is of immense physiological importance in the regulation of lipogenesis. SREBP-1a is encoded by the same gene as SREBP-1c and differs from the SREBP-1c isoform only at the N-terminus. SREBP-1a is highly expressed in cell culture models. SREBP-2 is encoded by a separate gene and regulates the expression of genes involved in cholesterol synthesis (Osborne, 2000).

The activation of SREBPs is regulated not only at the level of transcription, but also at the level of mRNA stability and protein maturation. The precursor SREBP proteins are inserted into the membrane of the endoplasmic reticulum with the COOH domain bound to the COOH domain of an escort protein, SREBP cleavage-activating protein (SCAP). When cellular sterol levels drop, SCAP escorts SREBP to the Golgi apparatus where it undergoes a two-step proteolytic cleavage through the actions of a site 1 and site 2 protease. The resultant smaller mature protein subsequently transits to the nucleus (Figure 29.1). Here, the SREBP protein binds to SREs in the promoter regions of target genes to affect transcription of genes involved in fatty acid and TG synthesis as well as cholesterolgenesis (Horton et al., 2002).

There is ample evidence, both *in vitro* and *in vivo*, suggesting that PUFAs repress lipogenic gene expression by affecting SREBP maturation in a posttranslational manner (Worgall et al., 1998; Kim et al., 1999). Incubation of CHO, HepG2, and CV-1 cells with PUFAs decreases expression of SRE containing genes in a dose-dependent manner. However, this regulation by PUFAs is lost in a cell line that has lost sterol regulation of SREBP, suggesting that the regulation of SREBP by PUFAs occurs upstream of SREBP binding to SREs on target genes (Worgall et al., 1998). Furthermore, *in vivo* studies have established that SREBP nuclear content is repressed by fasting and is greatly increased upon refeeding; this pattern corresponds to the expression pattern of various lipogenic SREBP-target genes such as ACC and FAS (Horton et al., 1998). However, the expression of these target genes as well as the nuclear abundance of SREBP-1c protein are greatly decreased by n-3 or n-6 PUFA supplementation in rats (Yahagi et al., 1999). In addition, rats overexpressing SREBP in liver do not display as great a reduction in lipogenic gene expression upon PUFA supplementation. These results suggest that PUFAs inhibit maturation of SREBP, possibly through increasing cellular cholesterol levels (Worgall et al., 2002).

In addition to affecting protein maturation, PUFAs, especially eicosapentanoate and arachidonate, decrease hepatic mRNA abundance of SREBP-1a and -1c, but not of SREBP-2. This reduction in SREBP mRNA has been attributed to changes in both mRNA stability as well as decreased SREBP transcription (Jump, 2002). Overall, the net effect of PUFA action on SREBPs is a reduction in

expression of lipogenic genes (Figure 29.1). This reduction in lipogenesis, along with the increase in fatty acid oxidation via PPARs, synergistically contributes to the hypolipidemic effect of PUFAs.

SREBP-1c is a very weak transactivator on its own and requires the presence of coactivators for maximal induction of gene expression (Magaña et al., 2000). One such coactivator is the transcription factor, PPARγ coactivator-1β (PGC-1β). Lin et al. (2004) have recently shown that treatment of cells with a variety of fatty acids, both saturated and unsaturated, results in increased expression and activation of PGC-1β. PGC-1β then functions as a coactivator of SREBP-1c, consequently activating downstream target genes such as FAS. However, treatment of these cells with a saturated fatty acid such as stearic acid also results in activation of the coactivator PGC-1α (Lin et al., 2004), which has been shown to be linked to pathways associated with the fasting response in liver (Puigserver, 2005). Hence, the physiological significance of the coordinate induction of both PGC-1α as well as PGC-1β by saturated fatty acids, and its consequent effect on SREBP-1c activity, is not yet clear.

Although the regulation of SREBP by PUFAs and expression of target lipogenic genes are closely correlated, there are also some emerging examples of SREBP-independent regulation of lipogenic genes. For example, Teran-Garcia et al. (2002) demonstrated that while dietary PUFAs reduce both hepatic SREBP as well as FAS content, mutation of the SRE in the promoter region of FAS results in only a 25% loss of PUFA regulation of FAS. On the other hand, mutating a region corresponding to the nuclear factor-Y (NF-Y) responsive site in the FAS promoter resulted in a 50% loss of PUFA repression of FAS and an 85% reduction in promoter activity, suggesting that PUFA regulation of FAS may be mediated more by NF-Y than by SREBP as previously assumed (Teran-Garcia et al., 2002).

Another example of SREBP-independent regulation of a lipogenic gene is seen in the regulation of the delta-9 desaturase, SCD1. SCD1 contains an SRE in its promoter region and is induced by SREBP-1. Treatment of HepG2 cells with PUFAs such as arachidonic, linoleic, and eicosapentanoic acids results in inhibition of transcription of SCD1, as predicted. However, overexpression of a mature SREBP protein does not circumvent this inhibition of SCD1 by PUFA treatment. These results suggest that the regulation of SCD1 by PUFAs is independent of SREBP maturation (Ntambi, 1992; Ntambi and Bene, 2001), and possibly mediated through another nuclear receptor such as LXR.

VI. LIVER X RECEPTORS

Similar to PPARs, LXRs belong to the superfamily of steroid nuclear receptors and include two isoforms, LXRα and LXRβ (Hannah et al., 2001; Ou et al., 2001). LXRs have been well characterized for their ability to bind oxysterols and consequently regulate genes involved in fatty acid and cholesterol transport and metabolism as well as bile acid synthesis. Similar to the PPARs, the LXRs function by heterodimerizing with RXRα and then bind DR4 elements in the promoters of target genes. LXRs have been shown to induce expression of genes such as Cyp7α, FAS, ACC, SCD1, lipoprotein lipase, as well as SREBP-1c (Repa et al., 2000; Lu et al., 2001).

PUFAs are thought to exert an inhibitory effect on LXR activation (Figure 29.1). For example, it has been proposed that PUFAs compete with oxysterols for binding to LXRs and that PUFA binding inhibits LXR activation of target genes (Hannah et al., 2001; Yoshikawa et al., 2001). Although it had previously been suggested that regulation of SREBPs by PUFAs was dependent on LXR inhibition by PUFAs (Yoshikawa et al., 2002), there is also some evidence suggesting that PUFAs can inhibit SREBP-1c expression independent of LXRs (Pawar et al., 2003).

Another proposed mechanism of PUFA regulation of LXR involves activation of PPARs α and γ by PUFAs (Yoshikawa et al., 2003). As discussed above, the PPARs are activated by PUFAs and dimerize with RXRα to bind to PPREs on target genes. Since LXRs also heterodimerize with RXRα, it has been proposed that inhibition of LXR activity by PUFAs occurs due to activation of PPARs and sequestration of RXRα away from LXR. In these studies, supplementation of PUFA-treated cells with RXRα decreased the level of PUFA-repression of LXR, suggesting that PPARs and LXRs may in fact compete for RXRα and therefore be reciprocally regulated by PUFAs (Yoshikawa et al., 2003).

VII. HEPATOCYTE NUCLEAR FACTOR-4α

Another player in PUFA regulation of gene expression is an orphan member of the hepatocyte nuclear factor family, HNF-4α (Hayhurst et al., 2001). This nuclear receptor binds DR1 sequences on target genes as a homodimer (Figure 29.1) and is involved in regulating hepatocyte differentiation as well as key hepatic functions such as lipoprotein secretion and cholesterol efflux (Hayhurst et al., 2001). Studies in cell culture models have demonstrated that HNF-4α is important for transcription of a variety of genes including apolipoprotein A-1 (ApoA-I), ApoA-II, ApoB, ApoC-II, ApoE, and ApoC-III, as well as CYP7A1. HNF-4α has also been shown to be important in iron metabolism through regulation of transferrin, and carbohydrate metabolism through regulation of PEPCK, glucose-6-phosphatase, and L-type pyruvate kinase (L-PK) (Hayhurst et al., 2001). Fatty acyl CoAs modulate HNF-4α activity through changes in phosphorylation via protein kinase A (Hayhurst et al., 2001). Fatty acyl CoAs can also bind directly to the ligand-binding domain (Figure 29.1) of HNF-4α (Hertz et al., 1998; Hertz et al., 2001), but the result of this binding varies depending on the specific fatty acid. For example, binding of 16:0-CoA seems to increase HNF-4α activity, while binding of 18:0-CoA and 18:3-CoA seem to have the opposite effect (Hertz et al., 1998). The regulation of the gluconeogenic gene, glucose-6-phosphatase by PUFAs via HNF-4α suggests yet another mechanism of regulation. PUFAs inhibit HNF-4α binding to its DNA recognition sites on the promoter of glucose-6-phosphatase (Figure 29.1), thereby decreasing transcription of this gene (Rajas et al., 2002). Another putative mechanism of action for PUFA regulation of HNF-4α is through activation of PPARα, which then competes with HNF-4α for binding to DNA recognition sites (Hertz et al., 2001). Xu et al. (2006) have shown in a recent study that while n-3 PUFA treatment of rat primary hepatocytes has no effect on transactivation of HNF-4α, treatment with WY14643, a PPARα activator, represses HNF4α. These results suggest that fatty acids and PPAR agonists may regulate gene expression through divergent mechanisms (Xu et al., 2006).

VIII. AMP-ACTIVATED PROTEIN KINASE AND CARBOHYDRATE RESPONSE ELEMENT BINDING PROTEIN

AMP-activated protein kinase (AMPK) is a heterotrimeric protein consisting of catalytic α-subunits and regulatory β- and γ-subunits. In recent years, AMPK has emerged as a key sensor and regulator of cellular energy status. AMPK is activated in response to increased AMP:ATP ratios. Activation of AMPK results in increased flux through catabolic processes such as glucose uptake, glycolysis, and fatty acid oxidation in order to generate ATP. This is accompanied by a downregulation of anabolic processes such as fatty acid and cholesterol synthesis (Kahn et al., 2005). AMPK can be activated in the presence of dietary fat due to increased cellular AMP concentrations generated by the synthesis of fatty acyl CoAs. In addition, both PUFA feeding and AMPK activation result in a favorable hypolipidemic phenotype, making it tempting to speculate that the cellular effects of PUFAs may be mediated by AMPK activation.

However, the topic of AMPK activation by PUFAs remains controversial at best. A study by Suchankova et al. (2005) showed that while refeeding after a fasting period results in inactivation of AMPK, addition of PUFAs to the diet stunts this inhibition, albeit not completely. The authors therefore suggest that PUFAs enhance AMPK activity *in vivo*. In contrast to these results, Dobrzyn et al. (2005) have reported that while feeding mice a high-PUFA diet causes decreased lipogenesis and increased oxidation as expected, this is not accompanied by increased AMPK activity. Furthermore, Dentin et al. (2005) also observed that the presence of PUFAs does not alter AMPK activity *in vivo* or *in vitro*. Thus, while a role for AMPK as a sensor of energy status has been firmly cemented, the issue of its regulation by PUFAs and the physiological significance of any such regulation remain to be ironed out.

Although regulation of AMPK by PUFAs remains questionable, one of the transcription factors known to be regulated by both AMPK and PUFAs is ChREBP. ChREBP is a recently identified

transcription factor belonging to the helix–loop–helix family of transcription factors and is expressed in liver. ChREBP is responsive to nutritional status in that its expression and nuclear translocation are both induced by glucose and insulin. Using ChREBP–/– mice, Iizuka et al. (2004) have shown that ChREBP is a transcription factor essential in regulating the expression of key glycolytic and lipogenic genes, especially in response to high-carbohydrate feeding. Deficiency of ChREBP has been shown to decrease adiposity as well as plasma glucose levels in leptin-deficient ob/ob mice that are normally obese and hyperglycemic (Iizuka et al., 2006). Dentin et al. (2005) have suggested that this transcription factor may also be the missing link in PUFA regulation of genes such as L-PK that are repressed by PUFAs (Figure 29.1) but do not contain any putative SREs in their promoter regions. These authors show that addition of linoleic acid to cultured hepatocytes results not only in SREBP-1c mRNA decay, as expected, but also in decreased half-life of ChREBP mRNA (Dentin et al., 2005). Furthermore, linoleate treatment also prevents nuclear translocation of ChREBP in response to glucose and insulin (Dentin et al., 2005). This is at least partly mediated by decreased expression of genes, encoding rate-limiting enzymes of the glycolytic and pentose phosphate pathways, which are required for promoting nuclear translocation of ChREBP (Dentin et al., 2005). Conversely, the decreased translocation of ChREBP in response to PUFAs is not mediated by AMPK, as neither the presence of the AMPK inhibitor AICAR, nor the genetic deletion of the α1 or α2 AMPK subunits abolishes the repressive effect of PUFAs on ChREBP (Dentin et al., 2005).

Overall, these studies indicate that ChREBP, at least partially, mediates the effect of PUFAs on genes such as L-PK (Dentin et al., 2005, 2006), albeit in an AMPK-independent manner. It remains to be determined whether this SREBP-independent regulation by PUFAs extends to other genes that are regulated by both PUFAs and ChREBP, notably SCD1.

IX. NUCLEAR FACTOR-κB

Although the nuclear receptors and transcription factors outlined above remain the best characterized mechanisms for fatty acid regulation of gene expression, there are several other potential players that are being identified. One such candidate is the transcription factor, nuclear factor-κB (NF-κB). NF-κB regulation of target genes is mainly regulated by sequestering it in the cytoplasm through association with the protein, inhibitor-κB (I-κB). Phosphorylation of I-κB in response to cellular signals results in its degradation, leaving NF-κB free to translocate to the nucleus, where it can regulate transcription of various genes including Cox-2 and interleukin-6 (Calder, 1997; Khalfoun et al., 1997). PUFAs have been shown to both increase (arachidonate) as well as decrease (arachidonate and eicosapentanoate) nuclear translocation of NF-κB (Camandola et al., 1996; Stuhlmeier et al., 1997; Zhao et al., 2004). In humans, fish-oil supplementation seems to decrease transcription of NF-κB target genes such as tumour necrosis factor-α (Zhao et al., 2004). More recently, it has been shown that this decrease in target gene expression (Figure 29.1) is mediated by decreased NF-κB DNA-binding activity. In addition to docosahexanoic and α-linolenic acids, linoleic acid has also an anti-inflammatory effect in human monocytic cells, suggesting a role for both n-3 and n-6 fatty acids in this pathway (Zhao et al., 2005). It is likely that PUFAs function synergistically with NF-κB as well as certain PPAR isoforms such as α and γ to decrease the inflammatory response (Lehmann et al., 1997; Ricote et al., 1998; Delerive et al., 1999; Jump and Clarke, 1999a; Zhao et al., 2005). Activation of the NF-κB pathway is involved in the formation of atherosclerotic lesions. PUFAs as well as some other fatty acids such as CLA and oleic acid blunt NF-κB activation and thereby reduce the inflammatory response in atherosclerotic lesions. These effects are similar to those achieved by the statin drugs and could therefore be of therapeutic importance in reducing inflammation and consequently, the risk for cardiovascular disease (Zhao et al., 2005).

X. CONCLUSION

Although certainly far from complete, our understanding of how fatty acids affect gene expression has progressed by leaps and bounds over the past decade. This research has not only provided

a clearer understanding of the roles of specific fatty acids, but also helped unravel some of the mechanisms involved in nutrient regulation of gene expression. Some of the better characterized mechanisms of fatty acid action remain modulation of gene expression via numerous transactivating factors including PPARs, LXRs, SREBP, ChREBP, HNF-4α, and NF-κB. The emergence of new players such as PGC-1α and PGC-1β have opened up further avenues for exploring the physiological significance of fatty acid mediated gene regulation.

Given that fatty acids represent an integral part of our diets, it is imperative that we continue to increase our understanding of how various fatty acids are capable of differentially modulating cellular metabolism. In the face of looming public health crises such as obesity and lipid-related disorders including diabetes and cardiovascular disease, understanding the molecular mechanisms that can lead to or prevent such conditions has no doubt become a scientific priority.

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30 Fatty Acids, Lipids, and Cellular Signaling

Geza Bruckner

CONTENTS

I. INTRODUCTION/BACKGROUND

Fatty acids are important constituents of membrane phospholipids and when fatty acids are released by cellular phospholipases and lipases, or made available to the cell from the extracellular environment, they serve as important cellular signaling molecules. These released fatty acids can serve as second messengers for transduction of internal and external signals. Furthermore, they function as cellular modulators, because their action can be reversed at a precise intra- and extracellular location; they can serve to amplify, attenuate, or alter a cellular signal. Among their many actions fatty acids can modify the action of protein kinases, G-proteins, adenylate and guanylate cyclases, and phospholipases, as well as modulate ion channels and events involved in stimulus-response coupling mechanisms. Many of the cellular signaling events related to fatty acids are via their conversion to eicosanoids, which are potent bioactive lipid molecules and these are reviewed in Chapter 28. Furthermore, the recently discovered lipid mediators lipoxins (LPX) and the two new families of lipid mediators, resolvins (resolution phase interaction products) and protectins, which derive from omega-3 polyunsaturated fatty acid (PUFA) provide for better understanding of the cellular signaling in the inflammatory process (see Chapter 28) (Schwab and Serhan, 2006). These resolution-active lipid mediators are small molecules that derive from essential n-6 and n-3 eicosapentaenoic acid and docosahexaenoic acid (EPA and DHA) PUFAs in the diet. Among the n-6 PUFAs, arachidonic acid (AA)-derived LXA_4 and aspirin-triggered LXA_4 (ATL) evoke anti-inflammatory protective actions in physiologic and pathophysiologic processes. These two molecular series have emerged as prototypic members of the first class of endogenous lipid/chemical mediators that are "switched on" in the resolution phase of an inflammatory response, thus acting as "braking signals" in inflammation (see Chapter 28).

In addition to their direct effects, fatty acids are also messenger and modulator molecules to extracellular signals. The generation and release of extracellular lipid mediators are generally cell

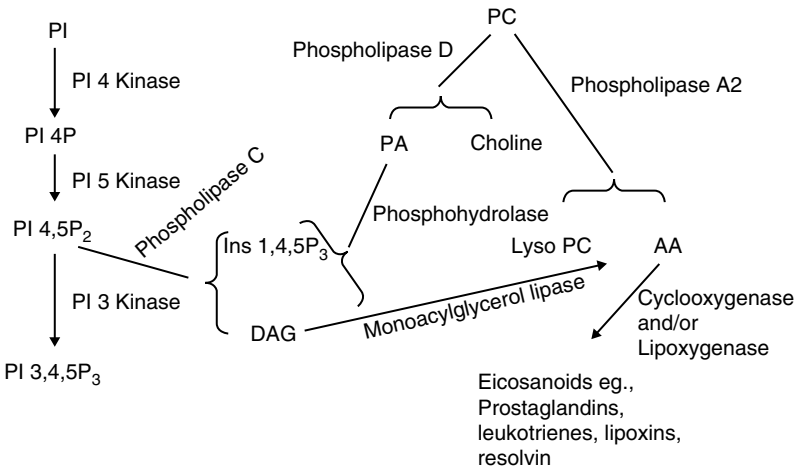

FIGURE 30.1 Signaling molecules include the protein kinase C regulator diacylglycerol (DAG), the calcium signal inositol 1,4,5-trisphosphate (Isn 1,4,5P3), and phosphorylated forms of phosphatidylinositol. The phospholipase C system depicted shows generation of DAG and Isn 1,4,5P3, from phosphatidylinositol 4,5-bisphosphate (PI 4,5P2) whereas phospholipase D catalyzes the hydrolysis of phosphatidylcholine to form phosphatidic acid and choline. The phosphatidic acid may itself act as a signal molecule or it can be hydrolyzed to form DAG by the enzyme PA phosphohydrolase. DAG when acted on by monoacylglycerol lipase can also release arachidonic acid (AA).

and receptor specific, whereas the intracellular lipid mediators appear to be ubiquitous regarding cellular transduction. These lipid mediators are involved in a wide range of processes involving cell-to-cell communication. The activation of specific phospholipases within individual cell types, via extracellular signals, that is, eicosanoids, cytokines, is critical in the biosynthesis of multiple cellular lipid mediators (Serhan et al., 1996; Chiang and Serhan, 2006). Lipid and nonlipid molecules can be divided into two groups regarding their second messenger function: (1) a group of molecules dedicated to signal transduction, that is, cyclic AMP, inositol triphosphate, various eicosanoids; and (2) a group of molecules that may function either as second messengers or lipid metabolic intermediates, that is, diacylglycerol (DAG), phosphatidic acid (PA), AA, and so forth (see Figures 30.1 and 30.2). The distinction between these groups as previously stated is difficult to make since many serve in both roles. Most likely intercellular compartments, concentrations, and receptors play a role as to their given function at any given time. For example, the role of AA, both as an eicosanoid or as a constituent of DAG in cellular signaling mechanisms illustrates this complexity.

Another example of signaling complexity is the phosphatidylinositol-cycle (PtdIns-cycle), an intracellular signaling pathway, which includes the receptor-mediated activation of GTP-binding protein (G-protein) that is associated with (PtdIns(4,5)P_2) specific phospholipase C-β (PLC-β) and tyrosine phosphorylation-regulated PLC-γ. Activation of PLC-β and PLC-γ results in the formation of the inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) and the activator of the protein kinase C (PKC) isoenzymes and 1,2-diacylglycerol (1,2-DAG) (deJonge et al., 1996; Heilmeyer et al., 2003). This signaling pathway of the PtdIns-cycle links various stimuli, such as hormone, growth factor, and mechanical stretch to their final responses at the transcriptional level through formation of the PKC and Ca^{2+}-calmodulin-dependent PKC activators: 1,2-DAG, Ca^{2+}, and Ca^{2+}-calmodulin (Straub et al., 2004). Many processes, believed to be involved in atherogenesis, such as expression of leukocyte adhesion molecules, adhesion and infiltration of blood cells to the vasculature, platelet aggregation, mechanical stress effects on the endothelium, secretion of endothelium-derived factors, and mitogenic responses of vascular smooth muscle cells are partially mediated by activation of the PtdIns-cycle. The PtdIns-cycle activity could be affected by the modification of the fatty acyl composition of the membrane phospholipids at several steps of the signaling cascade.

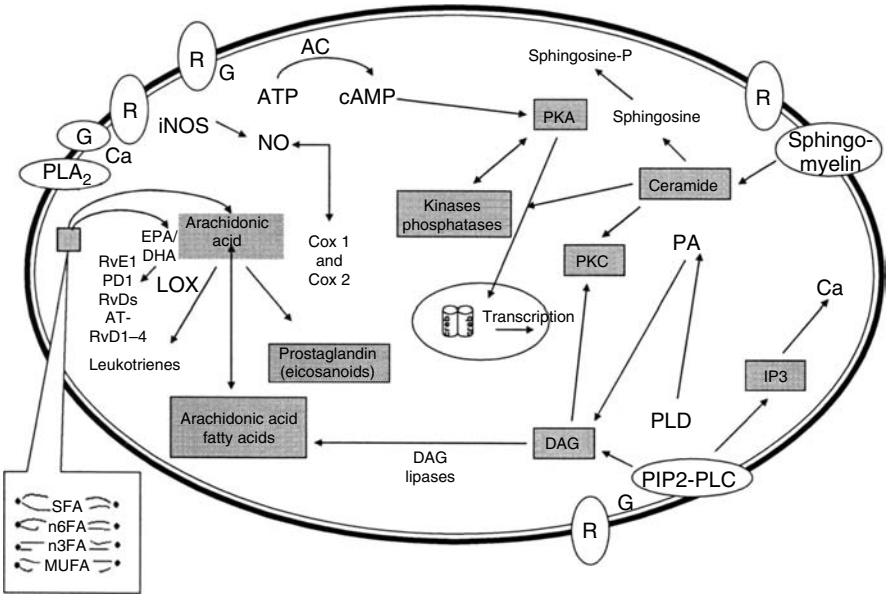

FIGURE 30.2 Cellular fatty acid and lipid signaling. iNOS, nitric oxide synthase; NO, nitric oxide; PK, protein kinase; PA, phosphatidic acid; DAG, diacylglycerol; PLD, phospholipase D; PLC, phospholipase C; LOX, lipoxygenase; COX, cyclooxygenase; PLA2, phospholipase A2; PIP2, phosphotidylinositol-4,5-bisphosphate; RvE1, resolvin E1; PD1, protectin D1; AT-RvD1–4, aspirin triggered resolvin 1–4; CERB, c-AMP receptor element binding protein; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; R, receptor.

The minimum requirements for a lipid to function as a direct second messenger, as stated by Khan et al. (1995) are as follows: (1) the demonstration of changes in concentration of the lipid in response to physiologic stimulation, (2) the demonstration of a direct *in vitro* or *in vivo* target (receptor) for this lipid, (3) the ability of this lipid to activate the target, and (4) establishing a true biological role for this lipid in mediating a biochemical and/or physiological response. Two characteristics are generally attributed to modulator molecules: (1) a reversible modification of the action of signaling molecules at a precise location and (2) a very short action that amplifies, attenuates, or deviates a signal.

For AA, the regulation of its intracellular concentrations and activation of cellular targets, that is, PKC, have been clearly demonstrated; however, whether these effects are direct or mediated via other cellular signaling is less clear. For example, the intracellular concentrations of AA have been reported to be in the range of 50–100 μM following stimulation of pancreatic cells or platelets (McPhail et al., 1984; Wolf et al., 1986; Nishikawa et al., 1988); however, the range of concentration for free fatty acids *in vivo* is less clear and the resulting effect on cellular signaling will certainly require further study. It has been widely accepted that AA and lysophospholipid generated by calcium-independent phospholipase A2 (iPLA2) act as a signaling molecule in cellular functions. These include eicosanoid production, glucose-induced insulin secretion, Fas-induced apoptosis, cellular proliferation, membrane trafficking in fusion, contribution to myocardial ischemia, and others. The functional role of iPLA2 in cellular responses upon stimulation has been recently reviewed by Akiba and Sato (2004).

Transmembrane mechanisms may be involved in the transduction of external signals (agonists that bind to plasma membrane receptors) and thus mediate the rapid production of intracellular second messengers (Berridge, 1987; Rana and Hokin, 1990). Two of these mechanisms, as mentioned, are the inositide phospholipid pathway that generates inositol 1,4,5-trisphosphate (IP3) and sn-1, 2-DAG (Berridge, 1984) and the cyclic AMP (cAMP) pathway that produces cAMP. Via these second messengers, the initial signal can be delivered to the nucleus to regulate a specific physiological

cell process. Perhaps the best examined nuclear lipid pathway is the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI4,5P(2)) by PLC resulting in activation of nuclear PKC and the subsequent production of inositol polyphosphates. However, phosphoinositides appear to function not only as precursors for soluble inositol phosphates and DAG, but may also act as second messengers themselves. In this role, as recently reviewed by Bunce et al. (2006), they modulate important nuclear signaling events such as cell cycle progression, apoptosis, chromatin remodeling, transcriptional regulation, and mRNA processing.

A number of studies have shown that fatty acids (particularly unsaturated fatty acids) act as second messengers and modulators, as defined above, through their capacity to modulate protein kinases, phospholipases, G-proteins, adenylate, guanylate cyclases, and ion channels activities (Khan et al., 1991, 1995; Zhu et al., 2006).

II. PHOSPHOLIPASES AND FATTY ACID MODULATION

The *in vivo* fatty acid composition of membrane phospholipids can be modified by dietary means and by alteration of cellular desaturation and acylation reactions. Most cellular membranes appear to reflect the dietary fatty acid constituents, including the endoplasmic reticulum, plasma, and mitochondrial membranes (King et al., 1977; Hammer and Wills, 1979). Cell signaling is accompanied by rapid remodeling of the membrane lipids following activation of the various lipases. This remodeling can occur on both the inner and outer membranes but affects different membrane lipid domains in these membranes. Sphingolipids are located primarily in the outer leaflet of the plasma membrane bilayer, while glycerophospholipids such as phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine occur mainly in the inner membrane. Cholesterol is believed to occur in roughly equal proportions in both leaflets. Cholesterol with sphingomyelin and other sphingolipids are located in an intimate association in specific sub-domains or "rafts" of membranes. These are segregated regions that form between sphingolipids and membrane proteins, which act to compartmentalize the latter and thereby separate different biochemical functions. Lipid "rafts" are defined as small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein–protein and protein–lipid interactions. These sphingolipid rafts contain primarily long-chain saturated fatty acids (Zeyda and Stulnig, 2006).

Fatty acids can be released from membrane phospholipids by cellular lipases and phospholipases, producing nonesterified free fatty acids (NFFA) (Irvine, 1982; Burgoyne and Morgan, 1990; Jenkins and Frohman, 2005). Agonists that stimulate these lipases and/or phospholipases appear to release endogenous fatty acids that then act directly as second messengers (Irvine, 1982; Akiba and Sato, 2004). As the regulation of membrane fatty acid composition appears to be tightly controlled, cellular function is therefore closely associated with membrane lipid form. Although the overall contents of the different phospholipid classes are mostly unchanged, a dynamic distribution of the different molecular species of the phospholipids or the lipid rafts embedded in the lipid bilayer are needed for enzymatic activity (Stubbs and Smith, 1984; Lamers et al., 1992; Rajendran and Simons, 2005). The rate of conversion of membrane lipids, for example, phosphatidylethanolamine methylation to phosphatidylcholine and PtdIns into PtdIns (4,5)P, may be indirectly stimulated by α_1- and β-adrenergic receptor stimulation of the methylating or phosphorylating membrane-associated enzymes. Phospholipids contain information in addition to their important structural role in membrane function. For example, the hydrolysis of phosphatidylethanolamine by PLA_2 from rat brain synaptosomes released primarily 20:4n6 despite the high abundance of 22:6n3 in these membrane lipids, suggesting that the role of 22:6n3 in neuronal membranes is required more as part of the structure and not as the free fatty acid (Kim et al., 1996). However, the release of other fatty acids, in other phospholipid molecules may be mediated by receptor-G-protein- and receptor nonG-protein-coupled activation of phospholipases A, C, and D (Zhu et al., 2006). Therefore, the hydrolysis of phospholipids remains a major contributor to transmembrane signaling; as slight changes

in phospholipid fatty acid composition are induced, membrane function, for example, fluidity and transport, might be altered in either a positive or negative sense. Membrane lipid composition has been shown to influence functions as diverse as insulin receptor sensitivity, phenylalanine transport, succinate oxidase activity, transport of hexose sugars, sodium and potassium transport, and Ca ATPase activity (Im et al., 1979; McMurchie and Raison, 1979; Legendre et al., 1980; Innis and Clandinin, 1981; Ginsberg et al., 1982; Spector and Yorek, 1985; Poling et al., 1996; Haugaard et al., 2006; Turner et al., 2006).

Not only the enzyme activities but also membrane receptor affinities may be altered by dietary fatty acids and thus affect cellular signaling; differences in fatty acid distribution have been demonstrated in native acetylcholine receptor-rich membranes with significantly more 24:1 found in the outer vs. the inner membrane (Bonini et al., 2002). Upon appropriate stimuli, the fatty acids are released from phospholipid membranes, and the levels of free fatty acids, for most tissues, thus reflect the nature of the membrane composition. It is in large part the amount of free fatty acid released that determines the final concentration of the eicosanoids produced. Thus, the rate-limiting step for eicosanoid production and other lipid signaling molecules appears to be PLA_2, C, or D activity. The free fatty acids that are released have different binding affinities for the cyclooxygenase (COX) and lipoxygenase enzymes, and the n-3 series (20:5n3, 22:6n3) are especially potent competitive inhibitors (Lands et al., 1973). For this reason, the fish oil fatty acids can alter eicosanoid biosynthesis and thus alter cellular response by increasing the 3-series eicosanoids (TXA_3, PGI_3) and decreasing the amount of n-6 products formed (TXA2, PGE2, etc.). The 3-series eicosanoids appear to have different biopotencies than the 2-series products. For example, TXA_2 is a more potent platelet aggregatory agent than TXA_3. The eicosanoid-mediated functions, via the release of fatty acids by phospholipases, may be enhanced by several agonists (thrombin, growth factors, adenosine, cholinergic agonists, α-adrenergic agonists, serotonin, vasopressin, angiotensin, bradykinin, etc.) that generally bind to plasma membrane receptors.

The cellular phospholipases that are triggered by various agonists are classified according to the bond cleaved in the phospholipid molecule. Thus, PLA_1 and PLA_2 selectively remove fatty acids from the sn-1 and sn-2 positions, respectively. Phospholipase B (PLB) cleaves both the 1- and 2-lysophospholipids produced by PLA_1 and PLA_2, respectively. Phospholipase C (PLC) cleaves the bond between glycerol and a phosphate, while phospholipase D (PLD) hydrolyzes the amino alcohol moiety from a phospholipid (see Figures 30.1 and 30.2).

PLA_2 is primarily responsible for the direct release of AA, which is predominately esterified in the sn-2 position of membrane phospholipids. However, highly unsaturated fatty acids such as DHA and EPA fatty acids can displace AA from the sn-2 position (Glaser et al., 1993; Murakami et al., 1997). AA can also be released by the activation of (see Figures 30.1 and 30.2) (1) PLC, followed by DAG and monoacylglycerol (MAG) lipase; or (2) PLD, followed by PA phosphohydrolase, DAG and MAG lipase; or (3) PLA_1, followed by PLB (less common). Other *cis*-unsaturated fatty acids such as oleic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids can also be liberated from phospholipids by lipases; therefore, the dietary fatty acid intake can influence membrane composition and, thus, the ratios and types of second messengers formed (Mitchell et al., 2003).

III. FATTY ACIDS AND PHOSPHOLIPASE MODULATION

A. Phospholipase A_2

PLA_2 plays a crucial role in diverse cellular responses and provides precursors for the generation of eicosanoids, platelet-activating factor (PAF), when the sn-1 position of the phosphatidylcholine contains an alkyl ether linkage, and some bioactive lysophospholipids, such as lysophosphatidic acid (LPA). As overproduction of these lipid mediators causes inflammation and tissue disorders, it is extremely important to understand the mechanisms regulating the expression and functions of PLA_2. PLA_2 isozymes that are regulated independently and exert distinct functions are classified

into four broad categories: (1) the group IV cytosolic PLA_2 ($cPLA_2$) and its paralogues; (2) the low molecular weight, secretory enzymes ($sPLA_2$); (3) the Ca^{2+}-independent, group VI enzymes $iPLA_2$; and (4) the selective acetyl hydrolases of PAF, groups VII and VIII PLA_2. These groups of PLA_2 enzymes differ in their Ca^{2+} requirements, overall structure, pH optima, substrate specificity, and susceptibility to pharmacologic inhibition (Dennis, 1997). One of the putative actions of group VI PLA_2 is the regulation of membrane remodeling to control the pool of available AA (Winstead et al., 2000). Groups VII and VIII PLA_2 degrade PAF and oxidized lipids, thereby downregulating oxidative injury and inflammation.

Most importantly, the $sPLA_2$ family is composed of a group of calcium-dependent lipolytic enzymes containing a conserved calcium-binding loop. To date, the following ten $sPLA_2$ isozymes have been identified in mammals: groups IB, IIA, IIC, IID, IIE, IIF, V, X, III, and XII. $sPLA_2$ isozymes generally exhibit tissue-specific and species-specific expression, suggesting that their cellular behaviors and functions differ. $sPLA_2$-IB is abundant in pancreatic secretions, and its main function is thought to be the digestion of dietary phospholipids. $sPLA_2$-IIA is the prototypic inflammatory PLA_2, found in inflammatory rheumatoid arthritic fluids; the two groups can be distinguished by the distribution of disulfide bonds and differences in primary structure. Since the genes for $sPLA_2$-IIA, -IIC, -IID, -IIE, -IIF, and -V are found at the same chromosomal locus, they are often referred to as the group II subfamily. Not only $sPLA_2$-IIA, but also the other group II subfamily $sPLA_2$ isozymes (i.e., IID, IIE, IIF, and V) are stimulus inducible (Murakami and Kudo, 2003).

Cytokines such as tumor necrosis factor (TNF), interleukin-1 and -6 (IL-1, IL-6) cause increases in expression of the group II $sPLA_2$s in a variety of cells that result in greater eicosanoid production; glucocorticoids via macrocortin suppress these effects.

$cPLA_2$ is an 85 kDa molecule and is the first arachidonic selective PLA_2 identified (Clark et al., 1995). $cPLA_2$ also exhibits relatively high-lysophospholipase activity and can completely diacylate diacylphospholipids and thus prevent toxic accumulation of lysophospholipids. A Ser228 site is essential for both PLA_2 and lysophospholipase activity and the N-terminal, Ca^{2+}, and phospholipid-binding domain is needed for the Ca^{2+}-dependent membrane association (Sharp et al., 1994), $cPLA_2$ is regulated by both transcriptional and posttranscriptional mechanisms (Shimizu et al., 2006). The ability of individual cytokines to induce different forms of PLA_2 and second messenger lipids demonstrates the complexity of the mechanisms involved. Posttranscriptional regulation depends on both serine phosphorylation and Ca^{2+}. In a variety of cells, phosphorylation occurs from a diverse group of agonists among that are thrombin, mitogens, zymosan, endotoxin, TNFα, Il-1, calcium ionophore, acetic acid, and phorbol esters; these resulted in increased PLA_2 activity and a gel shift on SDS gels (Serhan et al., 1996). The mechanisms involved in specific targeting of $cPLA_2$ to the nuclear membrane and exclusion from the plasma or other membranes are not clearly understood, however, it seems that colocalization of $cPLA_2$, 5-lipoxygenase activating protein (FLAP), and PGHS-II on the nuclear envelope represents a coordinated regulation of enzymes that are involved in fatty acid release and subsequent eicosanoid synthesis (Shimizu et al., 2006).

Membrane-bound PLA_2 can be inhibited by oleic, arachidonic, linoleic, linolenic, and eicosapentaenoic acids depending on the concentration of the fatty acid. AA has been found to be the most inhibitory fatty acid and behaves kinetically as a competitive inhibitor (K_I of about 5 μM). However, the saturated fatty acid palmitate causes no enzyme inhibition (Lister et al., 1988).

Calcium-dependent PLA_2, purified from snake venom was strongly inhibited *in vitro* by micromolar concentrations of *cis*-unsaturated fatty acids (oleic and arachidonic acid) and to a much lesser extent by *trans*-unsaturated or saturated fatty acids (Raghupathi and Franson, 1992); *cis*-unsaturated fatty acids bound directly to the isolated enzyme but did not affect the enzyme–substrate interaction. This suggests that the inhibition of PLA_2 activity *in vitro* may be mediated by the formation of an enzymatically inactive enzyme–substrate–fatty acid complex that represents a negative feedback regulation for PLA_2 activity. On the other hand, there is ample evidence that nonesterified fatty acids, particularly AA, can increase the activity of various phospholipases. For example, AA was shown to stimulate the hydrolysis of membrane PI by soluble PLC and oleic acid-activated cytosolic PIP2-PLC in human

peripheral monocytes (Irvine et al., 1979; Guan, 1994; Sternfeld et al., 2000); in addition, unsaturated fatty acids can stimulate PLD which in turn leads to other lipid mediators (Jackson, 1997).

Little attention has been given to understanding how modification of the membrane phospholipid fatty acid composition may modulate the membrane-bound PLA_2 activity and conversely how $cPLA_2$ plays a role in maintaining membrane fatty acid composition (Asai et al., 2003).

It has been reported that the catalytic activity of pancreatic PLA_2 was altered by small changes in the interfacial lipid architecture, such as incorporation of unsaturated fatty acids, and that these changes modulate the intensity of the hydrophobic interactions at the active site of the enzyme (Vergal et al., 1972). Ventricular myocytes treated with n6 fatty acid exhibited 34% greater PLA_2 activity than n3 fatty acid supplemented cells and it was concluded in these studies that it was the membrane alteration that brought about the change in enzyme activity (Grynberg et al., 1992). Other studies using n3 vs. n6 fatty acids, both *in vivo* and *in vitro*, indicate that marked changes in eicosanoid production can be brought about by n3 fatty acids, but it is unclear whether these changes are a result of altered PLA_2 activity, decreased COX, or lipoxygenase substrate availability or fatty acid binding affinity for the enzymes. Recently, Asai et al. (2003) showed that overexpression of cPLA(2)γ increased the proportion of PUFAs in phosphatidylethanolamine, suggesting that the enzyme modulates the phospholipid composition. They propose that cPLA(2)γ is constitutively expressed in the endoplasmic reticulum and plays an important role in remodeling and maintaining membrane phospholipids under various conditions, including during oxidative stress.

B. Phospholipase C and D

Several studies support that dietary PUFAs affect the receptor mediated activation of PLC as reviewed by deJonge et al. (1996). Phosphoinositide-specific phospholipase C is a key enzyme involved in inositide metabolism, leading to the formation of the second messengers IP_3, and DAG after binding of agonists to the cell surface (see Figure 30.2) (Berridge, 1984; deJonge, 1996). Numerous studies have reported on the modulating effects of fatty acids on PLC activity. Irvine et al. (1979) demonstrated that oleic acid or AA markedly activated the hydrolysis of membrane-bound phosphatidylinositol in rat liver microsomal fraction. The effect of AA on PLC activity appears to be indirectly mediated by some of its eicosanoid metabolites. In intact human platelets, the AA-induced stimulation of phosphatidylinositol-specific PLC is due in part to endoperoxides or thromboxane A_2, and this effect could be blocked by COX inhibitors (Siess et al., 1983). Other studies have shown that α_1-adrenergically stimulated PLC-β in ventricular myocytes was reduced in n3 fatty acid pretreated cells while the PLC-β in the same cells was stimulated by endothelin-1 (deJonge, 1996). *Cis*-unsaturated free fatty acids (oleic and palmitoleic acids at 10–100 μM), but not *trans*-unsaturated or saturated fatty acids, blocked the stimulatory effect of epidermal growth factor (EGF), bradykinin and bombesin on elevating cytosolic calcium concentration in diverse cell lines (Casabiell et al., 1991). It appears that unsaturated *cis* fatty acids block signal transduction by interfering with the PLC receptor but do not alter PKC-mediated mechanisms. These effects appear to be related to their chemical configuration rather than chain length or the hydrophobicity of the fatty acid.

PLC-β hydrolysis of $PtdIns(4,5)P_2$ results in two messengers: $Ins(1,4,5)P_3$ which is known to release calcium from intracellular stores (Berridge, 1993) and 1,2-DAG which is known to activate members of the PKC family, some of which are also dependent on calcium (Mori et al., 1982; Berridge, 1984). Pretreatment of rat submandibular acinar cells with 100 μM AA produced a 75% inhibition of IP3 (Chung and Fleming, 1992). The AA effect on the carbachol-IP_3 response was not mediated by eicosanoid metabolites and did not involve PKC. As suggested by these authors the action of AA was most likely due to an inhibition of phosphoinositide synthesis and not due to PLC inactivation. AA (10–100 μM) induced a dose-dependent inhibition of inositol incorporation into phosphoinositide of rat pancreatic acinar cells (Chaudry et al., 1987). It is therefore suggested that in these tissues, AA might act as a negative feedback regulator of phosphoinositide turnover via inhibition of inositol phospholipid synthesis.

Evidence has accumulated over the past decade, and as cited above, that there is a distinct nuclear phosphatidylinositol pathway. Nuclear lipid pathways showing the hydrolysis of PI4,5P(2) by PLC results in activation of nuclear PKC and production of inositol polyphosphates. Recent findings suggest that not only are they precursors for soluble inositol phosphates and DAG, instead they can act as second messengers themselves. They have been implicated to play a role in different important nuclear signaling events such as cell cycle progression, apoptosis, chromatin remodeling, transcriptional regulation, and mRNA processing. This has been reviewed by Bunce et al. (2006) with a focus on the role of PI4,5P(2) in the nucleus as a second messenger as well as a precursor for PI3,4,5P3, inositol polyphosphates, and DAG.

By changing the PUFA composition of membrane phospholipids, the endogenous substrates for the membrane-associated phospholipase C-β and A_2 are changed. It appears that the hydrolytic action of the phospholipases on these substrates is also changed and thus results in altered products (1,2-DAGs and nonesterified PUFAs) which in turn evoke changes downstream in the signaling cascades such as activation of distinct PKC isoenzymes, formation of distinct eicosanoids, and regulation of Ca^{2+} channels. Membrane physicochemical properties, in terms of fluidity and cholesterol content of the bilayer, might also undergo changes due to altered PUFA incorporation into membrane phospholipids. As mentioned previously, these changes could alter receptor function, receptor-GTP-coupling, and GTP-binding protein-phospholipase C-β or A_2 coupling as well (deJonge et al., 1996).

It has been suggested that high-dietary fat might enhance colon carcinogenesis through the modulation of colonic mucosal PLA_2 and phosphatidylinositol-specific phospholipase C (PI-PLC). These authors state that colon carcinogen treatment increased the activities of colonic mucosal PLA_2 and PI-PLC and the formation of prostaglandins and thromboxane A_2 from AA through COX compared to saline-treated rats fed on similar diets. The activities of PLA_2, PI-PLC, and COX were significantly higher in colon tumors compared to colonic mucosa and this effect was related to the amount of corn oil (Rao et al., 1996).

PLC activation generates primarily polyunsaturated species while PLD activation generates saturated/monounsaturated species. In endothelial cells, PLD when activated by LPA independent of PLC showed no activation of PKC. These authors also suggest that only polyunsaturated DAGs and saturated/monounsaturated phosphatidates function as intracellular messengers and that their interconversion products are inactive (Pettitt et al., 1997). PLC- γ isozymes when activated involve various agonists resulting in tyrosine phosphorylation of the effector enzymes. Further PLC-γ isozymes are also activated by PA, (PI3,4,5P(3)), and AA in the absence of PLC γ tyrosine phosphorylation. These lipid-derived messengers are the products of phosphatidylinositol 3-kinase, phospholipase D, and phospholipase A_2, enzymes that are often stimulated along with PLC-γ in response to an agonist. Additionally, PI4,5P(2) may act as a substrate for both PLC-γ and phosphatidylinositol 3-kinase and can activate PLD and PLA_2. Sekiya et al. (1999) have shown that there is an elaborate mechanism of cross-talk and mutual regulation between four effector enzymes that participate in receptor signaling by acting on phospholipids.

It has been shown by several investigators that PLD activity is stimulated by PKC and PKCα, small G-protein ADP-ribosylation factor (ARF), small G-proteins of the Rho family, $PtdIns(4,5)P_2$ (and as cofactor) and by fatty acids, particularly by oleic acid (Hattori and Kanfer, 1984; Shukla and Halenda, 1991; Cockcroft, 1997). To this effect, PLD activity was shown to be dependent on the concentration of oleate (4 mM giving maximal activity) in rat brain microsomal fraction (Gustavsson and Alling, 1987).

Chalifour and Kanfer (1982) reported that monounsaturated fatty acids were the most effective activators of rat brain microsomal PLD; PUFAs stimulated it to a lesser degree, while SATs were ineffective. A consequence of PLD activation by FAs is that PA is mainly formed by the hydrolysis of phosphatidylcholine due to the activation of PLD; PA can be further degraded by PA phosphohydrolase to DAG (Dhalla et al., 1997). PA is also generated by phosphorylation of DAG due to the action of DAG kinase and is converted to DAG under the action of PA phosphohydrolase. PA has

been implicated in the regulation of protein kinases, GTPase activating proteins, PI kinases, adenylate cyclase, and other signaling proteins. The growth factor-like effect of PA has been recognized in a wide variety of tissues and the positive inotropic agents that are known to stimulate cardiac hypertrophy have been shown to increase the level of PA in cardiac sarcolemma. Thus, the signaling of PLD by FAs can induce other second messengers. Some of the mechanisms by which unsaturated fatty acids can exert their effects on signal pathways are best demonstrated by their effects on PKC.

IV. PROTEIN KINASE C

PKC has been implicated in the regulation of a variety of physiological and pathological cellular processes, including proliferation, differentiation, and release of hormones and neurotransmitters. PKC isozymes constitute a family of ubiquitous phosphotransferases and to date, at least 11 different PKC isotypes have been identified. Lipid cofactors play a key role in the activation of PKC isoforms and this area has been recently reviewed by Martelli et al. (2006). PKC appears to reside in the cytoplasm in an inactive form and to translocate to the plasma membrane or cytoplasmic organelles upon cell activation. However, PKC is also capable of translocating to the nucleus and several PKC isoforms actually resident within the nucleus.

PKC normally requires phosphtidylserine and either DAG and/or Ca^{2+} depending on the isoform of PKC. DAG generally increases the affinity for Ca^{2+} and thus facilitates full PKC expression. Unsaturated fatty acids may activate PKC directly, independently of DAG, phosphtidylserine or Ca^{2+} or enhance its DAG-dependent activation at relatively low-Ca^{2+} concentrations. Thus, *cis* linoleic acid, but not the *trans* linoleate isomer or oleic or stearic acids, activated PKC in human platelets (Seifert et al., 1987; Jackson, 1997).

Incorporation of linoleic and AA into lymphocyte plasma membranes phospholipids resulted in long-term activation of PKC that was linked to IL-2 release; the incorporation of palmitic, oleic, or *trans* linoleic did not induce a PKC response. In T-helper cells, linoleic but not AA activated PKC (Jackson, 1997).

Diacylglycerol must have the 1,2-sn-configuration to act as an activator of PKC and the various fatty acyl moieties determine the kinetic characteristics of PKC activation (Go et al., 1987). This implies that the molecular species of 1,2-DAG may be of importance for its potency to activate PKC. In most tissues the sn-2 position of the PtdIns is mainly occupied by 20:4n-6, which as mentioned previously appears to play a significant role in signal transduction processes. The α, β, and γ isoforms of PKC were synergistically activated by the simultaneous addition of DAG and AA (50 mM). Activation did not require Ca^{2+}, but micromolar concentrations of Ca^{2+} enhanced activity. Synergistic activation of PKC by unsaturated fatty acids, and not the saturated fatty acids, was most evident for the PKC isoenzyme (Graber et al., 1994).

Both monounsaturated and PUFAs may promote a time- and dose-dependent redistribution of PKC activity. In rat hepatocytes, oleate or arachidonate (in the presence of Ca^{2+}) caused a translocation of cytosolic PKC toward the plasma membrane. Oleate-induced translocation was dose-dependent (between 0.5 and 0.75 mM oleate) for PKC activity found in the plasma membrane.

PKC could also be activated by other 18 and 20 carbon PUFAs with either n3 or n6 configuration in the absence of other lipid activators. However, when PKC was in its activated state in the presence of phosphatidylserine and DAG, the n3 or n6 fatty acids inhibited phosphatidylserine/DAG-stimulated PKC activity. DHA acid was noted an exception as it did not stimulate PKC activity in the absence of phosphatidylserine and DAG but was the most potent inhibitor of phosphatidylserine/DAG-stimulated activity. These observations indicate that the fatty acids of fish oil behave differently from other long chain PUFAs with respect to PKC.

The impact of FFA on activating novel PKCs (nPKC) has received a great deal of interest regarding their major role in inducing insulin resistance in insulin target cells. FFAs are also found to be involved in activating nPKCs associated with the impairment of insulin sensitivity (Dey et al., 2006).

V. PLATELET-ACTIVATING FACTOR AND LPA

Eicosanoids, PAF, and LPA are ubiquitous lipid mediators that play important roles in inflammation, cardiovascular regulation, and immunity and also modulate gene expression of specific proinflammatory genes. These lipids are dependent on their specific plasma membrane receptors belonging to the superfamily of G-protein-coupled receptors (GPCR).

PAF and LPA fall into a subclass of intercellular lipid messengers that are released from cells and transmit signals via these GPCR. Increasing evidence suggests the existence of a functional intracellular GPCR population. PAF, similar to LPA, can activate a variety of cells by binding to the membrane receptor. It has been proposed that the immediate effects of these bioactive lipids are mediated via cell surface receptors whereas long-term responses are dependent on intracellular receptor effects. It has recently been reviewed by Zhu et al. (2006) that receptors for PAF, LPA, and PGE (2) (specifically EP(1), EP(3), and EP(4)) localize at the cell nucleus of cerebral microvascular endothelial cells of newborn pigs, rat hepatocytes, and cells overexpressing each receptor. Stimulation of isolated nuclei with these lipids reveals biological functions including transcriptional regulation of major genes, namely c-fos, COX-2, and endothelial as well as inducible nitric oxide synthase. It has been shown that certain oxidized phospholipids can elicit a response similar to PAF, suggesting that oxidized phospholipids are mediators of inflammation in a mechanistic manner similar to that of PAF (Liscovitch and Cantley, 1994).

LPA binds to GPCR of the EDG family, inducing proliferation and migration in many cell lines. The characterization and purification of lysophospholipase D (lysoPLDs) have been recently reviewed by Xie and Meier (2004). Some proposed routes for LPA production involve the sequential actions of PLD and PLA_2. Another route involves the sequential actions of PLA_2 and lysoPLD. LysoPLD is defined as an enzyme that hydrolyzes lysophospholipids to produce LPA and both microsomal and extracellular forms of lysoPLD have been reported. The microsomal lysoPLD plays an important role in the metabolism of PAF since it has a preference for alkyl-phospholipids and shows a close linkage between these two active lipid molecules.

PAF activity is also markedly altered by fatty acids. It has been shown that the phosphoinositide (PI)-signaling pathway through the activation of G-proteins, stimulated a higher Ca^{2+} mobilization in macrophages from mice fed n-3 compared to n-6 fatty acids. In addition, the response of macrophages from n-3 fed mice to PAF was less sensitive to PLC inhibition than that of macrophages from those fed n-6 diets. The activity of PLC in macrophages from mice fed n-3 diets was significantly higher than that of macrophages from mice fed diets containing n-6 fatty acids. These results show that n-3 fatty acids can enhance the PAF-signaling pathway in macrophages by increasing the activation potential of PLC, without affecting PAF receptor number and affinity (Chakrabarti et al., 1997). For an excellent review on the effects of fatty acids of PKC and PAF see Graber et al. (1994) and Jackson (1997).

VI. OTHER FATTY ACID SIGNALING MECHANISMS

A. Involvement in Ion Channel Regulation

It has been shown by many investigators that certain fatty acids may be regulators of ion channels. This may involve direct interaction of the fatty acid with the ion channel itself or by altering the interaction of channels with the lipid bilayer.

1. *Potassium channels* Ordway et al. (1989, 1991) have shown that *cis*-mono and polyunsaturated, *trans*-polyunsaturated and saturated fatty acids are capable of directly activating K^+ channels in gastric smooth muscle cells. Others have noted similar effects in cardiac atrial cells (Kim and Clapham, 1989) and in aortic or pulmonary artery smooth muscle cells (Bregestovski et al., 1989; Kirber et al., 1992). In membrane patches from toad smooth muscle cells, both myristic and AAs at micromolar concentrations promptly activated a K^+ selective channel (Ordway et al., 1991). Poling et al. (1995, 1996) showed

that DHA produced a concentration-dependent inhibition of the sustained outward current in isolated neocortical neurons. These authors suggest that the fatty acid interacts directly with an external domain of the ion channel and serves to selectively modulate certain voltage-gated K^+ channels in a zinc-dependent fashion. However, concentrations of 5 μM, unsaturated and medium chain length saturated fatty acids accelerated the inactivation of voltage-dependent K^+ channels in neuroblastoma cells (Rouzaire-Dubois et al., 1991).

As recently reviewed by Besana et al. (2005), two-pore domain K^+ channels make up the newest branch of the K^+ channel super-family and modulation of these channels is thought to underlie the function of physiological processes as diverse as the sedation of anesthesia, regulation of normal cardiac rhythm and synaptic plasticity associated with simple forms of learning. Lipids, including arachidonate and its lipoxygenase metabolites (12(S)-HPETE) as well as PAF have been identified as important mediators of these channels.

2. *Calcium channels* It has been shown that AA-induced Ca^{2+} mobilization from pancreatic islets that occurred rapidly, within 2 min, was not due to the metabolites of AA (Wolf et al., 1986). A similar effect of AA on intracellular Ca^{2+} efflux was also observed in T and B human lymphocytes and in MA-lO cultured Leydig tumor cells (Graber et al., 1994).

   On the whole, as suggested by Graber et al. (1994) AA and other unsaturated fatty acids play the role of second messengers in transducing activation signals by inducing Ca^{2+} mobilization from intracellular stores or by modulating plasma membrane Ca^{2+} channels. However, many of the Ca^{2+} channel regulatory effects of AA appear due to eicosanoid metabolites.
3. *Sodium and chloride channels* AA and other *cis*-unsaturated fatty acids (including oleic and linoleic acids) in human fetal tracheal epithelial cells has been shown to block secretory chloride (CV) channels in a dose-dependent manner (1–25 μM), whereas *trans*-unsaturated and saturated fatty acids do not inhibit the channel.

   The effects of fatty acids on sodium channels in muscle cells depend on the side of the membrane exposed to the fatty acids. It appears that fatty acids are modulators of skeletal muscle sodium channels by two different pathways. Extracellular fatty acid could be interacting with specific sodium channel receptors and intracellular fatty acid could be modulating PKC activity, but the mechanism of excitatory and inhibitory modulation of sodium channels by fatty acids remains to be elucidated. These mechanisms as they relate to Na-K-ATPase activity modulation are reviewed by Graber et al. (1994).

VII. CONCLUSIONS

This review has focused on some of the roles fatty acids contribute to cellular signaling directly as free fatty acids and indirectly as structural analogues to other lipid signaling molecules. Our understanding of these fatty acid–lipid signaling molecules has advanced tremendously in the past decade but much work still remains. It appears that many of the studies reviewed cite contradictory effects for many of the fatty acids depending on the cell type used and other co-factors needed (e.g., zinc). Furthermore, the studies cited are predominately *in vitro* studies and the *in vivo* conditions and concentrations are most likely very different and thus great care should be exercised in extrapolating these fatty acid effects to *in vivo* conditions. However, the study of lipid signaling molecules is growing exponentially and as more is understood new doors are opened and seemingly never ending new pathways revealed.

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31 Safety and Health Effects of *Trans* Fatty Acids

J. Edward Hunter

CONTENTS

I. INTRODUCTION

In recent years, *trans* fatty acids in foods have raised health concerns largely from reports that high levels in the diet, compared to high levels of *cis* fatty acids, have resulted in unfavorable effects on both low-density lipoprotein cholesterol (LDL-cholesterol) and high-density lipoprotein cholesterol (HDL-cholesterol). In response, many health professional organizations have recommended reduced consumption of foods containing *trans* fatty acids, and the U.S. Food and Drug

Administration (FDA) issued regulations requiring the labeling of *trans* fatty acids on packaged foods effective from January 1, 2006. In addition, many food manufacturers, who have used partially hydrogenated oils in their products, have developed or are considering ways to reduce or eliminate *trans* fatty acids from these products.

This chapter updates that of Hunter (2000) and covers the following topics: (1) occurrence of *trans* fatty acids in the U.S. food supply; (2) review of human studies relating dietary *trans* fatty acids to risk of coronary heart disease (CHD); (3) review of studies relating dietary *trans* fatty acids to other health conditions, such as cancer, maternal and child health, type 2 diabetes, and macular degeneration; (4) dietary recommendations regarding *trans* and saturated fatty acids by health professional organizations; and (5) alternatives for replacing or reducing *trans* fatty acids in foods.

II. OCCURRENCE OF *TRANS* FATTY ACIDS IN THE U.S. FOOD SUPPLY

Unsaturated fatty acids in foods can exist in either the *cis* or *trans* configuration (Figure 31.1). In the *cis* form, the hydrogen atoms are on the same side of the double bond. In the *trans* form they are opposite. As a result of these orientations around the double bond, the *cis* fatty acid has a bend in the carbon chain, whereas the *trans* fatty acid has a straight carbon chain resembling that of a saturated fatty acid. The term "positional isomer" is commonly used to refer to *cis* or *trans* fatty acids if one or more of the double bonds has migrated to a new position in the fatty acid chain. *Trans* fatty acids (with double bonds at various positions in the fatty acid chain) and *cis* positional isomers are formed during partial hydrogenation of fats and oils, a process used to impart desirable stability and physical properties to food products such as margarines and spreads, shortenings, frying fats, and specialty fats (e.g., for fillings, toppings, and candy). In addition, small amounts of *trans* fatty acids occur naturally in foods such as milk, butter, and tallow as a result of biohydrogenation in ruminants.

Widespread use of partially hydrogenated vegetable oils in the United States during the past four or five decades has raised questions about the health effects resulting from the consumption of *trans* fatty acids present in these products. Among isomeric fatty acids, interest has focused on *trans* fatty acids rather than on positional isomers of *cis* fatty acids. Accordingly, this chapter deals with effects of *trans* fatty acids rather than positional isomers of *cis* fatty acids.

Typical levels of *trans* fatty acids in food products containing partially hydrogenated oils are shown in Table 31.1. Frying oils used by restaurants and food service operations range in *trans* fatty acid content from 0% to about 35% of total fatty acids. Some restaurants and food service operations currently use unhydrogenated oils (such as those used as salad oils) for frying, and such oils are *trans*-free. Until a few years ago, the fats used in the manufacture of retail margarines and spreads contained, on average, about 15%–25% *trans* fatty acids. Currently, most tub, liquid, and spray products contain no *trans* fat. The total fat content of many retail spreads, both tub and stick

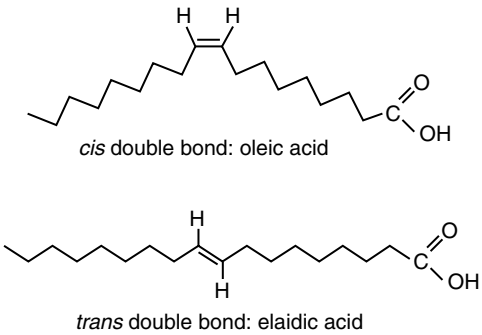

FIGURE 31.1 Structural formulas for the *cis* fatty acid, oleic acid, and its *trans* isomer, elaidic acid.

TABLE 31.1
Typical Levels of *Trans* Fatty Acids in Food Fats/Products

Food Fat/Product	*Trans* Fatty Acid Content
Frying fats	0%–35% of fatty acids
Margarines/spreads	Fat: 0%–25% of fatty acids Product: 0%–15% by weight
Shortenings	0%–30% of fatty acids
Beef and dairy fat	3% of fatty acids

The levels of *trans* fatty acids in various food products are expressed as a percentage of total fatty acids.

varieties, now averages about 55%–60%. Thus, these spread products contain between 0% and 15% *trans* fatty acids by weight. True margarines contain at least 80% fat (required by the FDA's standard of identity for margarines), but such products now represent only a small market share of the category of margarines and spreads. Most currently available baking shortenings typically contain about 15%–30% *trans* fatty acids, expressed as a percentage of total fatty acids. In 2005, at least one marketed shortening was *trans*-free. Beef and dairy fat typically contain about 3% *trans* fatty acids, also expressed as a percentage of total fatty acids.

With regard to levels of consumption, Allison et al. (1999) reported a mean intake of *trans* fatty acids by the U.S. population of 2.6% of energy, or 5.3 g/person/day. This estimate was based on 24-h recalls and 2-day food records by over 11,200 subjects as part of the USDA's Continuing Survey of Food Intakes by Individuals. Consistent with these results, Harnack et al. (2003) reported that the mean intake of *trans* fatty acids in a population of adult subjects in the Minneapolis–St. Paul, MN, metropolitan area decreased from 3.0% of total energy in 1980–1982 to 2.2% of total energy in 1995–1997. The estimates of Harnack et al. (2003) were based on 24-h dietary recalls by more than 7900 subjects participating in the Minnesota Heart Study, an ongoing observational epidemiologic study. These estimated intakes of *trans* fatty acids by Allison et al. (1999) and by Harnack et al. (2003) of 2%–3% of total energy are smaller than those of saturated fatty acids, which contribute 12%–14% of energy intake (Grundy and Denke, 1990; Kris-Etherton and Nicolosi, 1995; American Society for Clinical Nutrition/American Institute of Nutrition Task Force on *Trans* Fatty Acids, 1996; Ahuja et al., 1997). The intake of *trans* fatty acids in 14 European countries has been reported to range from 0.5% to 2.1% of energy (Hulshof et al., 1999), somewhat less than the intake reported for the United States. This intake of *trans* fatty acids in Europe also is considerably less than the intake of saturated fatty acids in Europe, estimated to be 10%–19% of energy (Hulshof et al., 1999).

The estimates of intake of *trans* fatty acids based on reported consumption of foods (Allison et al., 1999; Hulshof et al., 1999; Harnack et al., 2003) are considerably lower than estimates based on the availability from foods. The latter estimates consider market size, market share, and composition of various products made from partially hydrogenated fats and oils, and thus represent reasonable approximations of intake. Such estimates, however, do not take into account fats that are wasted or deliberately discarded. In 1986, Hunter and Applewhite (1986) reported an estimate of *trans* fatty acids available for consumption in the U.S. diet for 1984 of 7.6 g/person/day. A similar value, 8.3 g/person/day, was obtained independently by a *Federation of American Societies for Experimental Biology* (FASEB) Review Panel on *Trans* Fatty Acids (Senti, 1985). Hunter and Applewhite (1991) updated their estimate of *trans* fatty acid availability in the U.S. diet for 1989 to be 8.1 g/person/day. Current efforts by many food manufacturers to eliminate or drastically reduce levels of *trans* fatty acids in their products (to be discussed in Section VI of this chapter), are expected to reduce the intake of *trans* fatty acids to levels well below those reported to date.

III. HUMAN STUDIES RELATING DIETARY *TRANS* FATTY ACIDS TO RISK OF CORONARY HEART DISEASE

A. Review Articles

a. Reviews of Studies Reported Prior to 1990

The year 1990 marked a period of renewed interest by many health professionals in effects of dietary *trans* fatty acids. That year a study by Mensink and Katan (1990) reported that high levels of *trans* fatty acids in the diet increased the level of LDL-cholesterol and decreased the level of HDL-cholesterol compared to high levels of *cis* fatty acids. Details of this study will be discussed subsequently.

Prior to the study by Mensink and Katan (1990), there were numerous reviews and comprehensive studies on the nutritional and biological effects of *trans* fatty acids (e.g., Mattson, 1960; Alfin-Slater and Aftergood, 1979; Applewhite, 1981; Beare-Rogers, 1983; Kritchevsky, 1983; Emken, 1984; Hunter, 1987) that focused on whether or not *trans* fatty acids play a role in the development of atherosclerosis. In general, these studies showed that diets high in 18-carbon *trans* monoenes but adequate in essential fatty acids were not uniquely atherogenic. Many of these reviews and studies were considered in a comprehensive literature review by a special FASEB committee (Senti, 1985). This report concluded that there is "little reason for concern with the safety of dietary *trans* fatty acids *both* at their present and expected levels of consumption *and* at the present and expected levels of consumption of dietary linoleic acid … ." A 1987 British Nutrition Foundation report on the safety of *trans* fatty acids reached the same conclusion (British Nutrition Foundation, 1987).

b. Reviews of Studies Reported Since 1990

Reviews published after 1990 discussed the Mensink and Katan study (1990) and subsequent studies, suggesting that high dietary levels of *trans* fatty acids increased CHD risk. A review by Kris-Etherton and Nicolosi (1995, before much of the *trans* fat literature was published) suggested that the association between *trans* fatty acid intake and CHD risk is weak and inconsistent compared with the large body of evidence from epidemiologic observations and animal and human studies that support a direct effect of saturated fat on CHD risk. These authors commented that some clinical studies were difficult to interpret because it was unclear whether the reported responses were due to the addition of *trans* fatty acids to the diet or to a decrease in dietary unsaturated (i.e., cholesterol-reducing) fatty acids. Kris-Etherton and Nicolosi (1995) considered the current level of intake of *trans* fatty acids to be safe; however, they supported the reduction of total fat intake (including reduced intake of *trans* and cholesterol-raising saturated fatty acids) as part of national dietary guidelines to promote health and well-being.

Katan et al. (1995) concluded that the effects of *trans* fatty acids on raising plasma LDL-cholesterol levels were similar to those of saturated fatty acids. These authors recommended that diets aimed at reducing the risk of CHD be low in both *trans* and saturated fatty acids.

A comprehensive review by the American Society for Clinical Nutrition/American Institute of Nutrition Task Force on *Trans* Fatty Acids (1996) concluded that "compared with saturated fatty acids, the issue of *trans* fatty acids is less significant because the U.S. diet provides a smaller proportion of *trans* fatty acids and the data on their biological effects are limited." The Task Force recommended that data be obtained on the intake of *trans* fatty acids, their biological effects, mechanisms of action, and relation to disease that are comparable to those of saturated fatty acids.

Zock and Katan (1997) reviewed 20 dietary trials comparing effects of butter and margarine on blood lipids. The authors reported that replacing butter by soft, low-*trans* margarines favorably affected the blood lipoprotein profile, which in turn might reduce the risk of CHD. Replacing butter by hard, high-*trans* margarines appeared to offer no benefit over butter.

Ascherio et al. (1999) reported a linear relationship between the change in LDL/HDL ratio (a measure of CHD risk) vs. the percentage of energy from either *trans* fatty acids or saturated fatty acids in the diet. These authors also concluded that the adverse effect on LDL/HDL ratio of *trans* fatty acids appeared to be stronger than that of saturated fatty acids.

A subsequent review by Katan (2000) indicated that since around 1990, *trans* fatty acids went from a type of fat generally regarded as safe and possibly beneficial to one considered by many to be unfavorable for CHD risk. Katan felt that *trans* fatty acids were at least as unfavorable as cholesterol-raising saturated fatty acids.

A review by Schaefer (2002) suggested that hydrogenated margarines and butter should be avoided, and that either oil or soft margarine low in *trans* fatty acids should be used as a spread. Schaefer noted that in addition to having unfavorable effects on LDL- and HDL-cholesterol concentrations, there is evidence that increased *trans* fatty acid consumption, in contrast with consumption of saturated fat, increases plasma concentrations of lipoprotein(a) [Lp(a)], an independent risk factor for CHD.

Mensink et al. (2003) reported a meta-analysis of 60 controlled trials focusing on the effect of individual dietary fatty acids on the ratio of total cholesterol to HDL-cholesterol and on serum lipoproteins. These authors determined that this ratio was decreased most effectively (corresponding to decreased risk of CHD) when *trans* fatty acids and saturated fatty acids were replaced with *cis* unsaturated fatty acids.

In contrast to the above reviews, Wilson et al. (2001) concluded that the intake of *trans* fatty acids does not pose as much of a health concern for Americans as does the intake of cholesterol-raising saturated fatty acids. Although Wilson et al. (2001) encouraged efforts to minimize increased amounts of *trans* fatty acids in the diet, they felt that *trans* fatty acids at the current level of intake are a safe component of the diet. Wilson et al. (2001) suggested that consumers limit their intake of *trans* fatty acids by lowering their intake of total fat within the context of a varied and healthful diet.

The Institute of Medicine (IOM) of the National Academy of Sciences (2002) reviewed effects of dietary *trans* fatty acids as part of a report on reference intake levels of macronutrients. Referring to the article by Ascherio et al. (1999), the IOM report stated that similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and increased risk of CHD. The IOM report did not establish a "Tolerable Upper Intake Level" above which long-term consumption might be undesirable for some individuals. The IOM noted that *trans* fatty acids are unavoidable in ordinary diets, and that eliminating them from the diet might result in inadequate intakes of certain nutrients and increase certain health risks. The report recommended that consumption of *trans* fatty acids as well as saturated fatty acids and cholesterol be as low as possible from a nutritionally adequate diet.

The most recent review, by Mozaffarian et al. (2006), considers evidence for physiological and cellular effects of *trans* fatty acids as well as the feasibility and potential implications of reducing or eliminating the consumption of *trans* fatty acids in the United States. Consistent with other reviews, this article conveys risks associated with consumption of high levels of *trans* fatty acids. In contrast to other reviews, the authors state that even low levels of consumption (1%–3% of total energy intake) confer "a substantially increased risk … ." However, the references cited to support this statement were epidemiological and prospective studies and not controlled dietary trials in which the investigators fed *trans* fatty acids at such levels. A controlled study by Lichtenstein et al. (1999) found no significant effect on either LDL- or HDL-cholesterol when a diet containing *trans* fatty acids at 3.3% of energy was fed for 35 days and compared to a control diet with *trans* fatty acids at only 0.6% of energy. Mozaffarian et al. (2006) expressed their view that consumption of *trans* fatty acids at less than 0.5% of total energy may be necessary to avoid adverse effects. This would mean complete or near-complete avoidance of dietary *trans* fatty acids, an extreme position compared to that expressed in the IOM report (2002) and probably unlikely in the near future to meet with significant consumer acceptance.

B. Human Trials Reporting Changes in Blood Lipid and Lipoprotein Levels with Dietary *Trans* Fatty Acids

a. Experimental Studies

Prior to 1990, two comprehensive literature reviews (Senti, 1985; British Nutrition Foundation, 1987) had concluded that there was little basis for concern about the consumption of *trans* fatty acids at present and expected dietary levels. One study (of many) on which this conclusion was based was that of Mattson et al. (1975). This study was particularly significant because it was the first to control carefully the fatty acid composition of the diets. In this study, subjects were fed liquid formula diets high in *cis* or *trans* fatty acids for 28 days. The fatty acid intakes of both groups were the same except for the presence or absence of *trans* fatty acids. The key result was that the subjects fed the high-*trans* diet had the same blood total cholesterol and triglyceride levels as subjects fed the high-*cis* diet. At the time this study was conducted, methodology for measuring HDL- and LDL-cholesterol and their apoproteins was not well established.

In a subsequent study by Mensink and Katan (1990), blood lipoprotein levels were measured in young adult human subjects (mean age 26 years) fed conventional food diets high in either *cis*, *trans*, or saturated fatty acids. The study design resembled that used by Mattson et al. (1975) in that the diets containing *cis* and *trans* fatty acids had similar fatty acid compositions except for the presence or absence of *trans* fatty acids. In this study, the high-*trans* diet (10.9% of energy as *trans* fatty acids) raised total and LDL-cholesterol and lowered HDL-cholesterol compared to the high-*cis* diet after 3 weeks. Although the level of *trans* fatty acids in the high-*trans* diet was unrealistically high (nearly 11% of energy), the authors concluded that the effect of *trans* fatty acids on serum lipoprotein profiles was similar to that of cholesterol-raising saturated fatty acids.

Since the Mensink and Katan study (1990), numerous other human trials have been conducted, which included measurement of LDL- and HDL-cholesterol [and occasionally Lp(a)], after feeding diets moderate or high in *trans* fatty acids. These studies include Mensink et al. (1992), Nestel et al. (1992), Zock and Katan (1992), Lichtenstein et al. (1993, 1999), Wood et al. (1993a,b), Judd et al. (1994, 1998, 2002), Almendingen et al. (1995), Cuchel et al. (1996), Aro et al. (1997), Clevidence et al. (1997), Sundram et al. (1997), Muller et al. (1998a,b), Noakes and Clifton (1998), Tholstrup et al. (1998, 2006), Vidgren (1998), Louheranta et al. (1999), Denke et al. (2000), de Roos et al. (2001a,b), French et al. (2002), Han et al. (2002), Lovejoy et al. (2002), and Sanders et al. (2003). Key study design parameters and results are summarized in Table 31.2. In essence, these studies demonstrated that diets high in *trans* fatty acids raised LDL-cholesterol and lowered HDL-cholesterol compared to diets low in *trans* fatty acids.

Three noteworthy studies (Zock and Katan, 1992; Judd et al., 1994, 2002) are discussed in more detail below. Zock and Katan (1992) compared effects on blood lipoproteins of diets high in linoleic acid, *trans* fatty acids, and stearic acid. In this study, the intake of *trans* fatty acids was lower than that of the Mensink and Katan (1990) study (7.7% of energy vs. 10.9% of energy). The lower level of *trans* fatty acids raised total cholesterol and LDL-cholesterol and lowered HDL-cholesterol compared to the linoleic acid diet. The stearic acid diet similarly raised total and LDL-cholesterol and reduced HDL-cholesterol compared to the linoleic acid diet. Subsequently, Mensink et al. (1992) reported that this *trans* fatty acid diet raised Lp(a) levels compared to the linoleic acid and stearic acid diets.

In the first study by Judd et al. (1994), moderate- and high-*trans* diets (3.8% and 6.6% of energy, respectively) resulted in increased plasma LDL-cholesterol levels compared to an oleic acid diet. These increases, however, were less than those observed with a saturated fatty acid diet. The high-*trans* diet, but not the moderate-*trans* diet, resulted in a minor (statistically significant) reduction in HDL-cholesterol. The saturated diet, however, led to a slight increase in HDL-cholesterol.

Lp(a) levels were reported in a subsequent publication (Clevidence et al., 1997). Compared to the oleic diet, the *trans* diets had no effect on Lp(a) levels when all subjects were considered collectively.

TABLE 31.2
Human Trials Reporting Changes in LDL-Cholesterol, HDL-Cholesterol, and Lipoprotein(a) with Dietary *Trans* Fatty Acids (TFA)

Reference	Study Population	Period (Days)	TFA (% en) Treatment and Control Diets	Change in LDL-C vs. Control[a]	Change in HDL-C vs. Control[a]	Change in Lp(a) vs. Control[a]
Mensink and Katan (1990); Mensink et al. (1992)	34 women 25 men normocholesterolemic	21	10.9 0.0 (OL diet)	13.9% increase (2.67 to 3.04 mM)	12.0% decrease (1.42 to 1.25 mM)	40.6% increase (32 to 45 mg/L)
Zock and Katan (1992); Mensink et al. (1992)	30 women 26 men normocholesterolemic	21	7.7 0.1 (LA diet)	8.5% increase (2.83 to 3.07 mM)	6.8% decrease (1.47 to 1.37 mM)	23.2% increase (69 to 85 mg/L)
Nestel et al. (1992)	27 mildly hypercholesterolemic men	21	6.7 2.4 (OL diet)	9.5% increased (3.90 to 4.27 mM)	NS change	NS change
Judd et al. (1994); Clevidence et al. (1997)	29 women 29 men normocholesterolemic	42	3.8 (moderate) 6.6 (high) 0.7 (OL diet)	6.0% increase (3.34 to 3.54 mM) 7.8% increase (3.34 to 3.60 mM)	NS change 2.8% decrease (1.42 to 1.38 mM)	NS change NS change
Judd et al. (2002)	50 men normocholesterolemic	35	4.2 (moderate; TFA/STE) 8.3 (high) 0.1 (OL diet)	12.5% increase (2.95 to 3.32 mM) 13.9% increase (2.95 to 3.36 mM)	5.6% decrease (1.24 to 1.17 mM) 6.5% decrease (1.24 to 1.16 mM)	NR
Lichtenstein et al. (1993)	8 women 6 men moderately hypercholesterolemic	32	4.2 (margarine) 0.4 (CO diet)	8.0% increase (3.23 to 3.49 mM), significant at $p < .058$	NS change	NS change

Continued

TABLE 31.2 (Continued)

Reference	Study Population	Period (days)	TFA (% en) Treatment and Control Diets	Change in LDL-C vs. Control[a]	Change in HDL-C vs. Control[a]	Change in Lp(a) vs. Control[a]
Lichtenstein et al. (1999)	18 women 18 men moderately hypercholesterolemic	35	0.9 (semiliquid margarine)	NS change	NS change	NS change
			1.3 (butter)	15.1% increase (3.98 to 4.58 mM)	NS change	NS change
			3.3 (soft margarine)	NS change	NS change	NS change
			4.2 (shortening)	6.5% increase (3.98 to 4.24 mM)	NS change	NS change
			6.7 (stick margarine)	9.0% increase (3.98 to 4.34 mM)	NS change	NS change
			0.6 (SBO diet)			
Aro et al. (1997)	49 women 31 men normocholesterolemic	35	8.7	8.3% increase (2.89 to 3.13 mM)	14.1% decrease (1.42 to 1.22 mM)	14.1% increase (270 to 308 mg/L)
			0.4 (STE diet)			
Sundram et al. (1997)	9 women 18 men normocholesterolemic	28	6.9	20.2% increased (3.17 to 3.81 mM)	16.0% decrease (1.25 to 1.05 mM)	19.5% increase (12.8 to 15.3 mg/dL)
			not detected (OL diet)			
Wood et al. (1993a)	29 men normocholesterolemic	42	6.3 (hard margarine) NR for foods common to all diets	NS change	NS change	NR
Almendingen et al. (1995)	31 men normocholesterolemic	19–21	8.5 (PHSBO margarine)	6.0% decrease (3.81 to 3.58 mM vs. butter diet)	NS change vs. butter diet	22.7% increase (194 to 238 mg/L, vs. butter diet)
			8.0 (PHFO margarine)	NS change vs. butter diet	6.7% decrease (1.05 to 0.98 mM, vs. butter diet)	20.6% increase (194 to 234 mg/L, vs. butter diet)
			0.9 (butter)		Difference between PHSBO (1.05 mM) and PHFO (0.98 mM) diets significant at $p = 0.03$	NS difference between PHSBO and PHFO diets
Wood et al. (1993b)	38 men normocholesterolemic, free living (total diet not controlled)	42	6.7 minimum (hard margarine)	8.2% decrease (3.78 to 3.47 mM, butter vs. hard margarine diet)	NS change, butter vs. hard margarine diet	NR
			1.2 minimum (butter)	6.1% decrease (3.47 to 3.26 mM, hard margarine vs. soft margarine diet)	NS change, butter vs. soft margarine diet	
			0 minimum (soft margarine)	13.8% decrease (3.78 to 3.26 mM, butter vs. soft margarine diet)	NS difference between hard and soft margarine diets	

Noakes and Clifton (1998)	17 women 21 men mildly hypercholesterolemic, free living (total diet not controlled)	21	2.1 min, canola + TFA	12.1% decrease (4.14 to 3.64 mM, butter vs. canola + TFA group)	NS change, butter vs. canola + TFA group	NR
			0 min, canola + TFA-free	12.8% decrease (4.14 to 3.61 mM, butter vs. TFA-free canola group)	NS change, butter vs. TFA-free canola group	
			0.7 min, butter			
			2.1 min, PUFA + TFA	10.0% decrease (4.70 to 4.23 mM, butter vs. PUFA + TFA group)	NS change, butter vs. PUFA + TFA group	
			0 min, PUFA + TFA-free	15.3% decrease 4.70 to 3.98 mM (butter vs. TFA-free PUFA group	NS change, butter vs. TFA-free PUFA group	
			0.7 min, butter			
Judd et al. (1998)	23 men 23 women normocholesterolemic	35	3.9 TFA margarine	4.9% decrease (3.44 to 3.27 mM, butter vs. TFA margarine group)	NS change vs. butter group	8.6% increase (186 to 202 mg/L, butter vs. TFA margarine group)
			2.4 PUFA margarine	6.7% decrease (3.44 to 3.21 mM, butter vs. PUFA margarine group)	NS change vs. butter group	5.9% increase (186 to 197 mg/dL, butter vs. PUFA margarine group)
			2.7 butter	Significant difference, 3.27 vs. 3.21 mM, TFA vs. PUFA margarine groups, $p \leq 0.05$	NS difference between TFA and PUFA margarine groups	NS difference between TFA and PUFA margarine groups
de Roos et al. (2001a)	10 men 19 women normocholesterolemic	28	9.2 0.3 (sat diet)	NS difference after *trans* vs. after saturated diet	21% decrease (1.87 to 1.48 mM after *trans* vs. after saturated diet	NR
de Roos et al. (2001b)	11 men 21 women normocholesterolemic	28	9.3 0.3 (sat diet)	NS difference after *trans* vs. after saturated diet	23% decrease (1.89 to 1.46 mM after *trans* vs. after saturated diet	NR
Denke et al. (2000)	46 families: 92 adults, 134 children normocholesterolemic	35	1.5[b] margarine 0.5 butter	Adults: 11.5% decrease (3.39 to 3.00 mM, after margarine diet vs. after butter diet)	Adults: NS difference, butter vs. margarine diet	NR
				Children: 10.8% decrease (2.69 to 2.40 mM, after margarine diet vs. after butter diet)	Children: NS difference, butter vs. margarine diet	

Continued

TABLE 31.2 (Continued)

Reference	Study Population	Period (days)	TFA (% en) Treatment and Control Diets	Change in LDL-C vs. Control[a]	Change in HDL-C vs. Control[a]	Change in Lp(a) vs. Control[a]
Louheranta et al. (1999)	14 women normocholesterolemic	28	5.1 margarine 0 (OL diet)	NS change	NS change	NS change
Muller et al. (1998b)	16 women normocholesterolemic	14	7.7 (PHFO) 1.1 (vegetable oil)	9.1% increase (2.63 to 2.87 mM)	NS change	NS change
Cuchel et al. (1996)	14 men and women moderately hypercholesterolemic	32	4.2 (CO stick margarine) 0.4 (CO)	8.0% increase (3.24 to 3.50 mM, $p = 0.058$)	NS change	NR
Han et al. (2002)	19 men and women moderately hypercholesterolemic	32	6.7 (SBO stick margarine) 0.6 (SBO)	11.3% increase (3.88 to 4.32 mM)	6.0 % decrease (1.16 to 1.09 mM)	NR
French et al. (2002)	10 men and women normocholesterolemic	30	5.6 0 (SFA)	11.5% increase (3.23 to 3.60 mM, after TFA vs. after SFA diet)	NS difference between TFA and SFA groups	NR
Lovejoy et al. (2002)	25 men and women normocholesterolemic	28	7.3 0 (OL)	NS change	NS change	NR
Sanders et al. (2003)	29 men normocholesterolemic	14	9.6 0.1 (OL)	NS change	6.3% decrease (1.26 to 1.18 mM)	NS change
Muller et al. (1998a)	27 women normocholesterolemic	17	7.0 (PHSBO margarine) 0.2 (PUFA margarine)	13.1% increase (2.61 to 2.88 mM, after PHSBO margarine vs. after PUFA margarine diets)	7.7% decrease (1.43 to 1.32 mM, after PHSBO margarine vs. after PUFA margarine diets)	NS change
Vidgren et al. (1998)	14 women normocholesterolemic	28	5.1 0 (OL)	NR	NR	NR
Tholstrup et al. (2006)	42 men normocholesterolemic	35	2.1 (vaccenic acid-rich butter) 0.4 (low vaccenic acid butter)	NS change	9.7% decrease (1.54 to 1.39 mM after vaccenic acid rich diet)	NR

[a] Changes indicated were significant at $p \leq 0.05$ unless otherwise indicated.

[b] Based on 3-day food records.

Abbreviations: TFA, *trans* fatty acids; % en, % of energy; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Lp(a), lipoprotein(a); mM, millimoles/liter; OL, oleic acid; LA, linoleic acid; STE, stearic acid; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; CO, corn oil; SBO, soybean oil; SO, sunflower oil; PHSBO, partially hydrogenated soybean oil; PHFO, partially hydrogenated fish oil; min, minimum (i.e., values apply only to test fat, not to total fat in diet); NS, not significant; NR, not reported.

However, a subset with initially high levels of Lp(a) (≥30 mg/dL) responded to the high-*trans* diet with a slight (5%) increase in Lp(a) levels relative to the oleic and moderate-*trans* diets. Thus, the unfavorable effects on Lp(a) of replacing *cis* with *trans* fatty acids were restricted to high-*trans* intakes in subjects with high levels of Lp(a).

The second study by Judd et al. (2002) included a high-carbohydrate diet to assess whether direct addition of *trans* fatty acids to the diet had an independent cholesterol-raising effect. In addition, a high-stearate diet was included to determine whether stearate and *trans* fatty acids were neutral with regard to their effects on blood lipoproteins. Compared to a high-oleic diet, a high-*trans* (8% of energy) diet raised LDL-cholesterol to a greater extent than a high-saturated diet despite similar levels of *trans* plus saturated fatty acids. In contrast, in the previous study (Judd et al., 1994), the increase in LDL-cholesterol after the high-*trans* diet was directionally (but not significantly) less than that observed after the high-saturated diet. Compared to the high-carbohydrate diet, both the moderate-*trans* (4% of energy) and high-*trans* diets and the saturated diet resulted in increased LDL-cholesterol levels. Both *trans* diets lowered HDL-cholesterol compared to the oleic diet but not compared to the carbohydrate diet. Also compared to the carbohydrate diet, the stearic acid diet had no effect on LDL-cholesterol but lowered HDL-cholesterol. The oleic acid diet had no effect on LDL-cholesterol but raised HDL-cholesterol.

The studies by Mensink and Katan (1990) and Zock and Katan (1992) and the two studies by Judd et al. (1994, 2002) were consistent in that in all four studies, the *trans* and saturated fatty acid treatments raised LDL-cholesterol levels compared to the control diet. The changes in LDL-cholesterol levels by *trans* fatty acids and by saturated fatty acids were similar. HDL-cholesterol levels in these studies were reduced consistently when the level of *trans* fatty acids in the diet was 6.6% of energy or higher. In the second study by Judd et al. (2002), the moderate-*trans* diet showed a reduction in HDL-cholesterol, which was not seen in the first study (Judd et al., 1994).

b. Collective Consideration of Human Trials

Other human studies (Nestel et al., 1992; Lichtenstein et al., 1993, 1999; Aro et al., 1997; Sundram et al., 1997) have also reported increases in LDL-cholesterol levels and decreases in HDL-cholesterol levels when diets high in *trans* fatty acids were compared to diets high in either oleic acid or linoleic acid (Table 31.2). Either of these control diets contained only small levels of *trans* fatty acids (from 0% to 0.7% of energy).

Nine such studies (Mensink and Katan, 1990; Nestel et al., 1992; Zock and Katan, 1992; Lichtenstein et al., 1993, 1999; Judd et al., 1994, 2002; Aro et al., 1997; Sundram et al., 1997) have been compared collectively by Ascherio et al. (1999). The latter investigators graphed the changes in LDL/HDL ratio reported in these studies against the percentage of energy from either *trans* fatty acids or saturated fatty acids (Figure 31.2). Two best-fit regression lines were plotted through the origin, one line representing the percentage of energy from *trans* fatty acids and the other line, the percentage of energy from saturated fatty acids. Six of the nine studies (Mensink and Katan, 1990; Nestel et al., 1992; Zock and Katan, 1992; Judd et al., 1994, 2002; Sundram et al., 1997) allowed a comparison between *trans* and saturated fatty acids. Both regression lines had positive slopes, indicating a positive association between intake of either category of fatty acids and CHD risk. However, the slope of the regression line for *trans* fatty acids was larger (by about twofold) than that for saturated fatty acids. This difference in slopes led to the conclusion that *trans* fatty acids have a more adverse effect on CHD risk than saturated fatty acids (Ascherio et al., 1999). The conclusion was supported further by the greater effect on LDL/HDL ratio of *trans* fatty acids compared to saturated fatty acids in the six studies that allowed such a comparison.

Another way to look at these studies collectively is to consider the relationship between simply the change in LDL-cholesterol level or the change in HDL-cholesterol level (rather than the change in their ratio) with increasing level of *trans* fatty acids in the diet (Hunter, 2005). This is reasonable because changes in the LDL/HDL ratio do not permit determination of whether LDL, or HDL, or

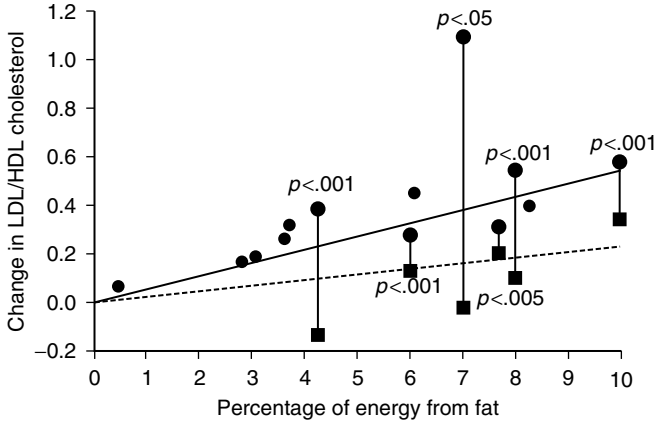

FIGURE 31.2 Change in LDL/HDL ratio with level of dietary *trans* fatty acids or saturated fatty acids. This graph was reported by Ascherio et al. (1999) and compares collectively the LDL/HDL ratios against the percentage of energy as either *trans* fat (solid circles, solid line) or saturated fat (solid squares, dashed line) for nine studies (see text).

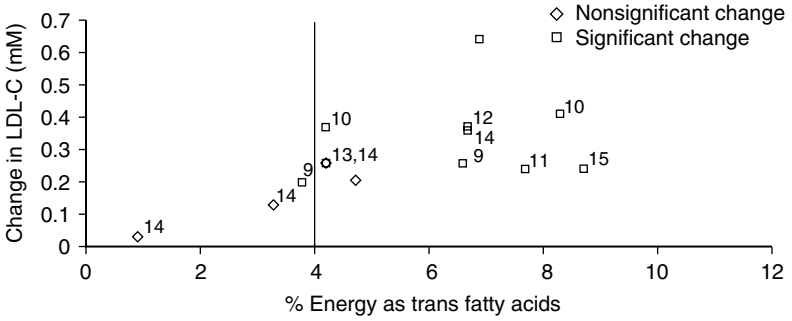

FIGURE 31.3 Scatter diagram of the change in LDL-cholesterol (expressed as mM) with dietary level of *trans* fatty acids (expressed as % energy) using data from the nine studies compared collectively by Ascherio et al. (1999). Changes in LDL-cholesterol were calculated from the difference between a *trans* fatty acid treatment and its corresponding control treatment. Open diamonds (◊) represent changes in LDL-cholesterol that were reported NOT to be statistically significant. Open squares (□) represent changes in LDL-cholesterol that were reported to be statistically significant. The number to the right of each point indicates the reference from which the data were taken (see Hunter, 2005).

both were changing at a particular dietary level of *trans* fatty acids. In addition, considering the ratio, saturated fatty acids tend to increase both LDL- and HDL-cholesterol, thus appearing to be "healthy" compared to *trans* fatty acids.

Using data from the same nine studies, Figure 31.3 is a scatter diagram of the change in LDL-cholesterol with dietary level of *trans* fatty acids. The relationship is directionally similar to that seen when the LDL/HDL ratio was plotted (Figure 31.2). Importantly, however, considering these nine studies, the change in LDL-cholesterol was not statistically significant unless the dietary level of *trans* fatty acids was around 4% of energy or higher. The change in HDL-cholesterol with increasing level of *trans* fatty acids in the diet (Figure 31.4), with the exception of one study, was not statistically significant unless the dietary level of *trans* fatty acids was higher than about 5%–6% of energy. [*Note:* Numerous other human studies (Table 31.2) that have evaluated effects of *trans* fatty acids on LDL- and HDL-cholesterol levels were excluded from consideration here because they did not compare effects of *trans* fatty acids with those of isocaloric amounts of *cis* fatty acids.]

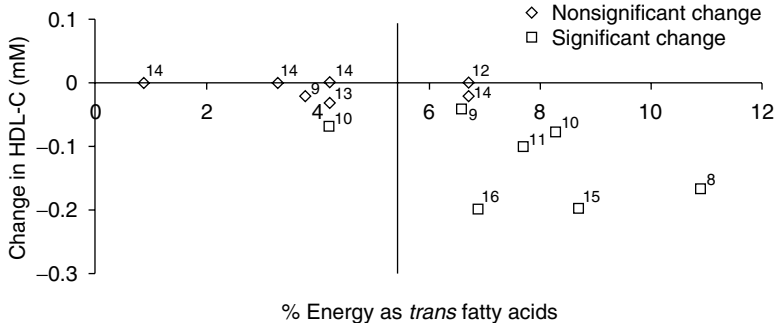

FIGURE 31.4 Scatter diagram of the change in HDL-cholesterol (expressed as mM) with dietary level of *trans* fatty acids (expressed as % energy) using data from the nine studies compared collectively by Ascherio et al. (1999). Changes in HDL-cholesterol were calculated from the difference between a *trans* fatty acid treatment and its corresponding control treatment. Open diamonds (◊) represent changes in HDL-cholesterol that were reported NOT to be statistically significant. Open squares (□) represent changes in HDL-cholesterol that were reported to be statistically significant. The number to the right of each point indicates the reference from which the data were taken (see Hunter, 2005).

In an effort to better define the dietary level of *trans* fatty acids that raises LDL-cholesterol and lowers HDL-cholesterol, weighted regression break point analysis and chi-squared analysis have been considered (Hunter, 2005). For LDL-cholesterol, a visual break point (the minimum level of *trans* fatty acids resulting in a significant increase in LDL) appeared to exist at or below about 4% of energy as *trans* fatty acids (Figure 31.3). Weighted regression analysis, however, did not show quantitatively that 4% of energy is the break point, probably in part because there were only two data points below 3.8% of energy. Nevertheless, it seemed reasonable to expect that a break point might exist in the range of 3.3%–4.2% of energy, where the difference between treatment and control LDL values was greatest. This range is consistent with a possible visual break point at or below 4% of energy.

In the case of HDL-cholesterol, more data were available below a visual break point estimated to be between 5% and 6% of energy (Figure 31.4). However, the absence of data in the range of 4.2%–6.6% of energy did not permit more precise estimation of a break point other than to suggest that it might fall in the 5%–6% of energy range.

Chi-squared analyses indicated reasonable break points for LDL and HDL to be about 3.5% and 7.0% of energy, respectively (Hunter, 2005). These latter values are similar to the visual break point estimates of 4% of energy for LDL and 5%–6% of energy for HDL. It is recognized that the confidence in these visual and estimated break points is limited because of the small amount of data (14 treatments) available from the nine studies considered. In particular from Figure 31.3, there were only two dietary treatments below 4% of energy as *trans* fatty acids. This is an insufficient amount of evidence at present to say whether or not *trans* fatty acids at less than 4% of energy are harmful to humans, and possible biological effects below 4% of energy cannot be ruled out.

An additional way to consider the same studies is to compare the levels of *trans* fatty acids and of linoleic acid used in the *trans* fatty acid diet treatments (Hunter, 2005). A scatter diagram (Figure 31.5) indicates that higher dietary levels of *trans* fatty acids were associated with lower levels of linoleic acid and vice versa. In some cases where high levels of *trans* fatty acids (i.e., more than 6% of energy) were fed, the corresponding levels of linoleic acid (2%–4% of energy) may have been nutritionally inadequate. For example, an adequate intake of linoleic acid for men 31–50 years old is 17 g/day (Institute of Medicine, 2002). For men in this age range consuming 2800 kcal/day, an adequate intake of linoleic acid would be 5.5% of energy, well above the 2%–4% of energy used in several studies.

Figure 31.6 plots the change in LDL/HDL ratio from the *trans* fatty acid diet treatments (Ascherio et al., 1999) against the percentage of energy as linoleic acid instead of against the percentage of

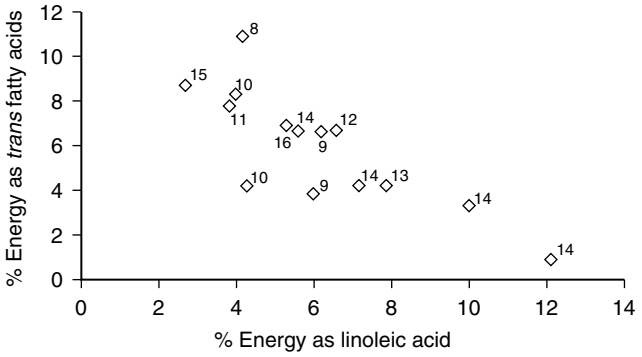

FIGURE 31.5 Scatter diagram comparing the levels of *trans* fatty acids and of linoleic acid used in the *trans* fatty acid diet treatments of the nine studies compared collectively by Ascherio et al. (1999). Each open diamond represents the level of dietary *trans* fatty acids and the corresponding level of linoleic acid used in that same treatment. The number to the right of each point indicates the reference from which the data were taken (see Hunter, 2005).

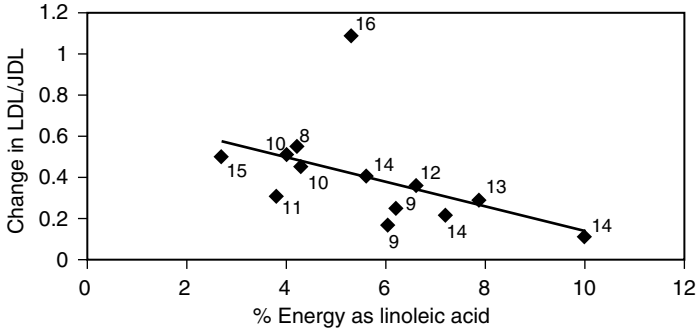

FIGURE 31.6 Change in the ratio of LDL-cholesterol/HDL-cholesterol vs. the dietary level of linoleic acid using data from the nine studies compared collectively by Ascherio et al. (1999). The slope for the best-fit regression line is –0.059, similar in magnitude (0.056) to that determined for the graph presented by Ascherio et al. (1999) (LDL/HDL ratio vs. % energy as *trans* fatty acids) but opposite in sign. The number to the right of each point indicates the reference from which the data were taken (see Hunter, 2005).

energy as *trans* fatty acids (Hunter, 2005). The regression line obtained is opposite in direction compared to that for the percentage of energy as *trans* fatty acids (Ascherio et al., 1999). One interpretation of this difference in direction of the regression lines is that at a sufficient level of linoleic acid in the diet (i.e., 5%–6% of energy or higher), *trans* fatty acids may be less effective in increasing the LDL/HDL ratio. Alternatively, increasing dietary linoleic acid may lower the LDL/HDL ratio through the well-known effect of lowering LDL-cholesterol. Thus, at sufficiently high levels of linoleic acid, there may be little effect on the LDL/HDL ratio due to *trans* fatty acids.

In summary, it appears that at a sufficiently high dietary level, *trans* fatty acids indeed raise LDL-cholesterol and reduce HDL-cholesterol levels compared to a diet essentially free of *trans* fatty acids. The dietary levels of *trans* fatty acids necessary to increase LDL-cholesterol appear to be around 4% of energy and higher and to decrease HDL-cholesterol, around 5%–6% of energy or higher compared to control diets. Statistically, based on limited available data, it was not possible to estimate precise break points for LDL or for HDL. Nevertheless, it appears that high dietary levels of *trans* fatty acids indeed increase CHD risk.

C. Studies on Clotting Tendency, Blood Pressure, and LDL Oxidation

Results of studies assessing the effect of dietary *trans* fatty acids on blood clotting tendency have been inconsistent. Wood et al. (1993a) reported that blood levels of thromboxane B_2, an indicator of increased platelet aggregation (i.e., a measure of increased clotting tendency), were unchanged in a group of human subjects fed high levels of hard margarine compared to subjects fed a baseline diet. On the other hand, blood levels of 6-keto-prostaglandin $F_{1\alpha}$, an indicator of antiaggregatory activity, were reduced in the margarine group compared to the baseline group, suggesting that *trans* fatty acids might have increased clotting tendency. Almendingen et al. (1996) found that a diet containing 8.5% energy from a margarine made from partially hydrogenated soybean oil resulted in higher levels of plasminogen activator inhibitor type 1 than a diet containing 8.0% energy from a margarine made from partially hydrogenated fish oil. This finding suggested that partially hydrogenated soybean oil had unfavorable antifibrinolytic effects compared to partially hydrogenated fish oil. There were no differences between the diets in levels of factor VII, fibrinogen peptide A, β-thromboglobulin, D-dimer, or tissue plasminogen activator.

In other studies, Mutanen and Aro (1997) reported no differences in coagulation and fibrinolysis between a high-stearic acid diet (0.4% of energy as *trans* fatty acids) and a high-*trans* fatty acid diet (8.7% of energy as *trans* fatty acids). The same high-stearic acid and *trans* fatty acid diets also produced similar effects on platelet aggregation and endothelial prostaglandin I_2 production (Turpeinen et al., 1998). Sanders et al. (2000), saw no significant differences in factor VII coagulation activity after feeding single meals high in either oleic acid (0.07% of energy as *trans* fatty acids) or elaidic acid (24.7% of energy as *trans* fatty acids). A subsequent study (Sanders et al., 2003) in which normocholesterolemic men were fed a high-oleic acid diet (0.1% of energy as *trans* fatty acids) or a high-*trans* fatty acid diet (9.6% of energy as *trans* fatty acids) for 2 weeks resulted in no differences in known hemostatic risk markers (fibrinogen and D-dimer concentrations, factor VII coagulant, plasminogen activator inhibitor type 1, and tissue plasminogen activity) for cardiovascular disease. Feeding a diet high in the ruminant *trans* fatty acid, vaccenic acid (1.1% of energy), similarly had no effect on the hemostatic variables factor VII coagulant activity and plasminogen activator inhibitor type 1 (Tholstrup et al., 2006).

With regard to blood pressure, two studies with normotensive subjects showed no effect of *trans* fatty acids (fed at up to 10.9% of energy) compared to oleic acid on either systolic or diastolic blood pressure (Mensink et al., 1991; Zock et al., 1993). Similarly, high-*trans* diets containing up to 8.5% of energy as *trans* fatty acids, consistently have reported little or no effect on the susceptibility of LDL to oxidation (Nestel et al., 1992; Cuchel et al., 1996; Halvorsen et al., 1996; Sorensen et al., 1998).

In summary, a limited number of studies to date have reported no consistent effects of dietary *trans* fatty acids on blood clotting tendency and no significant effects on blood pressure or LDL oxidation compared to dietary *cis* fatty acids.

D. Epidemiologic and Case–Control Studies

Several epidemiologic and case–control studies have reported associations between the intake of *trans* fatty acids and the risk of developing CHD. These studies include Troisi et al. (1992), Willett et al. (1993), Ascherio et al. (1994, 1996), Kromhout et al. (1995), Hu et al. (1997), Pietinen et al. (1997), Tavani et al. (1997), Oomen et al. (2001), and Lemaitre et al. (2002). Key study design aspects and results of these studies are summarized in Table 31.3. Results of the studies were consistent in that *trans* fatty acid intake was positively associated with risk of CHD or with a major coronary event, particularly when relative risks between the highest and lowest quintiles of intake were considered.

On the other hand, in at least one study (Ascherio et al., 1994), individuals consuming intermediate levels of *trans* fatty acids had a lower risk of CHD compared to individuals consuming the *lowest*

TABLE 31.3
Epidemiological/Case–Control Studies Relating Dietary *Trans* Fatty Acids and Risk of CHD

Reference	Study Design	Subjects	TFA Intake	Results	Comments
Troisi et al. (1992)	Cross-sectional	748 men, aged 43–85 years	Food frequency questionnaire; for entire sample, mean TFA intake was 1.6% of total energy	TFA intake directly related to total serum ($r = 0.07$, $p = 0.04$) and LDL-C ($r = 0.09$, $p = 0.01$)	Authors suggested increasing TFA intake from 2.1 to 4.9 g/d would correspond to a 27% increase in risk of MI
Willett et al. (1993)	Prospective cohort study among 85,095 women	431 women experiencing new CHD (nonfatal MI or death from CHD) over 8 year follow-up period	Food frequency questionnaire; quintiles of mean TFA intakes: 1.3, 1.8, 2.2, 2.6, and 3.2% of energy (2.4, 3.2, 3.9, 4.5, and 5.7 g/d)	TFA intake directly related to risk of CHD (relative risk for highest vs. lowest quintile was 1.50 ($p = 0.001$ for trend)	Authors suggested consumption of partially hydrogenated vegetable oils may contribute to occurrence of CHD
Ascherio et al. (1994)	Case–control	239 MI patients, 282 population controls	Food frequency questionnaire; quintiles of mean TFA intake: 1.69, 2.48, 3.35, 4.52, 6.51 g/d	TFA intake directly related to risk of MI (relative risk for highest vs. lowest quintile was 2.44 ($p < 0.0001$ for trend). Increased risk evident only among individuals in top quintile of intake	Patients not asked whether they changed their dietary intakes after their MI. LDL-C in this group may reflect recent dietary intakes rather than diet before MI
Kromhout et al. (1995)	Cohort study among men from seven countries	12,763 men, aged 40–59 years, over 25-year follow-up period	Food composites collected and analyzed for TFA. Average TFA intakes: 0.05% to 1.84% of energy	Strong positive associations between 25-year death rates from CHD and average intake of TFA (elaidic acid, $r = 0.78$, $p < .001$)	Authors suggested dietary TFA (also SFA and cholesterol) are important determinants of differences in population rates of CHD death
Ascherio et al. (1996)	Cohort study	43,757 men, aged 40–75, over 6 year follow-up period	Food frequency questionnaire; median intakes (g/d) of quintiles: 1.5, 2.2, 2.7, 3.3, 4.3 (0.8% to 1.6% of energy, lowest to highest quintiles)	TFA intake directly associated with risk of MI (nonfatal and fatal). Relative risk for quintiles: 1.0, 1.20, 1.24, 1.27, 1.40, $p = 0.01$	The association was attenuated after adjustment for dietary fiber
Hu et al. (1997)	Prospective cohort study	939 women with MI, aged 34–59 years	Food frequency questionnaire; median intakes of quintiles (% of energy): 1.3, 1.7, 2.0, 2.4, 2.9	TFA intake directly associated with risk of CHD. Relative risk for quintiles: 1.0, 1.07, 1.10, 1.13, 1.27 ($p = 0.02$ for trend)	Authors reported that replacing 2% of energy intake from TFA with unhydrogenated, unsaturated fats would reduce CHD risk by 53%

Pietinen et al. (1997)	Cohort study	21,390 male smokers, aged 50–69 years, over 6.1 year follow-up period	Food frequency questionnaire; median intakes (g/d) of quintiles: 1.3, 1.7, 2.0, 2.7, 6.2	TFA intake significantly associated with risk of major coronary event. Relative risk for quintiles: 1.00, 1.10, 0.97, 1.07, 1.14 (p = 0.158 for trend)	Authors also reported positive association between TFA intake and risk of coronary death (p for trend = 0.004)
Tavani et al. (1997)	Case–control	Italian women, aged 18–74 years, 429 MI cases, 866 controls	Margarine intakes reported as no or low, medium or high	Medium or high intake of margarine associated with increased risk of MI (multivariate odds ratio [OR] = 1.5) vs. no or low intake). Association stronger for women aged 60–74 years (OR = 2.4) and in current smokers (OR = 2.3)	Authors suggested the association with margarine could explain about 6% of MI in this population. Major limitation of this study: low intake of margarine by these Italian women
Oomen et al. (2001)	Prospective study	667 elderly Dutch men, aged 64–84 years	Dietary surveys; average TFA intakes fell from 10.9 to 6.9 to 4.4 g/d from 1985 to 1990 to 1995 (4.3%, 2.9%, and 1.9% of energy)	TFA intake positively associated with 10-year risk of CHD. Relative risk for a difference of 2% of energy in TFA intake at baseline was 1.28	Authors suggested that high intake of TFA contributes to risk of CHD
Lemaitre et al. (2002)	Case–control	174 cardiac arrest patients, 285 controls, aged 25–74 years	TFA levels in RBC membranes used as indicator of intake	Higher TFA levels in RBC membranes associated with increased risk of primary cardiac arrest (relative risk = 1.47). *Trans* isomers of oleic acid not associated with risk, but those of linoleic acid associated with threefold increase in risk	It seems unlikely that *trans* 18:2 isomers are present at sufficient levels in typical U.S. diets to contribute significantly to risk of primary cardiac arrest

Abbreviations: CHD, coronary heart disease; MI, myocardial infarction; TFA, *trans* fatty acids; SFA, saturated fatty acids; LDL-C, LDL-cholesterol; g/d, grams per day; r = partial correlation coefficient (adjusted for various parameters including energy intake, age, BMI, waist-to-hip ratio, smoking status, and alcohol intake); RBC, red blood cell.

level of *trans* fatty acids. This study did not support a dose–response relationship. A limitation of epidemiologic studies is that they may show associations between two variables but do not prove cause and effect relationships. The relatively low risk values reported for associations of *trans* fatty acid intake and CHD risk (typically around 1.2–1.5, Table 31.3) are considered weak associations by many epidemiologists (Lilienfeld and Stolley, 1994; Rothman and Greenland, 1998).

The study by Lemaitre et al. (2002) reported that *trans* isomers of oleic acid were not associated with CHD risk, whereas higher levels of *trans* isomers of linoleic acid were associated with a three-fold increase in risk. It seems doubtful, however, that *trans* 18:2 isomers are present at sufficient levels in typical U.S. diets to contribute uniquely and significantly to the risk of primary cardiac arrest (Expert Panel on *Trans* Fatty Acids and Coronary Heart Disease, 1995).

A further limitation of epidemiologic studies is that self-reported dietary intake data are likely inaccurate. Mertz et al. (1991) found that recall and food record data usually underestimate intake. A further complicating factor is that the *trans* fatty acid contents of similar appearing foods, such as tub margarines, can be highly variable among brands and even with the same brand over time. Thus, an assumed *trans* fatty acid level for the category of "tub margarines" may not result in accurate calculation of *trans* fatty acid intake levels over time.

In addition, a recent report by Schatzkin et al. (2003) does *not* recommend use of a food frequency questionnaire for evaluating relationships between absolute intake values and disease. This is because repeated applications of the questionnaire did not lead to improvement in proposed relationships being considered. The study by Schatzkin et al. (2003) was part of the National Cancer Institute's "Observing Protein and Energy Nutrition" (OPEN) Study designed to assess dietary measurement error by comparing results from self-reported dietary intake data with four dietary biomarkers: doubly labeled water and urinary nitrogen, sodium, and potassium. The study was conducted from July 1999 to March 2000 among 484 men and women, aged 40–69 years old. A key finding was the significant underreporting of energy and protein intakes by both men and women. While the study by Schatzkin et al. (2003) did not assess fatty acid intake, this study nevertheless would raise questions about the strength of the proposed relationship between CHD risk and *trans* fatty acid intake because most of the studies reporting such a relationship used a food frequency questionnaire to assess *trans* fatty acid intake. (Further information about the OPEN study may be found at the NCI website: http://riskfactor.cancer.gov/studies/open/status.html)

E. Other Studies

Muller et al. (2001) developed regression equations to predict effects on serum total and LDL-cholesterol levels of dietary *trans* fatty acids as well as individual saturated fatty acids. The regression equations were based on consideration of four controlled studies. The food sources of *trans* fatty acids were partially hydrogenated soybean oil and partially hydrogenated fish oil. *Trans* fatty acids from these two sources differ with respect to chain length and number of *trans* bonds. The authors concluded that myristic acid (C14:0) is the most hypercholesterolemic fatty acid and that *trans* fatty acids are less hypercholesterolemic than the saturated fatty acids, myristic and palmitic (C16:0) acids. Hydrogenated fish oil was felt to be slightly more hypercholesterolemic than hydrogenated soybean oil.

Kritchevsky (1999) cited human data indicating that if the diet contains a high level of linoleic acid (e.g., 22%–37% of total dietary fatty acids) and a relatively low level of *trans* fatty acids (e.g., 8%–18% of total dietary fatty acids), the change in total serum cholesterol is much smaller than if the diet contains a low level of linoleic acid and a high level of *trans* fatty acids. Such a relationship would be predicted according to equations presented by Muller et al. (2001). The relationship is supported by data cited in Figures 31.5 and 31.6 (see also Hunter, 2005).

The *trans* fats used in studies considered thus far have contained principally *trans* 18:1 isomers as well as low levels of *trans* 18:2 isomers. These fats have been essentially free of *trans* 18:3 isomers. Vermunt et al. (2001) recently investigated the effect of *trans* α-linolenic acid formed by deodorization

of canola oil (Sebedio et al., 2000) on plasma lipids and lipoproteins. Eighty-five healthy men from three European countries consumed a *trans* fatty acid-free diet for 6 weeks followed by either a diet "high" or "low" in *trans* α-linolenic acid for 6 weeks. Daily mean total *trans* α-linolenic acid intake was 1410 mg in the high *trans* group and 60 mg in the low *trans* group. The high *trans* α-linolenic acid diet did not change total, LDL-, or HDL-cholesterol, but it significantly increased the plasma LDL/HDL ratio by 8.1% and the total/HDL ratio by 5.1% compared to the low-*trans* diet. The authors commented that whether the diet-induced changes in these ratios truly affects CHD risk remains to be established. These results, however, suggest the importance of selecting conditions for deodorization that minimize formation of *trans* α-linolenic acid isomers. A parallel study by Armstrong et al. (2000) using the same high dietary level of *trans* α-linolenic acid found no effect on platelet aggregation and blood coagulation factors, including platelet thromboxane production, fibrinogen levels, factor VII, activated factor VIIa, or plasminogen activator inhibitor activity.

The level of *trans* α-linolenic acid produced by deodorization of canola or soybean oil depends on the time and temperature of the deodorization process (Kellens and De Greyt, 2000). The canola oil used in the studies by Vermunt et al. (2001) and Armstrong et al. (2000) contained 4.5% *trans* α-linolenic acid and was produced by batch deodorization for 52.5 h at 205°C (Sebedio et al., 2000). In contrast, most canola oil produced in the United States has a *trans* α-linolenic acid level ranging from about 1.0% to 2.5% (personal communication, David Volker, J.M. Smucker Co., Cincinnati, OH). The lower (1.0%) level of *trans* α-linolenic acid is typically produced by a continuous deodorization process lasting up to about 5 min at about 240°C–270°C. The higher (2.5%) level is typically produced by batch deodorization for up to 2 h also at 240°C–270°C. Canola oil containing 2.5% *trans* α-linolenic acid would qualify for a label claim of zero *trans* fatty acids in the United States, because a 14 g (1 tablespoon) serving of the oil would provide 0.35 g of *trans* fatty acids. The U.S. FDA permits a label claim of zero *trans* fatty acids if the food product contains less than 0.5 g of *trans* fatty acids per serving.

In summary, since 1990, controlled human studies have found that sufficiently high levels of dietary *trans* fatty acids increase LDL-cholesterol and decrease HDL-cholesterol compared with diets high in *cis* monounsaturated or polyunsaturated fatty acids. The dietary levels of *trans* fatty acids necessary to do this appear to be approximately 4% of energy or higher to increase LDL-cholesterol and approximately 5%–6% of energy or higher to decrease HDL-cholesterol, compared with control diets essentially *trans*-free. There are very limited data at lower levels of intake (less than 4% of energy). In the United States, the intake of saturated fatty acids is reported to be substantially higher (about 10%–12% of energy) than the intake of *trans* fatty acids (about 2%–3% of energy). In addition, since 1992, several epidemiologic and case–control studies have reported a positive association between dietary *trans* fatty acids and the risk of CHD. A major limitation of the epidemiologic studies may be inaccurate *trans* fatty acid intake data. To date, no consistent effects of dietary *trans* fatty acids compared to *cis* fatty acids have been reported on blood clotting tendency, blood pressure, or LDL oxidation.

IV. STUDIES RELATING DIETARY *TRANS* FATTY ACIDS TO CANCER, MATERNAL AND CHILD HEALTH, TYPE 2 DIABETES, AND MACULAR DEGENERATION

A. *Trans* Fatty Acids in Relation to Cancer

In contrast to the extensive literature on *trans* fatty acids in relation to CHD, relatively few investigators have studied *trans* fatty acids with respect to cancer. Ip and Marshall (1996) published a comprehensive review of more than 30 reports addressing the strength and consistency of available scientific data on *trans* fatty acids and cancer. One reviewed study (Sugano et al., 1989) involved the treatment of a colon cancer-sensitive strain of rats with the colon carcinogen dimethylhydrazine and then compared tumor development after feeding diets containing either partially hydrogenated corn

oil or high-oleic safflower oil. Overall, there was no evidence that cancer of the intestinal tract was differentially affected by fat type given to animals of either sex.

Ip and Marshall (1996) also evaluated epidemiologic studies since around 1990 that have considered the intake of various types of fatty acids on the development of cancer at different sites. For breast cancer, Ip and Marshall noted that the epidemiologic evidence shows only a slight to negligible effect of fat intake in general on breast cancer risk and no strong evidence that the intake of *trans* fatty acids is related to increased risk. For colon cancer, evidence exists of increasing risk with the intake of saturated fat or animal fat and decreasing risk with increased intake of fiber and vegetable products. However, no evidence indicated that the intake of *trans* fatty acids is related to an increased risk of colon cancer or rectal cancer. For prostate cancer, Ip and Marshall noted evidence that fat intake might be related to risk. Most of this evidence has focused on the consumption of saturated and animal fats. The intake of *trans* fatty acids has not been correlated with prostate cancer risk.

Since the review by Ip and Marshall (1996), two case–control studies have investigated an association between dietary *trans* fatty acids and the development of cancer. Kohlmeier et al. (1997) investigated the relationship between adipose tissue levels of *trans* fatty acids and postmenopausal breast cancer in European populations in 209 cases and 407 controls. Adipose tissue levels of *trans* fatty acids were used as a biomarker of dietary exposure. The adipose concentration of *trans* fatty acids showed a positive association with breast cancer, suggesting a relationship between adipose stores of *trans* fatty acids and postmenopausal breast cancer risk in European women. Dietary fatty acid data, however, were not provided to relate to adipose tissue stores.

The other case–control study was conducted by Slattery et al. (2001) to examine a possible association between dietary *trans* fatty acids and colon cancer incidence in 1993 cases and 2410 control subjects. Dietary data were collected using a diet history questionnaire. The authors found a weak association between dietary *trans* fatty acids and colon cancer risk in women (odds ratio = 1.5) but not in men (odds ratio = 1.2). Slightly stronger associations were observed in subjects aged 67 years or older (odds ratios = 1.4 for men, 1.6 for women). The authors concluded that it seems prudent to avoid consuming partially hydrogenated fats, because no increased risk was observed for *cis* fatty acids.

In recent years, a specific category of *trans* fatty acids, conjugated linoleic acid (CLA), has been reported to have human health benefits, including controlling body fat gain, enhancing immunity, and reducing inflammation (reviewed by Pariza, 2004). These and other health effects associated with CLA are covered by a separate chapter in this book.

In summary, current scientific evidence does not support a convincing relationship between *trans* fatty acid intake and the risk of cancer at specific sites. Two recent case–control studies have reported an association between the intake of *trans* fatty acids and breast cancer or colon cancer risk. However, the accuracy of the intake values is subject to question.

B. *Trans* Fatty Acids in Relation to Maternal and Child Health

Concerns about a possible relationship between dietary *trans* fatty acids and maternal and child health have focused on whether or not *trans* fatty acids may affect human fetal growth and infant development. It is well established that n-6 and n-3 long-chain polyunsaturated fatty acids play significant roles in growth and development. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are essential fatty acids (i.e., they cannot be synthesized by humans) that are precursors of other important long-chain polyunsaturated fatty acids. Specifically, arachidonic acid (20:4n-6) is derived from linoleic acid by desaturation and chain elongation reactions and is a component of membrane phospholipids, a precursor for eicosanoids, and a stimulant of growth and cell division. Docosahexaenoic acid (22:6n-3) is obtained in the diet (e.g., from fish oils) or synthesized from α-linolenic acid (also by desaturation and chain elongation) and is a component of retinal and neural membrane phospholipids. As such, it is involved in visual and central nervous system functions.

According to Carlson et al. (1997), concerns have been expressed that *trans* fatty acids could decrease fetal and neonatal tissue concentrations of n-6 and n-3 long-chain polyunsaturated fatty acids, thereby interfering with normal growth and development. Carlson et al. (1997) concluded that a definitive answer to the question of whether *trans* fatty acids affect fetal development is not possible because there are few studies in this area, observed effects have been small, results have been inconsistent, and confounding factors have not been excluded.

Noting that *trans* fatty acids from the maternal diet can cross the placenta or be secreted into human milk, Craig-Schmidt (2001) indicated that developing fetuses and nursing infants in the United States and Canada generally would be exposed to larger amounts of *trans* fatty acids than would infants in other parts of the world. This is supported by larger amounts of *trans* isomers in human milk samples from the United States and Canada compared to human milk samples from various countries of Europe, Asia, or Africa.

Craig-Schmidt (2001) also cited studies in which plasma *trans* fatty acid levels were inversely related to birth weight and head circumference. She added that the hypothesis that dietary *trans* fatty acids could inhibit the biosynthesis of long-chain polyunsaturated fatty acids with 20- or 22-carbon atoms and thus affect infant development is supported by studies demonstrating an inverse correlation between plasma *trans* fatty acids and n-3 and n-6 long-chain polyunsaturated fatty acids in infants. However, no such relationship has been observed in human milk. Craig-Schmidt (2001) concluded that the evidence supporting an unfavorable effect of *trans* fatty acids on infant development is correlational and that the physiological implications of the reported relationships are largely unknown.

Larque et al. (2001) also reviewed studies on the effects of dietary *trans* fatty acids in early life. These authors added that multigenerational studies using animals have shown no correlation between birth weight or growth and dietary *trans* fatty acids. Citing reports of inverse relationships between plasma *trans* fatty acids and arachidonic and docosahexaenoic acids, Larque et al. (2001) suggested that such results may indicate an inhibitory effect of *trans* fatty acids on liver Δ-6 fatty acid desaturase activity. In contrast to blood and liver, the brain appears to be protected from *trans* fatty acid accumulation in experimental animals, but no data have been reported for human infants.

Decsi et al. (2001) evaluated previously reported inverse correlations between values of *trans* fatty acids and long-chain polyunsaturated fatty acids in plasma lipids of full-term infants. In this study, the fatty acid composition of venous cord blood lipids was determined in 42 healthy full-term infants. The investigators found significant inverse correlations between total *trans* fatty acids and both arachidonic and docosahexaenoic acids in phospholipids, cholesterol esters, and nonesterified fatty acids, but not in triglycerides. Because *trans* fatty acids in the fetal circulation originate from the maternal diet, the authors suggested that maternal exposure to *trans* fatty acids may inversely influence long-chain polyunsaturated fatty acid status in full-term infants at birth.

The IOM report (2002) recognized concerns that *trans* fatty acids have been suggested to have adverse effects on growth and development through inhibition of the desaturation of linoleic acid and α-linolenic acid to arachidonic acid and docosahexaenoic acid, respectively. However, considering available evidence, including experimental work showing that inhibition of the Δ6 desaturation of linoleic acid is not of concern with linoleic acid intakes above about 2% of energy, the IOM concluded that inhibition of essential fatty acid metabolism by *trans* fatty acids is unlikely to be of concern for practical human diets.

In summary, it appears unlikely that current average dietary levels of *trans* fatty acids in the United States and Europe will have unfavorable effects on growth and development, reproduction, or gross aspects of fetal development.

C. *Trans* Fatty Acids in Relation to Type 2 Diabetes

Salmeron et al. (2001) suggested that an increase in polyunsaturated fatty acid intake and a decrease in *trans* fatty acid intake would substantially reduce the risk of women developing type 2 diabetes. During a 14-year follow-up of 84,204 women with no diabetes, cardiovascular disease, or

cancer, the investigators documented 2507 new cases of type 2 diabetes. Dietary information was assessed by food frequency questionnaires. Total fat intake, compared to equivalent energy intake from carbohydrates, was not associated with risk of type 2 diabetes. In contrast, polyunsaturated fatty acid intake was inversely associated and *trans* fatty acid intake positively associated with risk. The authors estimated that replacing 2% of the energy from *trans* fatty acids with polyunsaturated fatty acids would reduce the risk of type 2 diabetes by 40%.

Commenting on this study, Clandinin and Wilke (2001) noted that the influence of dietary *trans* fatty acids on insulin resistance has not been studied, and epidemiologic evidence relating *trans* fatty acid intake to diabetes is lacking. There is no known functional or physiological relation to connect *trans* fatty acids to disease mechanisms involved with type 2 diabetes. Clandinin and Wilke also questioned the reliability of estimates of intake of *trans* fatty acids from food frequency questionnaires.

An additional complication is that some processed foods containing *trans* fatty acids also contain large amounts of refined carbohydrates (e.g., certain baked goods), which could exacerbate the insulin-resistant state in diabetes and/or contribute to increases in serum triglyceride levels. This association of *trans* fatty acids with simple and complex carbohydrates does not permit distinction about whether *trans* fatty acids alone, *trans* fatty acids plus carbohydrates, or carbohydrates alone in certain products would contribute to the risk of type 2 diabetes. Thus, the conclusions of Salmeron et al. (2001) still represent a hypothesis to be tested.

A follow-up study by Hu et al. (2001) looked at combined effects of dietary and lifestyle factors in relation to type 2 diabetes. Considering essentially the same subject population studied by Salmeron et al. (2001), Hu et al. (2001) documented 3300 new cases of type 2 diabetes during 16 years of follow-up. Overweight or obesity was the single most important predictor of diabetes. Lack of exercise, a poor diet, current smoking, and abstinence from alcohol use were all associated with increased risk of diabetes, even after adjusting for body mass index. In addition, the combination of an appropriate diet, moderate amount of exercise, and abstinence from smoking, even in overweight and obese subjects, resulted in reduced risk of type 2 diabetes. A "poor diet" was described as being high in *trans* fat and glycemic load, low in cereal fiber, and having a low ratio of polyunsaturated to saturated fat. Quantitative levels of *trans* fatty acids, sugars, cereal fiber, and polyunsaturated and saturated fatty acids associated with the "poor diet" were not specified, and *trans* fatty acids were not singled out as an individual risk factor.

A similar study by van Dam et al. (2002) examined dietary fat and meat intake in relation to risk of type 2 diabetes in 42,504 male subjects initially free of diabetes, cardiovascular disease, and cancer. Diet was assessed by food frequency questionnaire. During 12 years of follow-up, 1321 new cases of type 2 diabetes were observed. Intakes of total fat and saturated fat were associated with a higher risk of type 2 diabetes. However, these associations disappeared after adjustment for body mass index. In addition, intakes of oleic acid, *trans* fatty acids, long-chain n-3 fatty acids, and α-linolenic acid were not associated with diabetes risk after multivariate adjustment. Results of this study with men were not consistent with the Salmeron et al. (2001) study reporting that *trans* fatty acids were positively associated and polyunsaturated fatty acids negatively associated with risk of type 2 diabetes in women.

D. *Trans* Fatty Acids in Relation to Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness in the United States and other developed countries. Two recent studies from the same institution that evaluated a possible connection between dietary *trans* fatty acids and type 2 diabetes also addressed whether dietary fat, including *trans* fatty acids, is related to risk of AMD.

One of these studies, by Seddon et al. (2001), evaluated the relationship between intake of total fat and of specific fatty acids, including *trans* fatty acids, and risk of AMD in a case–control study at five U.S. clinical ophthalmology centers. Case subjects included 349 individuals (aged 55–80 years) with the advanced neovascular stage of AMD diagnosed within 1 year of their enrollment into the study. Control subjects included 504 individuals without AMD but with other

ocular diseases. Higher consumption of vegetable fat, monounsaturated fatty acids, polyunsaturated fatty acids, or linoleic acid was associated with an elevated risk of AMD. In contrast, higher consumption of saturated or *trans* fatty acids was not associated with higher risk of AMD.

The association between AMD and higher intake of vegetable, monounsaturated, and polyunsaturated fats and linoleic acid was suggested to be related to snack foods containing products that adversely affect the blood vessels of the choroids or retina. The authors felt that such foods might also increase oxidative damage in the macula, which is susceptible to oxidation because of high oxygen tension in the presence of light exposure. Thus, some individual dietary fats, but not necessarily total fat consumption, might increase or decrease the risk for AMD.

Cho et al. (2001) assessed a possible association between total fat and specific types of fat and AMD. This was a prospective study that at baseline included 42,743 women and 29,746 men, aged 50 years or older with no diagnosis of AMD. During the 12-year follow-up period, 567 subjects were diagnosed with AMD. The pooled (women and men) multivariate relative risk for the highest compared to the lowest quintile of total fat intake was 1.54, a value considered statistically significant. Unexplained was why the fourth quintile for fat intake had a relative risk equal to that of the first quintile and lower than that of the second and third quintiles. *Trans* fat intake was not related to risk of AMD, whereas α-linolenic acid intake was positively associated with risk of AMD. Docosahexaenoic acid, on the other hand, showed a slight inverse relationship with AMD, and more than four servings of fish per week was associated with a 35% lower risk of AMD compared with three or fewer servings per month.

In summary, one study has reported an association between *trans* fatty acid intake and type 2 diabetes, however, a follow-up study found no such association. In view of no known functional or physiological connection between *trans* fatty acids and type 2 diabetes, the reported association requires further testing. In the case of AMD, neither of the two studies reported an association between dietary *trans* fatty acids and risk of developing the condition. Considering the difficulty of conducting meaningful studies on effects of dietary fat on specific human disorders, such as type 2 diabetes and AMD, it is not surprising that no adverse effects were found in the few studies reported in these areas.

V. DIETARY RECOMMENDATIONS REGARDING SATURATED AND *TRANS* FATTY ACIDS

Current dietary recommendations by health professional organizations within and outside the United States (Anon, 2001a,b, 2003, 2005; Krawczyk, 2001; Institute of Medicine, 2002; Lichtenstein et al., 2006) address intake of cholesterol-raising saturated and *trans* fatty acids. These organizations are consistent in their recommendations of an upper limit of intake of saturated fatty acids of 10% of

TABLE 31.4
Dietary Recommendations for Saturated and *Trans* Fatty Acids: U.S. Organizations

Organization	Saturated Fatty Acids	*Trans* Fatty Acids
American Heart Association	<7% Energy (population) <7% Energy (those at risk)	<1% Energy (population)
Adult Treatment Panel III of the National Cholesterol Education Program	<7% Energy (those at risk)	Keep intake low
Health and Human Services/U.S. Department of Agriculture	<10% Energy (population)	Low as possible
Institute of Medicine of the National Academy of Sciences	Low as possible	Low as possible

Current dietary recommendations of U.S. health professional organizations for saturated and *trans* fatty acids.

TABLE 31.5
Dietary Recommendations for Saturated and *Trans* Fatty Acids: Non-U.S. Organizations

Organization	Saturated Fatty Acids	*Trans* Fatty Acids
Health Council of The Netherlands	Low as possible UL 10% energy	Low as possible UL 1% energy
Health Canada	<10% Energy	
Ministry of Agriculture, U.K.	<10% Energy	<2% Energy
Austria, Germany, Switzerland	<10% Energy	
Japan	6%–8% Energy	
World Health Organization/Food and Agricultural Organization of the United Nations	<10% Energy; <7% energy for high-risk groups	<1% Energy

Current dietary recommendations of non-U.S. health professional organizations for saturated and *trans* fatty acids.

energy and reduced intake of *trans* fatty acids. Recommendations from U.S. organizations are summarized in Table 31.4, and those from organizations in Europe and Japan, in Table 31.5. The consistency of these recommendations provides the basis for food manufacturers in the United States and globally to consider ways to decrease or eliminate *trans* fatty acids from their food products.

VI. ALTERNATIVES FOR REPLACING OR REDUCING *TRANS* FATTY ACIDS IN FOODS

Food manufacturers are using or developing basically four technological options to reduce or eliminate *trans* fatty acids in their products. These options include (1) modification of the hydrogenation process; (2) use of interesterification; (3) use of fractions high in solids from natural oils; and (4) use of trait-enhanced oils. Information on each of these topics was presented at a recent *AOCS* symposium on *trans* fat (Watkins, 2004). Frequently, some approaches are used in combination with others. Discussion of each option follows.

A. Modification of the Hydrogenation Process

Certain food products, such as spreads, margarines, and shortenings, require oils with specific levels of solids at different temperatures to achieve desired functionality. Solids normally come from either *trans* fatty acids (resulting from hydrogenation) or saturated fatty acids. Modifying the conditions of hydrogenation (e.g., pressure, temperature, and catalyst) affects the fatty acid composition of the resulting oil, including the amount of *trans* fatty acids formed, and properties such as melting point and solid fat content of the oil. It is possible to make equivalently performing low-*trans* fats by increasing the degree of hydrogenation, which reduces the level of *trans* fatty acids but increases the level of saturated fatty acids. Such fats will differ analytically from fats hydrogenated to a lesser degree because saturates now contribute most of the solids. This is illustrated in Figure 31.7 indicating that with progressive hydrogenation (decreasing iodine value) of soybean oil, the content of *trans* fatty acids increases to a maximum and then decreases essentially to zero. The corresponding level of saturated fatty acids increases gradually until the content of *trans* fatty acids is maximized and then increases rapidly.

Modification of the hydrogenation process can be used to prepare low-*trans* baking shortenings. The simple substitution of unhydrogenated (*trans*-free) fats for hydrogenated fats does not work well in baking applications. Some recipes for baked foods require a greater amount of saturated fat to replace a given amount of *trans* fat for the recipe to work (i.e., one-to-one substitution of saturated fat for *trans* fat often does not produce foods of equivalent consumer acceptability).

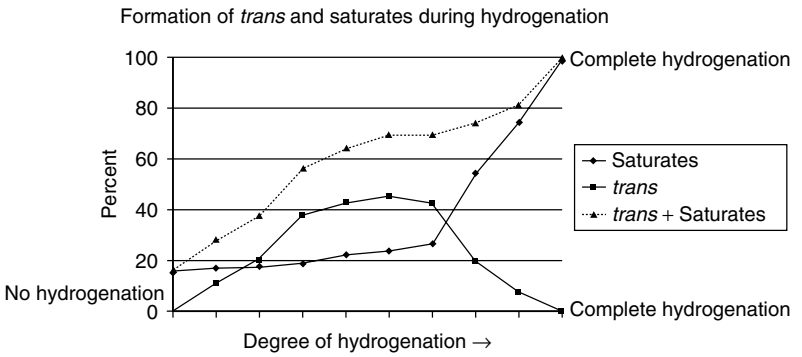

FIGURE 31.7 Changes in percentages of *trans* and saturated fatty acids with progressive hydrogenation of soybean oil. Progressive hydrogenation is indicated by decreasing iodine value. Corresponding levels of *trans* and saturated fatty acids, expressed as percent of total fatty acids, are shown. The graph was provided by Bob Wainwright, Cargill, Inc.

Low- or zero-*trans* baking fats may have increased levels of stearic acid from the hydrogenation of α-linolenic, linoleic, and oleic acids, and also significant levels of palmitic acid for functionality. Palmitic acid may be provided by incorporating a high palmitic acid fat, such as cottonseed oil, into the shortening.

B. Use of Interesterification

The interesterification process involves the rearrangement (randomization) of the fatty acids on the glycerol backbone of the fat (i.e., triglyceride) in the presence of a chemical catalyst or an enzyme. Interesterification modifies the melting and crystallization behavior of the fat, and the resulting fat is very low in *trans* fatty acids. One current application of this process is in the production of *trans*-free or low-*trans* fats for margarine, spread, and shortening applications. Several human studies have shown no significant effects of interesterified fats on blood lipid parameters (Nestel et al., 1995, 1998; Zock et al., 1995; Meijer and Weststrate, 1997; summarized in Hunter, 2001).

In chemical interesterification, an unhydrogenated liquid vegetable oil (such as a salad oil) and a fully hydrogenated vegetable oil (called a "hardstock," which has a low iodine value and a low amount of *trans* fatty acids) are mixed and heated. A typical blend may contain about 85% unhydrogenated oil and about 15% hardstock. Both components of the blend are essentially *trans*-free. A catalyst, commonly sodium methoxide, is added, and the interesterification process causes a random rearrangement of the fatty acids on the glycerol backbone. It is important to note that in such blended fat products, the term "hydrogenated fat" or "partially hydrogenated oil" would appear on the ingredient statement, but the product would be very low in *trans* fatty acids. Therefore, the listing of "hydrogenated fat" or "partially hydrogenated oil" on an ingredient statement does not always mean that the product contains a significant level of *trans* fat, as has been erroneously indicated by the popular press.

A recently modified interesterification process uses an enzyme instead of sodium methoxide to catalyze the reaction. The enzyme is a heat-stable, 1,3-specific lipase derived from a microorganism and immobilized on a granulated silica matrix. Because the lipase is specific for the 1- and 3-positions of the triglycerides, its use results in a fat that is partially randomized rather than being completely randomized, as occurs with use of sodium methoxide. One advantage of enzymatic interesterification is that there are no waste or by-product issues, for example, no catalyst residues that could be released into the environment.

Table 31.6 compares the fatty acid composition of a typical shortening fat prepared by partial hydrogenation with a newly developed product made by enzymatic interesterification. The interesterified fat is low in *trans* fatty acids and has a lower oleic acid content and higher levels of stearic,

TABLE 31.6
Fatty Acid Composition (Percentages) and Melting Points of Shortenings Made by Partial Hydrogenation and by Enzymatic Interesterification

Fatty Acid or Melting Point	Partial Hydrogenation (PHSBO, PHCSO)	Enzymatic Interesterification (SBO, FHSBO)
% C16:0	10.4	10.8
% C18:0	15.5	21.9
% C18:1 *cis*9	37.5	21.9
% C18:2 *cis*9,12	4.80	39.8
% C18:3 *cis*9,12,15	0.30	5.6
% Total *trans*	30.0	2.5–3.5 typical 5.0 max
% Total saturates	25.9	32.7
Melting point (°F)	117.5	110.0

Fatty acid compositions and melting points are shown for two shortening products, one made by conventional partial hydrogenation (using a blend of partially hydrogenated soybean oil [PHSBO] and partially hydrogenated cottonseed oil [PHCSO]) and one made by enzymatic interesterification (using a blend of unhydrogenated soybean oil [SBO] and fully hydrogenated soybean oil [FHSBO]).

Source: Data were provided by Tom Tiffany, Archer Daniels Midland Co.

linoleic, and α-linolenic acids compared to the partially hydrogenated fat. Although the fatty acid compositions of the two products differ from each other, the functional characteristics for shortening applications (e.g., melting points) of the two products are very similar. The low level of *trans* fatty acids (typically 2.5%–3.5%, with a maximum of 5.0%) in this interesterified fat may have resulted in part from deodorization and/or incomplete hydrogenation of the component hydrogenated oil. Nevertheless, considering the normal 12 g serving size for shortening, the product made by interesterification would qualify for a declaration of 0.5 g of *trans* fat per serving according to the FDA's final labeling rule. If this shortening were incorporated into a baked product, depending on the amount of fat per serving, the product may be able to declare 0 g of *trans* fat per serving.

C. Use of Fractions High in Solids

Fractions high in solids derived from natural oils, namely coconut, palm, and palm kernel oils, are not new to the food industry and have been components of functional ingredients for years. Many commercially available fractions come from palm and palm kernel oils. They can be used successfully either as single fractions or in combination with other fractions to meet specific needs. Examples of fractions from palm oil that can be used in preparing *trans*-free or low-*trans* shortenings and margarines include palm olein, palm mid-fractions, and palm kernel stearin. Such fractions can be blended with liquid oils, such as soybean and canola oils, to create either no-*trans* shortenings and margarines or low-*trans*, low-saturate shortenings and margarines.

Fractions high in solids usually are prepared by reducing the temperature of an oil sample so that a more saturated fraction solidifies and a more unsaturated fraction remains liquid. The solid fraction is then physically separated from the liquid fraction by filtration or centrifugation. Liquid fractions, sometimes referred to as "olein fractions," are high in oleic acid and also are good sources of antioxidants, such as tocopherols and tocotrienols. Olein fractions frequently are used in Pacific-rim countries as salad oils and frying oils. Fractions with higher solids content may be incorporated into margarine or shortening fats.

TABLE 31.7
Fatty Acid Composition (Percentages) and Melting Points of No-*Trans* Shortening and Margarine Oils Made with Fractions from Palm and/or Palm Kernel Oils

	No-*Trans* Shortening	No-*Trans* Shortening	No-*Trans* Margarine Oil
Label declaration	Safflower, palm, and/or palm kernel oils	Canola, palm, and/or palm kernel oils	Canola, palm, and/or palm kernel oils
% TFA	0	0	0
% SFA	35	23	26
% MUFA	55	53	35
% PUFA	10	24	39
Melting point (°F)	109	101	97

All products were made with either safflower or canola oil plus fractions of palm and/or palm kernel oils. Partial hydrogenation was not used in making these products. One shortening was made with safflower oil, and the other, with canola oil.

Abbreviations: TFA, *trans* fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Source: Data were provided by Carl Heckel, Aarhus United, USA, Inc.

Table 31.7 compares fatty acid compositions of two no-*trans* shortenings and a no-*trans* margarine oil made with specific fractions from naturally solid vegetable oils such as palm or palm kernel oil. All the products are *trans*-free, although there are differences in the levels of saturated, monounsaturated, and polyunsaturated fatty acids.

D. Use of Trait-Enhanced Oils

Trait-enhanced oils generally fall into three categories: high-oleic acid oils, such as high-oleic sunflower and canola oils; mid-range oleic acid oils, such as mid-oleic sunflower and soybean oils; and low-linolenic acid oils, such as low-linolenic canola and soybean oils. (The term "low linolenic" commonly refers to an oil containing about 1%–3% α-linolenic acid. Soybean oil typically contains about 7%, and canola oil, about 10% α-linolenic acid.) These types of oils are derived through traditional plant breeding or biotechnological methods. The high- and mid-range oleic acid oils are more stable oxidatively than traditional oils high in linoleic acid, such as soybean, corn, and sunflower oils. Low-linolenic acid oils have significantly reduced levels of oxidatively unstable α-linolenic acid. All these trait-enhanced oils have good oxidative stability (i.e., a relatively high content of oleic acid and comparatively low levels of linoleic and α-linolenic acids) making them suitable for frying, spraying, beverages, and some bakery applications.

At present, the plant varieties that produce high-oleic and low-linolenic as well as some mid-oleic oils are not widely cultivated, and these oils are more expensive to produce than the corresponding polyunsaturated oils. In addition, the high-oleic oils are liquid at room temperature, and thus would not be particularly useful in spread and baking applications that require a relatively high solids content.

Table 31.8 shows typical fatty acid compositions of several trait-enhanced oils. All have significant levels of oleic acid and comparatively lower levels of linoleic and α-linolenic acids. For salad oil and frying applications, these oils would not require hydrogenation and therefore would be *trans*-free.

The United Soybean Board (USB) has projected the future availability of low-linolenic soybean oil (having less than 3% α-linolenic acid) (Reeves, 2006). Seed for low-linolenic soybeans has been developed by duPont, Monsanto, and Iowa State University. For 2006, the USB expected 800,000 acres to be planted, which likely would have yielded about 32 million bushels of soybeans and 320 million

TABLE 31.8
Typical Fatty Acid Composition (Percentages) of Trait-Enhanced Oils

Fatty Acid	Canola	High-Oleic Canola	Sunflower	Mid-Oleic Sunflower	High-Oleic Sunflower	Soybean	Low-Linolenic Soybean
% C16:0	5	4	7	5	4	11	10
% C18:0	2	2	4	4	4	4.5	5
% C18:1 *cis*9	61	74	14	58	79	25	28
% C18:2 *cis*9,12	21	12	74	32	11	51	56
% C18:3 *cis*9,12,15	10	4	<0.5	<0.5	<0.5	8	1

Typical fatty acid compositions of high-oleic canola oil, mid-oleic and high-oleic sunflower oils, and low-linolenic soybean oil (low in α-linolenic acid) are compared to their respective conventional oils.

Source: Data were provided by Bob Wainwright, Cargill, Inc.

TABLE 31.9
Projected Availability of Low-Linolenic Soybean Oil

Year	Acres to be Planted	Production (Bushels)	Oil Production (Pounds)
2006	800,000	32 M	320 M
2008	4 M	160 M	1.6 B

Abbreviations: M, million; B, billion.

Source: Data were provided by the United Soybean Board.

pounds of oil (Table 31.9). By 2008, these totals are projected to be 4 million acres, 160 million bushels of soybeans, and 1.6 billion pounds of oil.

VII. CURRENT STATUS OF USAGE OF ZERO- AND LOW-*TRANS* FATS

There is considerable interest in zero- and low-*trans* fats among food manufacturers, and current usage of such products is increasing. For example, in frying applications, some restaurants and food service operations currently use unhydrogenated, *trans*-free oils. The use of such oils for frying is likely to increase as greater quantities of trait-enhanced oils, such as high-oleic and mid-range-oleic oils, become available in the future. With regard to spreads and margarines, low-*trans* and *trans*-free products are currently marketed. Considering shortenings, in 2005 at least one *trans*-free product was being marketed. Key challenges to manufacturers of zero- or low-*trans* fat products are to provide equivalent functionality at reasonable cost but without drastic increases in saturated fatty acid content.

VIII. SUMMARY AND CONCLUSIONS

In summary, dietary *trans* fatty acids at sufficiently high levels have been found to increase LDL- and decrease HDL-cholesterol levels compared to diets high in *cis* monounsaturated or polyunsaturated fatty acids. On the other hand, dietary *trans* fatty acids have not been strongly related to cancer risk at specific sites or to other health conditions, including fetal growth and development,

type 2 diabetes, and AMD. As a result of the effects of dietary *trans* fatty acids on LDL- and HDL-cholesterol, most health professional groups have recommended reduced consumption of *trans* as well as saturated fatty acids. Current efforts to replace or reduce *trans* fatty acids in food products focus on (1) modification of the hydrogenation process; (2) use of interesterification; (3) use of fractions high in solids from natural oils; and (4) use of trait-enhanced oils.

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32 Significance of Dietary γ-Linolenate in Biological Systems: Attenuation of Inflammatory and Proliferative Processes

Vincent A. Ziboh

CONTENTS

I. INTRODUCTION AND HISTORICAL PERSPECTIVES

A. Essential Fatty Acids

The first indication that dietary fat may be essential for healthy growing animals was presented in 1918 by Aron (1918) who proposed that butter has a nutrient value that cannot be provided by other dietary components. This report suggested that there was a special nutritive value inherent in fat apart from its caloric contribution and that this possibly was related to the presence of certain lipids. In 1929, Burr and Burr (1929) presented the first series of articles outlining a "new deficiency disease produced by the rigid exclusion of fat from the diet." In the series of conclusions put forth, they developed the hypothesis that warm-blooded animals, in general, cannot synthesize appreciable quantities of certain fatty acids. In 1930, both investigators significantly added to their earlier work by presenting evidence that the dietary inclusion of linoleic acid alone could reverse all deficiency symptoms resulting from a fat-free diet and thus linoleic acid (LA or 18:2n-6)[1] was heralded as an essential fatty acid (EFA) (Burr and Burr, 1930; Burr et al., 1940). The recognition that some unsaturated fatty acids could not be synthesized from endogenous precursors by mammals and were essential dietary elements led to the designation of essential and nonessential fatty acids. It was originally thought that there are only two EFAs, linoleic acid (9,12-octadecadienoic acid, LA, 18:2n-6) and γ-linolenic acid (9,12,15-octadecatrienoic acid, GLA, 18:3n-6), but continued nutritional studies revealed positive essential growth responses not only for linoleic acid and GLA, but also for arachidonic acid (AA) as well as the long-chain highly unsaturated fatty acids in fish oil [eicosapentaenoic acid (EPA), 20:5n-3 and docosahexaenoic acid (DHA), 22:6n-3]. More recent reports on the biological significance of the longer-chain n-3 polyunsaturated fatty acids (PUFAs) do qualify these long-chain fatty acids as essential PUFAs.

[1]Fatty acids and acyl groups are denoted 18:2n-6, 18:3n-3, and so on, with the first number representing the number of carbons in a straight chain and the number following the colon indicating the number of methylene interrupted *cis* double bonds. The number after "n" indicates the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

B. Deficiency Symptoms

With the discovery of the EFAs came the recognition of their deficiencies (Burr and Burr, 1929; Burr et al., 1940; Griffith, 1958; Marcel et al., 1968). The various deficiency symptoms apparent in the response to diets low or free from EFAs were first described by Bur and Burr (Burr and Burr, 1929; Burr et al., 1940). A salient feature of this syndrome is decreased growth rate, particularly in the male-fed animals. The most visible and striking feature of EFA-deficiency (EFAD) is scaly skin dermatosis, hair loss, tail necrosis with histological section of the skin revealing epidermal thickness or acanthosis as well as hyperproliferation of the stratum corneum. Other organs also undergo alterations such as fatty liver, kidney damage, impaired reproduction, fetal resorption in females, testicular degeneration in males, and reduced ability to form and maintain cell membrane integrity.

Interest to explore the mechanisms that produced these EFAD symptoms have prompted examination of the roles they play in (1) the homeoviscious control of the membrane bilayers of most cells (Rance et al., 1980; Esfahani and Devlin, 1982); (2) providing a controlling influence in processes by which small molecules are translocated in either direction across cell membranes via protein channel or pore (Read and McElhaney, 1976; Schindler and Nelson, 1982; Solomon et al., 1983); (3) providing lipid–protein interactions that affect membrane enzyme activity; and (4) serving as vital precursors for the biosynthesis of eicosanoids, prostaglandins, leukotrienes, and biologically active hydroxy fatty acids (Dunham et al., 1978; Hansen, 1981).

C. Desaturation/Elongation of EFA

The shorter-chain EFA, linoleic acid (LA, 18:2n-6), serves as the initial unsaturated precursor for the *in vivo* biosynthesis of the longer-chain PUFAs. Metabolism of the LA in most tissues involves an alternating sequence of Δ^6-desaturation, chain elongation, and Δ^5-desaturation in which two hydrogen atoms are removed to create a new double bond followed by the addition of two carbon atoms from glucose metabolism to lengthen the fatty acid chain (Marcel et al., 1968) (Figure 32.1). The desaturations are catalyzed by two separate enzymes: the Δ^6-desaturase catalyzing the transformation of 18:2n-6 (LA) to 18:3n-6 (GLA) and the Δ^5-desaturase catalyzing the transformation of 20:3n-6 dihomo-γ-linolenic acid (DGLA) to 20:4n-6 (AA). The elongase enzyme catalyzes the elongation of GLA to DGLA (Fujiwara et al., 1983). It is believed that the same enzyme catalyzes equivalent steps in the n-3 and n-9 pathways (Brenner, 1974). The PUFA families interact in such a manner that the n-3 PUFAs competitively suppress the bioconversion of the n-6 PUFAs. Both the n-6 and the n-3 PUFAs respectively do suppress the formation of the nonessential long-chain n-9 fatty acids, hence the negligible formation of the long-chain n-9 PUFA (20:3n-9) in the EFA-fed animals.

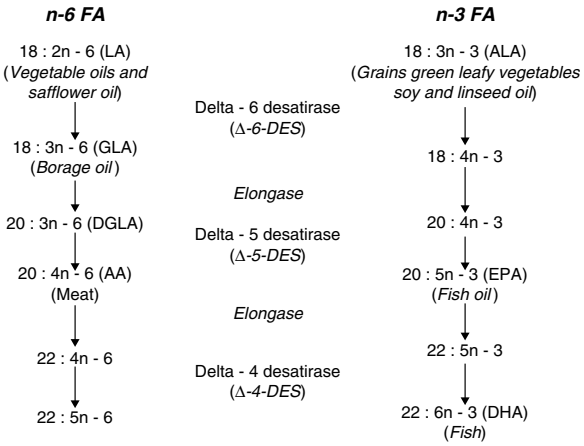

Figure 32.1 Oxidative desaturation/elongation of n-6 and n-3 PUFAs.

II. THE BIOLOGICAL SIGNIFICANCE OF γ-LINOLENIC ACID

A. Sources of γ-Linolenic Acid

γ-Linolenic acid (18:3n-6) is an important n-6 PUFA and a Δ^6-desaturase product of linoleic acid (18:2n-6). Most of the GLA currently being used in a variety of reported studies is extracted from three major sources:

1. *Evening Primrose* (*Oenothera biennis* L.), of the *Onagraceae family*, is a biennial crop with yellow flowers that blooms in the evening. It grows widely in North America. The seed oil contains GLA (7%–14%), LA (65%–80%), and negligible amounts of n-3 fatty acids (Wolf et al., 1983; Hudson, 1984).
2. *Borage* (*Borago Officianalis* L.), of the *Boraginaceae family*, is a herbaceous hardy annual crop with starlike bright blue flowers. It is found commonly in the Mediterranean region in North Africa. The seed oil contains high levels of GLA (20%–27% of total fatty acids), low levels of LA (35%–40%), and no n-3 fatty acids (Kleiman et al., 1964; Miller et al., 1968). Interestingly, the leaf lipids of Borage (Jamieson and Reid, 1969) contain high levels of α-linolenic acid (ALA, 18:3n-3) and stearidonic acid (STA, 18:4n-3).
3. *Blackcurrant* (*Ribes nigrum*), of the *Saxifragaceae family*, is a shrub that grows mainly in Europe. Its seed oil, unlike Evening Primrose and Borage contains high levels of GLA (15%–19%) (Traitler et al., 1984) and n-3 fatty acids ALA (12%–14%) and STA (2%–4%) (Gunstone, 1992).
4. *Others*: Although several *microorganisms* and *fungi* have been reported to contain GLA (Shaw, 1965; Hiruta et al., 1989; Shimitzu et al., 1989; Nakajima and Izu, 1992), these oils have not been used as much as those from plant sources in the conduct of dietary experiments.

B. Metabolism and Generation of Potent Biological Modulators

The metabolism of linoleic acid derived from corn oil and GLA derived from dietary Borage or Evening Primrose is illustrated in Figure 32.2. Specifically, GLA derived from LA (18:2n-6) by Δ^6-desaturase and directly ingested as GLA from the dietary oil is rapidly elongated to DGLA by the DGLA elongase enzyme. DGLA occupies a pivotal position in this cascade. On the one hand, DGLA is modestly metabolized into AA by the rate-limited enzyme Δ^5-desaturase. The Δ^5-desaturase

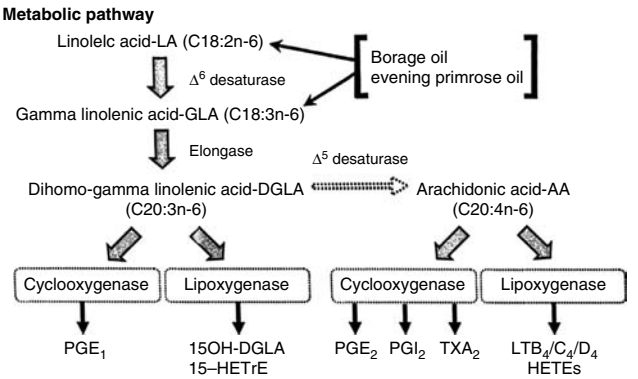

Figure 32.2 Metabolism of linoleic acid (LA)/γ-linolenic acid (GLA) into cyclooxygenase and lipoxygenase metabolites. *Abbreviations:* DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; PGE_1, prostaglandin E_1; 15-OH-DGLA, 15-hydroxyeicosatrienoic acid; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; TXA, thromboxane A; $LTB_4/C_4/D_4$, leukotrienes; HETEs, hydroxyeicosatetraenoic acid.

enzyme (as shown in Figure 32.2) is known to elicit significant species and tissue differences in enzyme activity. For instance, the rodent exhibits a striking active Δ^5-desaturase enzyme system whereas in both guinea pig and liver, the enzyme is less active and lower (Marcel et al., 1968). Thus, in any of the metabolic pathway of unsaturated fatty acids, the rates of desaturation are generally lower than those of chain elongation (Crawford et al., 1977).

In addition, the rate-limiting steps associated with the bioconversion of LA to AA are Δ^6-desaturation and Δ^5-desaturation. The AA (20:4n-6) that is biosynthesized by Δ^5-desaturation catalyzes and then undergoes oxygenation reactions catalyzed by oxygenase 1 and 2 (COX-1/COX-2) to generate (1) prostaglandins of the two series: PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and TXA_2; (2) via the lipoxygenase enzymes to generate leukotrienes (leukotriene B_4 or LTB_4, C_4, D_4); and (3) three major monohydroxy acids [5-, 12-, and 15-hydroxyeicosatetraenoic acids (HETEs)]. The DGLA (20:3n-6), which is derived from the dietary GLA-containing oils, is rapidly bioconverted by COX-1 either via the cyclooxygenase pathway to prostaglandin of the 1-series (PGE_1) as well as via the 15-lipoxygenase (15-LOX) pathway into 15-hydroxy-8,11,13-eicosatrienoic acid (15-HETrE). The latter metabolites derived from DGLA are known to exert anti-inflammatory/anti-proliferative properties.

III. GENERALIZED FUNCTIONAL ROLES OF γ-LINOLENIC ACID

The triacylglycerol stereospecific position of GLA varies with the source of the oils, and this is important in establishing their relative efficacies. For instance, GLA is concentrated in the *sn*-3 position of the glycerol bridge of the triacylglycerol in the Evening Primrose and Blackcurrant seed oil. In contrast, GLA is concentrated in the *sn*-2 position of the glycerol bridge in Borage oil and in the *sn*-2/*sn*-3 positions of fungal oil. Following ingestion of the triacylglycerol structures of Borage and Evening Primrose GLA in both humans and rodents, only a small fraction of the DGLA resulting from the elongation of GLA is converted by the Δ^5-desaturase to AA. This minor conversion (as shown in Figure 32.2) is due to the low activity of Δ^5-desaturase in most tissues. This metabolic consequence therefore argues against the often raised concerns that the ingestion of dietary GLA/DGLA does contribute to the excessive accumulation of AA and thus result in the excessive generation of proinflammatory AA metabolites such as PGE_2, LTB_4, LTC_4, and LTD_4. Indeed, in many tissues and cell types, DGLA, but not AA, is what accumulates in the tissues and cells after GLA supplementation in the diet. The accumulated DGLA is oxygenated via the COX-1 pathway to PGE_1 and via the 15-LOX pathway to 15-hydroxyeicosatrienoic acid (15-HETrE) as shown in Figure 32.2. These two major oxidative metabolites of DGLA (PGE_1 and 15-HETrE) have been reported to exert biological and clinical effects and notably the suppression of acute and chronic inflammation as well as hyperproliferation in a variety of systems and disease conditions. In addition, DGLA can compete with AA for COX-2 enzyme to decrease the production of PGE_2. In addition, DGLA is metabolized by the lipoxygenase enzyme 15-LOX to generate 15-HETrE (a potent inhibitor of the 5-LOX pathway and the suppression of synthesis of the proinflammatory mediators: LTB_4, LTC_4, and LTD_4 from AA) via the inhibition of 5-LOX activity (Vanderhoek et al., 1980; Miller et al., 1988).

IV. NUTRITIONAL/BIOLOGICAL SIGNIFICANCE OF γ-LINOLENIC ACID

The rationale for the dietary ingestion of GLA can be summarized as follows: (1) GLA will bypass the Δ^6 desaturation step in the n-6 EFA metabolism and be rapidly elongated to increase the *in vivo* level of DGLA; (2) the formed DGLA does compete to diminish the amount of AA for incorporation into membrane phospholipids for maintenance of the normal fluidity of the cell membrane; and (3) the DGLA most importantly serves as the precursor and substrate for the biosynthesis of potent oxidative metabolites via the COX-1 pathway to form most importantly, PGE_1 and via the 15-LOX pathway to form 15-HETrE as illustrated in Figure 32.2. These metabolites function *in vivo* to attenuate inflammatory and proliferative processes.

A. Significance of Dose of Dietary Intake of γ-Linolenic Acid

The results from a number of dietary studies of GLA [content of Evening Primrose oil (EPO) and/or Borage oil] have been variable resulting in contentious results and controversies as to whether or not the reported beneficial effects of GLA are valid. One likely reason for these controversies is the use of varying dietary doses presumably inadequate to elicit the biological effects being investigated. To address this concern, it is prudent to establish as a first step whether varying doses of dietary GLA can elicit selected biological effect in healthy normal individuals. Thus, in one study, one group of healthy normal human volunteers ingested Borage oil as capsules containing a total of 480 mg GLA/day as dietary supplement for 12 weeks. The second group in a parallel experiment received approximately threefold (a total of 1300 mg GLA/day) for 12 weeks. Using isolated polymorphonuclear (PMN) cells as the model to evaluate the effect of the dietary GLA, the *ex vivo* generation of proinflammatory LTB_4 by isolated PMNS after challenge with calcium ionophore was evaluated. The findings revealed that ingestion of the lower concentration of 480 mg GLA/day only moderately suppressed the *ex vivo* PMN-biosynthesis of LTB_4 (46%). In contrast, the group that ingested the higher concentration of 1300 mg GLA/day significantly exerted higher suppression of *ex vivo* PMN-induced biosynthesis of LTB_4 (65%) (Ziboh and Fletcher, 1992). These findings clearly demonstrated the relationship of the ingested dose of GLA and its biological efficacy. Although the trial of 1300 mg GLA/day was not maximal, the data support the view that the efficacy of GLA was dose-dependent; hence, it must be ascertained in any given study.

B. *In Vivo* Role of γ-Linolenic Acid in Normal Skin

Two PUFAs, linoleic acid (LA, 8:2n-6) and GLA (18:3n-6), are known to play important roles in the physiology and pathophysiology of the skin. For instance, the 18-carbon linoleic acid is the most abundant PUFA in human skin epidermis (Chapkin and Ziboh, 1984). A major functional role of LA in the skin is its involvement in the maintenance of the epidermal water barrier (Hansen and Jensen, 1985), which is one of the major abnormalities of cutaneous EFAD. This significant effect of linoleic acid (LA) was reported early in a series of studies by Prottey and coworkers who demonstrated that increased transepidermal water loss (TEWL) occurred in EFAD animals (Hartop and Prottey, 1976). Information derived from these findings supported the special role of LA in restoring the defect of skin epidermal water barrier, which has been induced by EFAD.

In humans, although deficiency of GLA has been associated with clinical disorders of the skin, information on its role in the normal physiology of skin is limited. This prompted an earlier study, which reported that intake of dietary Borage oil (containing GLA) by humans decreased skin roughness, TEWL, as well as increased the moisture in normal skin (Nissen et al., 1995). This, presumably, is a physiological effect of GLA. However, in a recent report of a randomized double-blind, placebo-controlled study of oral capsules of EPO (containing a total of 345 mg GLA/day) given to healthy human adults with normal skin for 12 weeks, the data revealed that significant skin moisture, firmness, and roughness were all improved as determined by biophysical techniques (Muggli, 2005). Taken together, these findings indicate that GLA does function as an EFA and can contribute to maintaining the normalcy of human skin. Further studies are, however, warranted, which will reveal its mechanism of action.

C. *In Vivo* Role of γ-Linolenic Acid-Enriched Diet in the Induction of the Biosynthesis of Potent Biologically Active Mediators

In an earlier study, it was shown that *in vitro* preparations from skin epidermis are active in the *in vitro* metabolism of GLA/DGLA (Miller et al., 1988). Using the guinea pig skin epidermis as a model, which is functionally similar to that of human epidermis, it was demonstrated that

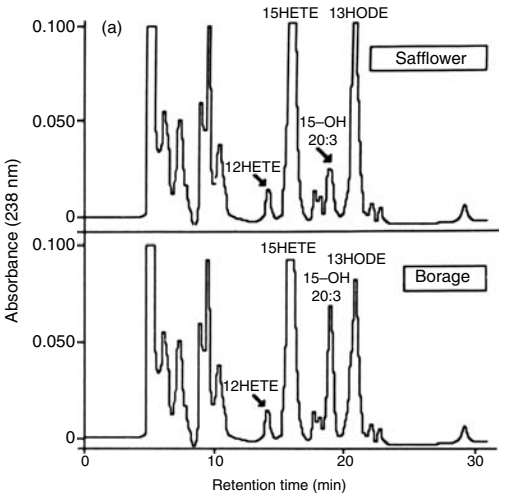

Figure 32.3 Reverse phase (RP)-HPLC profile of the effect of GLA-enriched diets on the *in vivo* generation of epidermal monohydroxy fatty acids by normal guinea pigs. 15-OH-(20:3), 15-HETrE (derived from dietary GLA); 15-OH-(20:4), 15-HETE (derived from dietary AA); and 15-OH-(18:2), 13-HODE (derived from dietary LA). (From Miller, C.C., and Ziboh, V.A. (1998). *Biochem Biophys Res Commun*. 154:3. With permission.)

supplementation of the guinea pig diet with GLA-containing Borage oil resulted in the major generation of PGE_1 and a 15-LOX metabolite (15-HETrE) from DGLA (Miller and Ziboh, 1988). A chromatographic representation of the skin epidermal extract from Borage oil-fed guinea pigs is shown in Figure 32.3. These *in vivo* findings were consistent with the *in vitro* metabolic results (Miller et al., 1988). Since the prostanoid (PGE_1) and monohydroxy acid (15-HETrE) have been reported to exhibit both anti-inflammatory/antiproliferative properties *in vitro* (Miller et al., 1988), supplementation of diets with appropriate amounts of Borage/Primrose or Blackcurrant could generate local *in vivo* anti-inflammatory/anti-proliferative modulators that can serve as adjuncts to standard therapy to attenuate inflammatory/proliferative skin disorders.

V. DIETARY ROLE OF γ-LINOLENIC ACID IN DISEASE SITUATIONS: MODULATION OF INFLAMMATORY/ IMMUNOLOGICAL DISORDERS

A. *In Vivo* Dietary Role in Rheumatoid Arthritis

Chronic disorders of inflammation are often associated with immunological activities and so it is appropriate to group these disorders together in evaluating the role of dietary GLA in the clinical course of the diseases. Rheumatoid Arthritis (RA) is a chronic systemic inflammatory disorder of unknown etiology. It is a human disease characterized by symmetrical polyarthritis and it is prototypical, immunologically induced, inflammatory arthritis. In an early clinical study of 20 patients given a relatively low dose of 360 mg GLA/day (as EPO) for 12 weeks, the observed beneficial effects were mixed due in part to administration of low concentrations of total GLA (360 mg/day) (Mork-Hansen et al., 1983). In a subsequent study, the dietary intake of patients with a higher concentration of Borage oil (equivalent to 1400 mg of GLA/day) for 24 weeks resulted in clinically significant reductions in signs and symptoms of rheumatoid disease activity (Leventhal et al., 1993), suggesting that responses to GLA were dose-dependent in contrast to placebo, which showed no change or worsening of disease. This finding is consistent with earlier *in vitro* findings in other systems, which demonstrated the dose-dependent effect of GLA. In a larger double-blind, placebo-controlled study, with the administration of higher total GLA content (2.8 g/day) for

6 months, the data revealed significant clinically relevant reductions in signs and symptoms of the disease activity (Zurier et al., 1996). Furthermore, patients who remained for an entire 12 months on the 2.8 g/day dietary GLA protocol exhibited continued improvement in symptoms with minimal deleterious effects. Indeed, the use of GLA at the above higher dose was reported to be well tolerated and effective in the treatment of active RA. In addition, it has been reported that GLA can be used as an adjunct with nonsteroidal anti-inflammatory drug (NSAID) in controlled clinical trial (Zurier, 1993) in the treatment of RA. Taken together, the data derived from these studies demonstrate that the amount of total GLA/day intake and the duration of GLA intake do correlate with the clinical efficacy. Both factors must therefore be considered in any dietary protocol.

B. *In Vivo* Dietary Role in a Model of Lupus Erythematosus

Since GLA has not been given to patients with systemic lupus erythematosus (SLE) in any elaborate study, the mechanism of efficacy is unknown. However, it has been reported that using the murine model of lupus, leukotrienes do contribute to the renal disease in MRL/lpr/lpr mouse model (Spurney et al., 1991). Since GLA has been reported to suppress leukotriene generation via the inhibition of 5-LOX pathway, it is likely that GLA can exert efficacy in this mouse model. In addition, dietary administration of GLA was also reported to enhance the generation of the renal protective COX-1 catalyzed metabolite from AA (prostacyclin, PGI_2), which has been shown to attenuate inflammatory effects in patients with RA and to suppress acute and chronic inflammation of joint tissue injury in several experimental animals (Zurier, 1993). These findings imply that GLA, which exerts anti-inflammatory and immune modulatory effects in patients with RA, may also be useful in the treatment of SLE patients. Thus, well-controlled human studies seem warranted in this disease.

C. *In Vivo* Dietary Role in Cardiovascular Disorders

Hypertension is considered as one of the major risk factors in cardiovascular disease because it has been associated with heredity, obesity, smoking, and diet. The interest in its control has triggered numerous studies involving pharmacological and nonpharmacological agents. Although pharmacological agents have been shown to exert beneficial effect by reducing blood pressure (BP), the downside of the protracted use of these agents has been associated with deleterious side effects, thus, diminishing the therapeutic potential of these chemical agents. For this reason, the use of nonpharmacological agents such as nutritional manipulation in recent years has seemed attractive. In particular, the role of dietary PUFAs has come to the forefront of dietary manipulation because of lesser tendency to toxicity.

Much of the evidence for the relationship between dietary intake of various levels and types of fatty acids and systemic BP has been derived from the work conducted on rats. Much of that work, in turn, has been derived from studies comparing the effects of different dietary fatty acids on BP development in the genetically spontaneously hypertensive rat (SHR), a commonly used animal model for human hypertension. In these animals, consistent differences in tissue fatty acid metabolism exist during hypertension (Watanabe et al., 1989). Numerous membrane abnormalities have been reported in SHR (Montenay-Garestier et al., 1981; Devynck et al., 1982; Tsuda et al., 1988). Consequently, the hypertension observed in SHR could be related to fundamental changes in lipid metabolism, affecting long-chain PUFA (LCPUFA) in cell membranes. For instance, it has been reported that Δ^{-6} and Δ^{-5} enzymes that catalyze the transformation of LA to AA activities are lower in the SHR when compared to normotensive rats (Narce and Poisson, 1995, 1996).

Another major contributor to BP regulation is the Renin–Angiotensin System (RAS) that has also been a focus of hypertension research. The determinants of arterial BP are cardiac output and systemic vascular resistance (Creager and Gerhard, 1999). The RAS plays a central role in BP regulation by increasing vascular resistance while promoting renal retention. Although various pharmacological agents have been developed to antagonize the RAS, their beneficial effects have often been limited by side effects. Thus the thrust to evaluate the role of PUFAs, particularly GLA, is prudent and attractive (Engler, 1993, 2000).

In the studies evaluating the role of PUFAs, the dietary administration of GLA-enriched diets to normotensive and hypertensive SHRs, GLA was reported to reduce BP in both animal groups, although, greater effect was exerted on the hypertensive animals. A suggested attractive and interesting mode of action is that dietary GLA does interfere with the RAS, which would lead to the production of angiotensin II (Creager and Gerhard, 1999). GLA is purported to alter the properties of the vascular smooth muscle cell (SMC) membrane, resulting in the inhibition of angiotensin II receptor binding to the adrenal glomerulosa cells (Goodfriend and Ball, 1986). This finding implies that dietary GLA blocks the primary effect of angiotensin II, which stimulates the synthesis and secretion of aldosterone by the adrenal cortex. This blockade prevents aldosterone's action on the kidney tubules that contribute to elevated sodium reabsorption and arterial BP. This suggested mechanism seems plausible and it implies that dietary GLA may function *in vivo* as a nonpharmacologic angiotensin II receptor antagonist or as a membrane modifier on the angiotensin II receptor-binding activity to suppress aldosterone's contribution to elevated BP.

Another possibility in the ability of dietary GLA to lower BP is the elongation metabolite (DGLA) and its tissue elevation. The elevated DGLA is then incorporated into vascular tissue membrane phospholipids. The DGLA–phospholipid complex undergoes phospholipase A_2 cleavage to release DGLA that is transformed by COX-1 to yield PGE_1. This prostanoid is biologically known to induce vascular smooth-muscle relaxation. This latter possibility is consistent with the *in vitro* findings that PUFAs incorporated into vascular tissue cell membrane phospholipids do influence signal transduction, ionic fluxes, and enzyme activities (Goodfriend and Ball, 1986; Oishi et al., 1990; Ordway et al., 1991).

D. *In Vivo* Dietary Role in Bleomycin-Induced Lung Fibrosis

Interstitial lung fibrosis (ILF) is a potentially lethal, chronic response of the lung to injury resulting from a wide range of processes. ILF is invariably associated with fibrosing alveolitis characterized by inflammation, fibroblast proliferation, and accumulation of extracellular matrix proteins. The hamster is a fitting animal model for the study of induced lung fibrosis (Snider et al., 1978). Although Bleomycin (BLM) is a glycopeptide known to cause pulmonary injury via the generation of reactive oxygen species and various inflammatory mediators, it is pharmacologically widely used as an antineoplastic drug in the treatment of human lymphoma, testicular and squamous cell tumor, which is often complicated by a dose-dependent induction of ILF (Crooke and Bradner, 1976). The possible efficacy of dietary GLA-containing oil (EPO) was tested in an animal model of BLM-induced lung fibrosis (Ziboh et al., 1997) since alterations in the levels of certain fatty acid metabolites had been associated with BLM-induced lung injury. The findings, after 14 days of dietary supplementation with GLA, revealed an increase in (1) GLA metabolite (DGLA) in the lung tissue, (2) enhanced COX-1 catalyzed generation of PGE_1, and (3) its 15-LOX catalyzed generation of 15-HETrE. The latter two metabolites are known to exert anti-inflammatory/antiproliferative properties. Associated with these biochemical events are the suppression of BLM-induced increase in tissue hydroxyproline (a marker for collagen synthesis) as well as the inhibition of LTB_4 (a proinflammatory mediator). These biochemical alterations parallel the attenuation of the lung fibrosis. Taken together, GLA-enriched diet may serve as an adjunct to diminish toxicity of standard therapy and for the management of interstitial lung fibrosis and the suppression of the peripheral inflammatory mediators induced by BLM.

E. *In Vivo* Dietary Role in the Management of Type I and Type II Diabetes

One of the major complications of diabetes mellitus is peripheral neuropathy characterized by reduction of nerve conduction velocity (NCV). Strict control of the hyperglycemia may delay the development of clinical diabetic neuropathy (Pirart, 1977) or improve nerve function in patients with clinical neuropathy (Brown and Asbury, 1984), but a complete cure has not been found. To explore the role of GLA in this serious disorder, the rat animal model of experimental diabetes was

used (Julu, 1988). Using this model of streptozotocin-induced insulin-dependent diabetic rats, the animals were administered GLA-enriched EPO orally to ascertain whether any efficacy would occur in this model of the gross lipid abnormality in the diabetic nerve. Interestingly, the results revealed marked increase of GLA in the diabetic plasma reflecting mostly the dietary intake. This increase paralleled the depletion of excess cholesterol in the diabetic nerve. Improvement of the NCV after EPO/GLA ingestion (Julu, 1988; Kuruvilla et al., 1998) correlated with conservation of AA in the nerve phospholipids.

Although human trials of GLA in the form of EPO in patients with neuropathy due to Type I or Type II diabetes are consistent with those of the animal studies, the effects after GLA ingestion did not attain complete normalization as seen in the animals (Jamal and Carmichael, 1990; Keen et al., 1993). These limited results in the human patients may be due, at least in part, to the fact that most of the human disease was Type II diabetes and not Type I, which predominantly occurs in the animal model. Furthermore, the dose of GLA administered to the humans may have been too low to elicit major beneficial effects. Thus, the administration of higher doses of GLA would be a prudent first step to establish the efficacy of dietary GLA in human diabetes. This investigation warrants further explorations.

F. *In Vivo* Dietary Role in the Management of Bronchial Asthma

In a previous study, the administration of EPO-containing GLA and fish oil-containing EPA/DHA was reported to be ineffective in the treatment of bronchial asthma (Stenius-Aarniala et al., 1989). Notably in that study, the amounts of GLA and EPA/DHA, which were contained in 15–20 mL and administered to patients, were not quantitated; thus, the actual amounts of GLA or EPA/DHA ingested were not known. The authors even concluded that the lack of detectable response in the patients could be attributed to insufficient amounts of EPO or fish oil administered.

However, in a recent trial with dietary GLA in asthma patients, the study design was based on the recognition that both humans and rodents exhibit no serious deleterious effects of the GLA-enriched oil had been described. Metabolically, only a small fraction of the DGLA derived from the elongation of GLA is converted by the Δ^5-desaturase to AA. This minor conversion has been demonstrated to be due to the low activity of Δ^5-desaturase in most tissues. This low metabolic desaturation of dietary GLA therefore does not support the often suggested concerns that dietary ingestion of GLA poses an undesirable risk because of its further elongation and Δ^5-desaturation to excessive generation and accumulation of AA, which in turn would result in the excessive generation of proinflammatory metabolites such as prostanoid (PGE_2) and lipoxygenase products such as LTB_4, LTC_4, and LTD_4. Indeed, in many tissues and cell types, this concern has not materialized; rather, it is DGLA that accumulates while AA shows a decrease after GLA supplementation in the diet. The accumulated DGLA becomes the substrate that is oxygenated via the COX-1 pathway to produce PGE_1 and via the 15-LOX pathway to produce 15-HETrE as shown in Figure 32.4. These two oxidative metabolites of DGLA (PGE_1 and 15-HETrE) have been reported *in vitro* to inhibit the generation of proinflammatory leukotrienes and *in vivo* to suppress acute and chronic inflammation in a variety of disease conditions. In addition, DGLA *in vitro* has been reported to compete with AA for the cyclooxygenase, thus, inhibiting the production of proinflammatory PGE_2. DGLA is additionally metabolized by 15-LOX to 15-HETrE (which is a potent inhibitor of the 5-LOX), the enzyme that catalyzes the synthesis of the proinflammatory LTB_4, LTC_4, and LTD_4. The inhibitory potential of 15-HETrE has been shown *in vitro* to markedly inhibit 5-LOX pathway and LTB_4 generation from AA by basophilic leukemia (RBL-I) cells (Vanderhoek et al., 1980; Miller et al., 1988; Ziboh and Fletcher, 1992). Also *in vivo*, the feeding of a diet supplemented with GLA-containing oil to hamsters revealed significantly elevated levels of DGLA and 15-HETrE biosynthesized *in vivo* in the hamster lung. This elevated 15-HETrE paralleled reduced LTB_4 generation by PMN-infiltrated hamster lung (Ziboh et al., 1997).

Because asthma is characterized by variable and reversible airflow obstruction and by bronchial hyperresponsiveness, as well as excessive airway narrowing in response to a variety of apparently

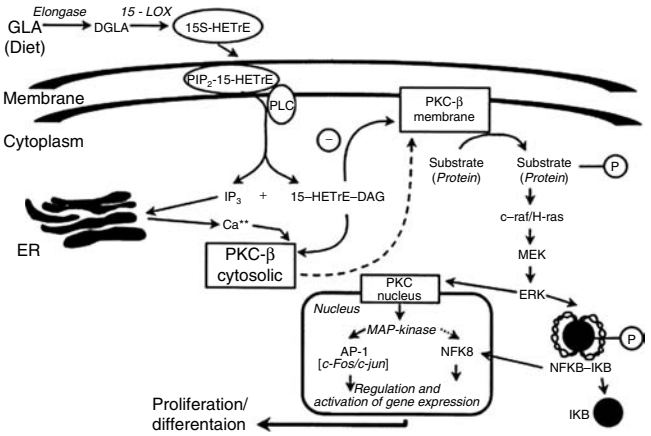

Figure 32.4 Speculative scenario of dietary GLA modulation of epidermal nuclear transcription factor. *Abbreviations*: 15-LOX, 15-lipoxygenase; 15-HETrE, 15-hydroxyeicosatrienoic acid; PIP, phosphatidylinositol phosphate; PKC, protein kinase C; DAG, diacylglycerol; MAPK, mitogen activated protein kinase; AP-1, activator protein 1. (From Ziboh, V.A., et al. (2002). *Arch Pharm Res*. 25:6. With permission.)

unrelated stimuli, contraction of the airway smooth muscle has been emphasized as an important mechanism contributing to asthmatic airway obstruction. Inflammation in the airway wall is therefore a prominent feature of fatal asthma attack (Dunnill, 1960; Glynn and Michaels, 1960). There is abundant experimental evidence that inflammation is also present in mild asthma and is related to bronchial hyperresponsiveness (Chung, 1986), a characteristic feature of asthma (Boushey et al., 1980). Taken together, the pathological changes are therefore likely to include the release of inflammatory mediators such as leukotrienes. Thus, in a randomized, double-blind comparison of dietary supplementation of GLA-containing Borage oil equivalent to the total of 2 g GLA/day and a placebo control receiving corn oil, in patients with asthma, the data revealed that the patients receiving dietary supplementation of the total of 2 g GLA/day for 52 weeks significantly suppressed the capability of their PMNs to generate LTB_4 (an inflammatory response characteristic of asthma) (Ziboh et al., 2004). The degree of beneficial clinical response by the patients, although moderate, was variable and not as highly correlated with the biochemical data. This variability is probably due to the multifactorial disease nature of asthma, which likely contributed other unknown mediators to the pathogenesis of asthma. Alternatively, and perhaps more importantly, the dose of γ-linolenate (2 g/day) given to patients with chronic moderate to severe asthma was inadequate in view of the severity of the disease and the need to generate sufficiently high *in vivo* inhibitor such as 15-HETrE to strongly inhibit 5-LOX pathway, diminish the inflammatory mediators, and attenuate the clinical course of asthma. Further higher dose-dependent study is therefore warranted.

VI. ROLE OF γ-LINOLENIC ACID-ENRICHED DIET IN HYPERPROLIFERATIVE PROCESSES

A. *In Vivo* Dietary Role in a Guinea Pig Model of Normal Skin Epidermis: Generation of Biologically Potent Metabolites

The excitement generated by reports of clinical improvement of patients with atopic eczema after oral administration of Primrose oil (Kunkel et al., 1981; Zurier, 1982) signaled a possible role for GLA-containing oil in cutaneous biology. To ascertain what form of GLA-containing oil could exert the beneficial effects of dietary GLA, the study included dietary supplementation of Safflower oil (containing predominantly 18:2n-6, LA) and Borage oil (containing predominantly 18:3n-6, GLA) to normal

guinea pigs for 8 weeks. The results revealed the generation of oxidative metabolites of the constituent LA/GLA/DGLA as shown graphically in Figure 32.4 (Miller et al., 1988). Extracts of the epidermis from the guinea pigs fed Safflower oil and Borage oil revealed no significant differences in AA-derived monohydroxy fatty acids such as 12- and 15-HETE in the two diets. In contrast, the epidermis of the Borage oil-fed animals revealed a significant increase in the DGLA-derived monohydroxy fatty acid (15-HETrE), indicating the *in vivo* metabolism of DGLA into 15-HETrE by epidermal 15-LOX. The skin epidermal tissue therefore generates significant 15-HETrE after dietary supplementation with GLA-enriched diet, which can contribute to maintain normal cutaneous homeostatis.

B. *In Vivo* Dietary Role in a Rodent Hyperproliferative Skin Model

The exclusion of EFA in guinea pig diet is known to result in a model of EFAD. This is associated with epidermal hyperplasia and scaling dermatosis of the skin. Replenishment of the diet of the EFAD animals with GLA-containing Borage oil resulted in reversal of the scaly dermatoses and suppression of hyperproliferation (Chung, 1986). Interestingly, the accumulation of DGLA and 15-HETrE (a potent antiproliferative metabolite derived from DGLA) was elevated in the EFAD-Borage oil-fed animals, suggesting that the antiproliferative effect of dietary GLA is due, at least in part, to the *in vivo* generation of 15-HETrE. This study further supports the view that metabolites of GLA are biosynthesized *in vivo* after dietary GLA-containing oils.

VII. ROLE OF γ-LINOLENIC ACID-ENRICHED DIET IN CARCINOGENESIS: MODELS, MECHANISMS, AND SPECULATIVE PATHWAYS

In vitro studies have been reported, which indicate that GLA exerts varying effects on a variety of malignant cells. For instance, the addition of GLA *in vitro* to malignant cell lines induced cytotoxity on the cells in a dose- and time-dependent manner (Begin et al., 1985, 1986), whereas normal cell lines are resistant to cytotoxity of GLA at low concentrations. Furthermore, it has been reported that the coculture of normal fibroblasts with human malignant cells results in the cancer cells completely overgrowing the normal fibroblasts. However, the coculture of the normal and malignant cells in the presence of GLA (10^{-4} M) resulted in the death of the malignant cells and being outgrown by the normal cells (Begin et al., 1986). These experiments indicate that *in vitro*, GLA/DGLA were presumably incorporated into the cellular membrane phospholipids and then alter the activity of malignant cells in culture. Although these *in vitro* findings are interesting, ultimately, it is the *in vivo* findings in animal models of carcinogenesis and in humans that will establish the beneficial effects of GLA-enriched diet in the management of hyperproliferative/carcinogenic disorders.

A. *In Vivo* Role in Animal Models of Human Breast Cancer

In vivo studies on the nutritional role of GLA-enriched diet in human breast carcinogenesis are sparse. A major problem has been, and is still, to develop a suitable animal model that mimics the biological and biochemical events in human breast cancer. As a consequence, many animal studies have been used, which yielded information that is either confusing or contrary to *in vitro* observations. For instance, in some animal observations it has been suggested that linoleic acid (an EFA) may be a tumor promoter in experimental cancer systems, notably, in rodent breast cancer models. The interpretation of some of these findings has been flawed because the amount of LA fed to these animals is excessively high, and there is no information of the normal level of LA in the animals and the dose-dependent effect of linoleic acid in normal breast tissue development and carcinogenesis.

Nevertheless, the use of athymic mouse model has found a niche in the study of human tumors because these animals lack T-cell mediated immunity and thus fail to reject foreign species xenografts. Using this type of model for transplantation of human breast cancer and melanoma, it has

been reported that dietary administration of adequate amounts of GLA (4–8 g/day) per day inhibited cancer growth (Pritchard et al., 1989). In a similar study with high dietary doses of GLA given as Evening Primrose to a small group of humans (21 patients) with advanced cancer stages of mainly liver, brain, and mesothelioma, GLA was reported to exert clinical improvement by reducing tumor bulk and pain (Van der Merwe et al., 1987, 1990). On the basis of these limited studies, there seems a valid rationale for investigating the role of GLA as a possible antitumor agent in a wide spectrum of human cancers. Although, the place of nutritional intervention at large in the management of human cancer still remains controversial, it is nonetheless an exciting prospect to consider that dietary EFA such as GLA, which has been reported to exert negligible *in vivo* toxicity, could serve at least in part, as an adjunct therapy to standard chemotherapeutic agents in the management of human cancers.

B. *In Vivo* Role of γ-Linolenic Acid as an Adjunct to Tamoxifen in Chemotherapeutic Management of Human Breast Cancer

Tamoxifen is a synthetic nonsteroidal antiestrogen. It is used extensively in the treatment of human breast cancer. The mechanisms of action are complex. However, the main mechanism is that it acts as a competitive antagonist to estrogen for binding with the estrogen receptor (ER). The resultant tamoxifen/ER complex prevents estrogen-induced effects on growth factors and proliferation (Kenny et al., 2000).

Because GLA has been proposed as a valuable adjunct in cancer therapy due to its *in vitro* selective antitumor properties with negligible systemic toxicity (Begin et al., 1985), orally administered GLA was investigated as an adjunct along with tamoxifen (a primary hormone therapy) in an endocrine-sensitive breast carcinogenesis. Specifically, the oral intake of capsules containing GLA along with tamoxifen (as primary therapy) by elderly patients (with primary, locally advanced, and metastatic breast cancer) significantly accelerated positive beneficial clinical response when compared to the group that was treated with tamoxifen alone (Kenny et al., 2000). Although the mechanism of action of the orally administered GLA in tamoxifen trial remains unclear, the dietary study revealed a significant suppression of ER expression in the patients treated with tamoxifen plus GLA than in patients treated with tamoxifen alone. This finding suggests that the biological actions of GLA in ER-breast cancer may be via the modulation of the ER pathway. Consistent with this finding, the suppression of ER binding by fatty acids has been reported in other studies (Borras and Leclercq, 1992). Specifically, GLA has been reported to induce apoptosis in human cervical carcinoma (HeLA) cells (de Kock et al., 1996). The important finding from this limited study is that the biological action of GLA in the human ER-driven breast cancer is mediated via the ER pathway. The modulatory effects of GLA on ER function and the possibility that GLA ingested with tamoxifen may be synergistic and can attenuate the growth of breast cancer via the enhanced downregulation of ER warrants further investigation.

C. Peroxisome Proliferator-Activated Receptor-γ: A Possible Target for γ-Linolenic Acid Mode of Action

Consistent with the biological effect of oral GLA plus tamoxifen in the attenuation of ER-driven breast cancer growth (Kenny et al., 2000), extensive *in vitro* studies of PUFAs on breast cancer cells have revealed that the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) by GLA and other PUFAs may function to suppress growth of breast cancer cells (Xu et al., 1999). This family of nuclear receptors has been reported to regulate gene-encoding enzymes for lipid metabolism (Schoonjans et al., 1996; Kilgore et al., 1997). In particular, ligands that activate PPAR-γ have been demonstrated to inhibit the proliferation of breast cancer cells *in vitro* (Elstner et al., 1998; Mueller et al., 1998; Yee et al., 1999). Among the several fatty acids studied, GLA was shown to induce the highest activation. Interestingly, this action of GLA was higher than ciglitazone (a known ligand for

PPAR) (Xu et al., 1999). In addition, *in vitro* studies revealed that GLA (derived from Borage oil), EPA (derived from fish oil), and ciglitazone (a pharmacological agent) significantly inhibited proliferation of all the human breast cancer cells tested. Interestingly, among the fatty acids tested, GLA exerted the highest activation of the reporter gene, which was higher than EPA (Xu et al., 1999). Taken together, the possibility exists that adequate oral intake of GLA or oil containing GLA could serve as a nontoxic dietary adjunct to standard chemotherapeutic agents to diminish toxicity during the management of breast cancer.

VIII. DIETARY ROLE OF γ-LINOLENIC ACID IN PROSTATE CARCINOGENESIS

A. *In Vivo* Role in a Rodent Model of Prostate Adenocarcinoma

Prostate cancer poses considerable threat to the aging male population as it has become a leading cause of cancer death to this group (Carter and Coffey, 1990). Due to the complexity of this age-related disease, the mechanism(s) and factors resulting in prostate cancer remain unclear. Reports associating the increased risk in prostatic cancer with increasing dietary fat have been contrasted by other studies suggesting that beneficial effects of certain PUFAs in the modulation of tumor development do exist. Similar to problems associated with the development of appropriate animal models for breast cancer, attempts to establish a fitting animal model for the different stages of prostate cancer development have been limiting. However, the animal model that best represents human prostate cancer disease is the Lobund–Wistar (L–W) rat model of prostatic adenocarcinoma. This experimental animal model of prostatic adenocarcinoma, which exhibits androgen dependency as in human beings, exhibits high disease spontaneity, with high incidences of clinically evident induced tumors, with long latency periods, and with the development of additional neoplasms in tissue other than the prostate, was developed in the L–W rats (Pollard, 1992). Experimentally, the induction of prostatic adenocarcinomas with high incidence (70%–90%) in these rats was accomplished within 10 months by the initial injection of *N*-nitroso-*N*-methylurea (NMU) (which serves as the initiator), followed by the implantation of testosterone propionate (TP) (which serves as the promoter) (Pollard and Luckert, 1987).

Although GLA has been reported to suppress tumor growth *in vitro* using androgen-primed prostatic hyperplastic and tumorigenic cells, which were derived from the L–W rats (Pham et al., 2003), the efficacy of GLA-enriched diet to attenuate prostatic carcinogenesis *in vivo* has not been demonstrated. Thus, using the L–W rat model of prostate carcinogenesis, Borage oil (highly enriched with GLA) was added to the diet of tumorigenic rodent (NMU/TP) group for 54 weeks. Result from this long study revealed (1) a significant reduction of prostate tumor size in the tumorigenic (NMU/TP) group when compared with the non-GLA-fed control (NMU/TP) group; (2) the dietary GLA-induced reduction of the tumor size and growth was accompanied by a significant suppression of the elevated biosynthesized PGE_2 and 5-HETE in the tumorigenic (NMU/TP) group (Pham et al., 2006). Both elevated PGE_2 and 5-HETE have previously been reported in human prostate cancer. This first long-range GLA dietary supplementation therefore revealed that dietary GLA can attenuate both the prostate tumor growth and the suppression of the dysregulated eicosanoid metabolism in the rodent model. This finding raises the possibility that dietary supplementation with highly GLA-enriched diet could serve as a nontoxic adjunct to standard therapy in the management of human prostate cancer. This possibility warrants testing in well-designed trials in human prostate carcinogenesis.

B. *In Vitro* Modulatory Role of GLA and 15-HETrE on Rodent Prostatic Carcinoma Cells in Culture

The excitement generated from the *in vivo* dietary efficacy of GLA in the L–W rodent model of prostate carcinogenesis prompted *in vitro* experiments of GLA and its 15-LOX metabolite (15-HETrE)

on the rodent prostatic cells in culture to delineate a possible mechanism (Pham et al., 2003). The preincubation of the isolated rodent (androgen-dependent prostatic) adenocarcinoma cells with GLA or 15-HETrE prior to incubation in culture revealed that 15-HETrE markedly inhibited growth of the prostatic cells in a dose-dependent manner. The *in vitro* inhibitory effect of precursor GLA was moderate on the mitogenic cells in culture, while 15-HETrE at the same concentration was markedly suppressive. This finding underscores the capacity of the *in vivo* generated 15-HETrE to suppress prostatic carcinogenesis. Further cellular exploration of growth of the prostatic tumor cells in culture paralleled the activation of PPAR-γ. The results from these *in vitro* studies support the *in vivo* observations that dietary GLA via its *in vivo* endogenous generation of 15-HETrE metabolite can attenuate prostatic adenocarcinoma in the L–W rodent model. It is therefore reasonable to speculate that dietary supplementation with sources containing GLA could serve as a nontoxic adjuvant to reduce toxic doses of standard chemotherapeutic agents to attenuate human prostatic carcinogenesis.

C. Comparison of the *In Vitro* Modulatory Roles of 15-LOX Metabolites of GLA/DGLA and EPA on Human Androgen-Dependent and Nonandrogen-Dependent Prostate Carcinoma Cells in Culture

The observed capability of dietary GLA to suppress growth of prostatic adenocarcinoma in the rodent model (Pham et al., 2006) prompted another experimental design to compare the effects of 15-HETrE (15-LOX metabolite of GLA/DGLA) and 15-HEPE (15-LOX metabolite of fish oil EPA), respectively, on the growth and AA metabolism of the human androgen-dependent (LNCaP) and nonandrogen-dependent (PC-3) prostatic cancer cells in culture. Both human prostatic cancer cells have been reported to have increased capability to biosynthesize PGE_2 and 5-HETE from AA. This capability is similar to the results observed in the L–W rodent prostatic cell model that also had an increased capability to biosynthesize PGE_2 and 5-HETE. Preincubation of both human prostatic cells with 15-HETrE or 15-HEPE revealed differential degrees of growth suppression by the cells as well as the decreased biosynthesis of PGE_2 and 5-HETE from AA, respectively (Ziboh et al., 2004). Notably, 15-HETrE exerted greater suppression of PGE_2 and 5-HETE biosynthesis than 15-HEPE. The parallel *in vitro* studies of the effects of GLA/15-HETrE and EPA/15-HEPE on the androgen-driven (LNCaP) and nonandrogen-driven (PC-3) cancer cells clearly suggest that dietary GLA derived from Borage oil may be more efficacious than dietary EPA derived from fish oil. This *in vitro* finding answers, in part, the often asked question as to which of the two PUFAs (GLA or EPA) is more beneficial in proliferative disorders. In addition, these findings imply that nutritional management of prostatic cancer via nontoxic dietary supplementation with GLA-containing oil, which *in vivo* could generate antiproliferative 15-HETrE, is a possibility and deserves to be explored.

IX. ATTENUATION OF CUTANEOUS HYPERPROLIFERATION BY GLA–15-LOX METABOLITE (15-HETrE): MODULATION OF EPIDERMAL CELL SIGNALING

A. *In Vitro* Role of 15-HETrE in Cutaneous Signal Transduction

In pursuit for possible mechanisms of action of dietary GLA and its metabolite on skin homeostasis, it was reported that incubation of 15-HETrE *in vitro* with skin epidermal homogenate resulted in its incorporation into phosphatidylinositol 4,5-bisphosphate (Ptd 4,5-P_2). When the Ptd 4,5-P_2 undergoes a catalytic cleavage by phospholipase-C (PLC) it yields novel 15-HETrE-containing diacylglycerol [1-acyl-2-15-HETrE-glycerol (15-HETrE-DAG)] and inositol trisphosphate (Ins-1,4,5-P_3)

(Schoonjans et al., 1996). Interestingly, 15-HETrE-DAG selectively inhibited membrane associated protein kinase C-β (PKC-β), a mediator of cell cycle proliferation in various cells. This finding raises the interesting speculation that the dietary GLA (from EPO or Borage oil) could elicit the *in vivo* generation of second messenger: 15-HETrE-substituted DAG that in turn could attenuate skin inflammation/proliferation.

B. Attenuation of Proliferation by 15-HETrE via Modulation of Downstream Signaling Transduction

Since cell growth and proliferation are regulated by biochemical events in the nuclear compartment of the cell, the modulatory role of 15-HETrE on cytoplasmic and nuclear signaling molecules was evaluated in an animal model of skin hyperproliferation (Xi et al., 2000a). Specifically, using this guinea pig skin model, topical application of DHA on guinea pig normal skin elicited severe hyperplasia and hyperproliferation in the skin epidermis. This skin lesion was accompanied by the suppression of the expression of activated protein-1 (AP-1) (Xi et al., 2000b). AP-1 has been associated with cell proliferation and apoptosis (Welter and Eckert, 1995). Furthermore, topical DHA application was also found to increase the expression of Bcl-2 (an antiapoptotic protein) while the expression of caspase-3 was found to decrease (an apoptotic protein). Interestingly, the topical application of 15-HETrE to the skin lesion did suppress the DHA-induced expression of Bcl-2 while enhancing the expression of caspase-3. Taken together, the reversal of DHA-induced suppression of AP-1 expression by 15-HETrE, which is derived from dietary GLA, does provide information of how to investigate the activity of new antiproliferative agents. Since 15-HETrE is a metabolite derived from dietary GLA, it is possible that increased dietary intake of this PUFA may result in the *in vivo* generation of biologically active metabolite (15-HETrE) that could alleviate the hyperproliferative skin conditions via the modulation of the transcription factor AP-1. A speculative scenario of how dietary GLA could modulate skin hyperproliferation is illustrated in Figure 32.4.

X. GLA/DGLA CATALYZED GENERATION OF PROSTAGLANDIN E_1 (PGE_1)

A. *In Vivo* Oral Intake of Natural PGE_1 or PGE_1 Analog

A plausible rationale for the dietary intake of GLA or GLA-containing oil is its rapid elongation to DGLA followed by the latter's oxygenation and catalyzed transformation by COX-1 to PGE_1 and 15-LOX transformation to 15-HETrE as illustrated in Figure 32.2. Interestingly, it is now evident that dietary content of fatty acids can alter/modulate membrane-bound receptors, enzyme activities, and eicosanoid biosynthesis (Brenner, 1974; Chapkin and Ziboh, 1984; Nakahara et al., 1990). The dietary modulation of eicosanoid generation has been well demonstrated in the following reports (Chapkin and Ziboh, 1984; Miller and Ziboh, 1988; Miller et al., 1990; Ziboh and Fletcher, 1992; Ziboh et al., 1997, 2004).

Biologically, PGE_1 (the prostanoid metabolite derived from dietary GLA) has been reported to exert a wide range of effects *in vivo*. For instance, PGE_1 has been reported to inhibit platelet aggregation via cyclic AMP (cAMP)-dependent mechanism. This in turn inhibits thrombus formation in the mesenteric microvessels as well as dilates the coronary arteries and lowers BP (Simmet and Peskar, 1988; Watanabe et al., 1989). Furthermore, PGE_1 infusion into humans after angioplasty has been shown to inhibit abrupt occlusion and early restenosis. Because of the systemic short half-life of natural PGE_1 in the body due to its *in vivo* metabolism into less biologically active products, the use of stable analogs of PGE_1 to circumvent the problem of short half-life of the natural PGE_1 has been studied. For example, PGE_1 analog (misoprostol) has been used to prevent acute Graft-Rejection in Renal-Transplant Recipients treated with cyclosporine and prednisone (Moran et al., 1990). These desirable effects of PGE_1 and/or analogs have considerable therapeutic potential. However, despite the promising use of the analogs of PGE_1, the possibility of their long-term use and side effects has

remained unresolved. Alternatively, a reasonable, nontoxic modality to counteract the drawbacks of exogenous use of natural or analog of PGE_1 for therapeutic purposes, particularly in the treatment of vascular diseases, is to enhance the endogenous biosynthesis of PGE_1 via the dietary intake of GLA.

PGE_1 analog has also been used in an interesting clinical disorder (Mork et al., 2004). Erythromelalgia (EM) is a rare condition characterized by red, hot, and painful extremities. Warmth, exercise, tight shoes, and gloves intensify discomfort (Thompson et al., 1979). The condition may be primary or secondary to another disease (Rance et al., 1980). Local skin cooling provides relief. The hypothesis to test oral PGE_1 analog (misoprostol) was based on previous reports of parenteral use of prostanoids (Kalgaard et al., 2003). In this trial, oral administration of misoprostol PGE_1 analog (0.4–0.8 mg/day) for 6 weeks to patients with symptoms, resulted in reduced EM symptoms such as microvascular arterionevous shunting in the affected skin. Although no major side effects were observed, the long-term oral effect of misoprostol has not been determined. Although the use of analogs of PGE_1 as a strategy to administer PGE_1 has shown promise, the possibility of side effects has remained unresolved. Therefore, a reasonable, nontoxic therapeutic modality to counteract any drawbacks of oral use of PGE_1 for therapeutic purposes, particularly in the treatment of vascular diseases, is to administer dietary GLA in order to enhance endogenous biosynthesis of PGE_1.

B. *In Vitro* Modulatory Role of PGE_1: A Speculative Scenario of *In Vivo* Mechanism of Action

The biological actions of the prostaglandins in tissues are known to be associated with changes in adenyl cyclase activity (Dayal et al., 1983). For instance, the binding of PGE_1 to its receptor (EP) on the cell surface modifies cyclase activity, depending on the nature of the regulatory G-proteins, which couple the receptor to the adenyl cyclase. The association of dietary GLA with the generation of PGE_1; the latter's modulation of cAMP and SMC proliferation are illustrated in Figure 32.5 in the investigation of the modulatory role of macrophage on SMCs that were harvested from mice previously fed GLA-enriched oil and the control of non-GLA-fed animals (Fan and Chapkin, 1998). Interestingly, exogenous PGE_1 restored the antiproliferative effect of GLA-enriched macrophages on SMC that were previously treated with cyclooxygenase inhibitor (indomethacin). These findings suggest that PGE_1 (derived from dietary GLA) is the primary mediator of macrophage–SMC interaction that involves the cAMP signaling cascade. A speculative scenario of dietary GLA-modulated macrophage/SMC interaction via G-protein coupled PG receptor (EP_2, EP_4, IP), which results in the elevation of intracellular cAMP/activation of PKA and ultimately modulation of nuclear transcription factor is illustrated in Figure 32.5 (Fan and Chapkin, 1998). The proposed model delineates that dietary GLA is elongated to DGLA and then incorporated into macrophage membrane phospholipids. Release of DGLA and metabolism by COX-1 results in the generation of PGE_1. The secreted PGE_1 binds to G-protein coupled prostaglandin receptor (EP_2/EP_4) or prostacyclin receptor (IP) on the SMCs. The binding results in stimulation of the adenyl cyclase resulting in the elevation of intracellular cAMP. The cAMP seemingly activates the PKA causing the phosphorylation of the nuclear cAMP response element-binding protein (CREB). CREB proteins do subsequently dimerize with other transcription factors to downregulate proliferation. This speculative scenario is nicely illustrated in Figure 32.5. Since PGE_1 is derived from GLA, the biological significance of dietary GLA as an EFA is further enhanced.

XI. CONCLUSION AND FUTURE PERSPECTIVES

γ-Linolenic acid (18:3n-6), although a Δ^6-desaturase product of linoleic acid, is normally found in great abundance in some plant seed oils, notably Evening Primrose, Borage, and Blackcurrant seed oils. It is also present in some storage oils of algae and various species of fungi. A notable metabolic feature of GLA is its rapid *in vivo* elongation to DGLA, followed by the latter's oxidative metabolism either via the cyclooxygenase (COX-1) pathway to PGE_1 or via the 15-LOX pathway into 15-HETrE. An increasing number of reports continue to emerge indicating that dietary GLA is

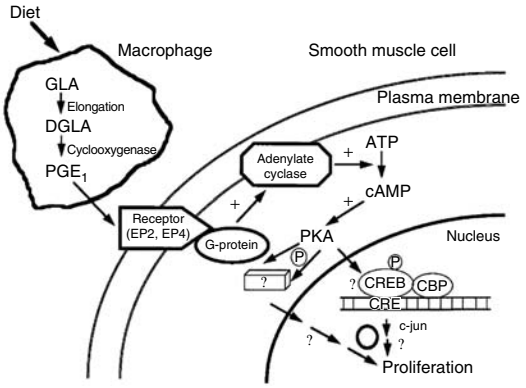

Figure 32.5 Speculative scenario of dietary GLA induced macrophage-DGLA derived PGE_1: Modulation of SMC nuclear transcription factor/gene expression. *Abbreviations:* cAMP, cyclic 3,5-adenosine monophosphate; CREB, cAMP response element-binding protein; CBP, CREB-binding protein. (From Fan, Y.Y., and Chapkin, R.S. (1998). *J Nutr*. 128:9. With permission.)

capable of modulating membrane bound receptors, enzyme activities, and *in vivo* eicosanoid biosynthesis. For instance, PGE_1 (the prostanoid metabolite of DGLA) does elicit an array of intracellular responses by binding to select G-protein coupled surface PGE receptors (EP) and the prostacylin receptor (IP). The receptors couple to adenyl cyclase resulting in increases in intracelluclar cAMP. Elevation of cAMP stimulates the expression of numerous genes via the PKA-mediated phosphorylation of the nuclear CREB. Through this mechanism, PGE_1 has been shown to inhibit vascular SMC proliferation *in vitro*. This modulatory effect *in vivo* would reduce the migration and proliferation of vascular SMC and retard development of the atherosclerotic plaque. In addition, PGE_1 has been associated with retarding platelet aggregation and lowering BP. PGE_1 has also been implicated with the inhibition of cancer cell growth. On the other hand, 15-HETrE the major 15-LOX metabolite derived from DGLA has been demonstrated to suppress both inflammation and cell growth, particularly in epithelial cells. A notable feature of this metabolite is its incorporation into epidermal phosphatidyl 4,5-bisphosphate (PtdIns 4,5-P_2) followed by its PLC catalyzed hydrolytic bifurcation into 15-HETrE-substituted diacylglycerol (15-HETrE-DAG) and the inositol trisphosphate (Ins-P_3). These GLA/DGLA metabolites exert anti-inflammatory/anti-proliferative effects via signal transduction pathways.

Taken together, dietary GLA, although a member of the n-6 PUFA family that is inappropriately deemed deleterious, serves as an important essential dietary fatty acid nutrient with the capacity to be transformed into potent metabolites that modulate pathways that enhance the excessive generation of proinflammatory prostanoids and leukotrienes as well as provide a nontoxic therapeutic adjunct to standard chemotherapy for the management of human inflammatory/proliferative disorders.

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33 Biological Effects of Alpha-Linolenic Acid

Luc Djoussé

CONTENTS

I. INTRODUCTION

Alpha-linolenic acid (ALA) belongs to the N-3 family of essential fatty acids for humans. ALA contains 18 carbons and 3 double bonds and may play an important role in human physiology. Long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are mainly found in fish have been shown to have health benefits (Burr et al., 1989; Siscovick et al., 1995; Valaugussa et al., 1999). For example EPA and DHA have been reported to possess antiarrhythmic effects (Kang and Leaf, 1996a; Billman et al., 1999; Leaf et al., 1999; Leaf et al., 2005) and to lower triglycerides in humans (Harris, 1989; Roche and Gibney, 1996, 2000). On the other hand, taste preference, vegetarian diet, and possibly mercury contamination are factors that influence the frequency of fish consumption—a major source of EPA and DHA. In contrast, since plants are major sources of ALA and a small amount of ALA can be converted to EPA *in vivo*, ALA may provide an alternative to obtaining adequate amounts of non-animal omega-3 fatty acids. Of note is that the conversion of ALA to EPA and DHA may be affected by the concentration of linoleic acid (18:2 n-6) because linoleic acid and ALA use the same set of enzymes for desaturation and chain elongation (Gerster, 1988). Americans consume on an average 0.8 g of ALA per day (Djousse et al., 2001; Koralek et al., 2006).

The physiologic effects of ALA are poorly understood as the majority of research has focused on long-chain omega-3 fatty acids (EPA and DHA). The current chapter will describe the sources of ALA and discuss current epidemiological evidence on biological effects of ALA as well as future directions in ALA research.

II. SOURCES OF ALA

ALA is provided in the food chain mostly by plants. Major sources of ALA are flaxseed oil (51% by weight), canola and soy oils (9% and 7% by weight, respectively), and walnuts (6% by weight)

(Connor, 1999; Kris-Etherton et al., 2000). ALA content of processed foods can be variable depending on the oil used for processing. ALA is found in lesser concentration in green leafy vegetables, corn oil, almonds, and hazelnuts (Connor, 1999; Kris-Etherton et al., 2000). Variable amounts of ALA are also available in red meat, poultry, eggs, and dairy products (Bourre, 2005; Koralek et al., 2006).

III. *IN VIVO* CONVERSION OF ALA TO EPA AND DHA

Because other chapters of this book are devoted to the metabolism of fatty acids including ALA, this section will only provide a brief overview of the ALA metabolism *in vivo* and refers the reader to appropriate chapters for further details. Theoretically, it is possible for humans to convert ALA into long-chain omega-3 fatty acids EPA and DHA. This process utilizes elongase and desaturase enzymes to transform 18:3 (n-3) into 20:5 (n-3) and to a lesser extent 22:6 (n-3). However, this path does not appear to be effective in humans to make ALA a substitute for EPA and DHA. Only a small portion of ALA is metabolized to EPA (Sanders and Roshanai, 1983; Gerster, 1988; Nettleton, 1991; Mantzioris et al., 1994; Karvonen et al., 2002; Wilkinson et al., 2005). The major rate-limiting step in the conversion of ALA to EPA and other long-chain n-3 is the first delta-6 desaturation. It is thus possible that some of the physiological effects of ALA are mediated through EPA. The reader is referred to Chapter 36 for details on biological effects of EPA and DHA.

IV. ALA AND ARRHYTHMIA

Epidemiological evidence suggests that ALA may play an important role in protecting against cardiac arrhythmia. ALA may raise the threshold for arrhythmia in cardiac muscle cells, thereby making it more difficult to develop and sustain severe arrhythmia. In an experimental model, intravenous infusion of ALA over 90 min just before the exercise and ischemia test resulted in a significant reduction of the incidence of ventricular flutter/fibrillation (Billman et al., 1999); in that study, six out of eight mongrel dogs failed to develop ventricular flutter/fibrillation after the induced ischemia ($p = .004$) while infusion of a control substance (soybean oil) did not protect any animal from severe arrhythmias. In another animal model, rats fed with an ALA-rich diet showed a reduced incidence of ventricular fibrillation and cardiac mortality (McLennan and Dallimore, 1995).

It is not clear whether observed antiarrhythmic effects of ALA are direct or are indirect through its conversion to EPA. However, the relatively short period of time between ALA infusion and observed antiarrythmic effects lends support to the hypothesis of a direct effect of ALA on cardiac myocytes. What is known at the molecular level to support antiarrhythmic effects of omega-3?

It has been suggested that ALA and other long-chain omega-3 fatty acids may prevent arrhythmia through inhibition of sodium and calcium channels (Kang and Leaf, 1995; Xiao et al., 1997, 1998). In an animal study using neonatal cardiac myocytes, it was shown that ALA as well as EPA and DHA inhibited the binding of [*benzoyl*-2,5-^{3}H]batrachotoxinin A 20α-benzoate (a toxin that binds to voltage-sensitive Na^+ channels) to Na^+ channels in a dose-response fashion (Kang and Leaf, 1996b). Sodium channels represent the major class of ion channels that predict cardiac excitability. In addition, ALA and other omega-3 fatty acids may prevent arrhythmia by modulating the conduction of the L-type Ca^{2+} channels (Xiao et al., 1997). The inhibition of calcium channel prevents excessive cytosolic calcium fluctuation (Kang and Leaf, 1996b; Xiao et al., 1997) that is responsible for triggered arrhythmias. It is thought that the inhibition of sodium channels by omega-3 fatty acids results in an prolongation of the inactivated state of the Na^+ channel, and thus to more negative potentials (Xiao et al., 1995).

Nevertheless, data on antiarrhythmic effects of ALA, EPA, and DHA remain inconsistent. Animal experiments have shown that both long-chain and short-chain omega-3 fatty acids can protect against ventricular fibrillation (McLennan et al., 1993; Billman et al., 1994; Billman et al., 1999). In a meta-analysis of 27 animal studies, intervention with fish oil, EPA, and DHA showed protective effects

on ventricular tachycardia and fibrillation, whereas ALA had no antiarrhythmic effects (Matthan et al., 2005). Similarly, studies examining the effects of omega-3 including ALA on sudden cardiac death and fatal myocardial infarction—diseases associated with ventricular fibrillation—have been inconsistent (Burr et al., 1989; Ascherio et al., 1995; Hu et al., 2001; Whelton et al., 2004; Iso et al., 2006). An earlier meta-analysis of randomized trials reported a 30% lower risk of fatal myocardial infarction, 30% lower risk of sudden death, 20% lower risk of total mortality, but only a statistically nonsignificant 20% lower risk of nonfatal myocardial infarction with omega-3 fatty acids compared with placebo (Bucher et al., 2002). These findings were corroborated by another meta-analysis of randomized control trials of omega-3 fatty acids (Yzebe and Lievre, 2004).

In contrast, other randomized control trials of EPA and DHA showed no significant effects of these fatty acids on ventricular premature complexes (Geelen et al., 2005; Matthan et al., 2005) or ventricular fibrillation (Raitt et al., 2005; Brouwer et al., 2006). Similarly, the effects of omega-3 fatty acids on heart rate (Geelen et al., 2005; Mozaffarian et al., 2005) or heart rate variability have been inconsistent (Christensen and Vaupel, 1996; Christensen et al., 1997, 2000, 2001, 2005; Christensen, 2003). These negative studies cast doubt on the validity of the antiarrhythmic hypothesis underlying a lower risk of sudden death and fatal coronary events supposedly conferred by intake of omega-3 fatty acids.

In light of current gaps in physiological mechanisms by which ALA and other long-chain omega-3 fatty acids may influence cardiac arrhythmias, additional studies are needed not only to help understand biological mechanisms of ALA, but also to clarify its effects in humans as a potential antiarrhythmic agent.

V. ALA AND INFLAMMATION

Inflammation plays an important role in the pathogenesis of coronary artery disease and other chronic diseases (Gimbrone et al., 2000; Libby et al., 2002). Activation of the vascular endothelium is an early event in the development of atherosclerosis (Cybulsky and Gimbrone, 1991). Increased expression of proinflammatory cytokines has been found in atherosclerotic lesions, and both macrophages and activated endothelium produce proinflammatory cytokines (Seino et al., 1994; Plutzky, 2001). The circulating cytokines stimulate endothelial expression of cell adhesion molecules—such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and E-selectin. These adhesion molecules mediate monocyte attachment to the endothelium and transmigration into the subendothelial space.

ALA is a precursor of EPA, which can displace arachidonic acid (20:4n-6) and reduce the production of proinflammatory eicosanoids prostaglandin E_2 (PGE_2) and leukotriene B_4. Epidemiologic data from animal and human trials suggest that ALA may indeed exhibit anti-inflammatory properties. In an animal experiment, a diet rich in ALA was shown to decrease PGE_2 and arachidonic acid in the hepatocyte membrane phospholipids and to increase circulating levels of tumor necrosis factor-alpha (TNF-α) (Chavali et al., 1998). In a study of healthy volunteers, the use of flaxseed oil in domestic food preparation for 4 weeks inhibited TNF-α and interleukin (IL)-1 beta production by approximately 30% (Caughey et al., 1996). An average daily intake of 9 g/day of ALA was associated with 36% decrease in thromboxane B_2, 26% decrease in PGE_2, and 20% decrease in IL-1 beta synthesis after 4 weeks of intervention (Mantzioris et al., 2000). In a randomized clinical trial of 76 dyslipidemic men, daily intervention with 15 mL of linseed oil was associated with a statistically significant decrease of C-reactive protein (CRP), serum amyloid A (SAA), and IL-6 concentrations after 3 months of intervention; the corresponding magnitude of decrease was 38% ($p = .0008$), 23.1% ($p = .0001$), and 10.5% ($p = .01$), for CRP, SAA, and IL-6, respectively (Rallidis et al., 2003). Contrary to linseed oil, safflower oil had no effects on inflammatory markers (Rallidis et al., 2003). Other studies have reported a decreased production of inflammatory markers that was attributable to long-chain omega-3 fatty acids (Endres et al., 1989; Kremer et al., 1990; Endres et al., 1993). In addition, other investigators have documented beneficial effects of ALA on inflammatory markers.

Supplementation with 15 mL of flaxseed oil per day for 12 weeks resulted in a statistically significant decrease in SAA, CRP, macrophage colony-stimulating factor, and IL-6 among 50 dyslipidemic men (Paschos et al., 2005). In a randomized crossover design, ALA-rich diet was associated with a significant decrease in CRP, ICAM-1, VCAM-1, and E-selectin compared with an average American diet after 6 weeks of intervention (Zhao et al., 2004). Compared with a control diet, ALA diet was associated with a decrease in CRP levels but had no effects on soluble ICAM-1, IL-6, or IL-10 levels in another randomized double-blind placebo controlled trial of 103 subjects over 2 years (Bemelmans et al., 2004).

Overall, there is sufficient data supporting anti-inflammatory effects of ALA in well-controlled trials, suggesting a favorable role of ALA on coronary heart disease (CHD), possibly mediated through an improved cytokine profile.

VI. ALA AND LIPIDS

Dyslipidemia is a major risk factor for CHD. Elevated low-density lipoprotein (LDL) cholesterol and triglycerides, low-levels of high-density lipoprotein (HDL) cholesterol individually or in combination are established risk factors for CHD (Kannel et al., 1965; Castelli, 1993; Kannel, 1995). Although it is possible that some of the reported effects of ALA on CHD might be mediated through lipid metabolism, limited and inconsistent data are available on the effects of ALA on lipids. Several studies did not find an effect of ALA on triglycerides (Annuzzi et al., 1991; Layne et al., 1996; McManus et al., 1996; Goh et al., 1997; Li et al., 1999; Rallidis et al., 2003; Goyens and Mensink, 2005), HDL (Layne et al., 1996; Li et al., 1999; Goyens and Mensink, 2005), and LDL or total cholesterol (Layne et al., 1996; Li et al., 1999; Bemelmans et al., 2002; Rallidis et al., 2003). In a randomized controlled trial, ALA-rich diet was associated with a significant increase in triglycerides and the ratio of total-to-HDL cholesterol compared with a controlled diet after 2 years of intervention (Bemelmans et al., 2002); in that study, ALA had no effects on total and LDL cholesterol.

In contrast, compared with the control diet, while an ALA-rich diet showed a significant reduction of total and LDL cholesterol, ALA had no effects on HDL and triglycerides after 6 weeks of intervention (Goyens and Mensink, 2005). In another trial, flaxseed oil was associated with a decrease in triglycerides, total, and LDL cholesterol (Wilkinson et al., 2005). Rallidis et al. (2003) reported a significant decrease in HDL-cholesterol; however, the magnitude of effect (1.3 mg/L) was not clinically meaningful. In a randomized crossover trial, an ALA-rich diet was shown to reduce total and LDL cholesterol by 11% each, triglycerides by 18%, HDL by 6%, and apolipoprotein B by 10% compared with an average American diet (Zhao et al., 2004). Data from the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study showed an inverse association between ALA and triglycerides (Djousse et al., 2003b).

In sum, despite inconsistent data on the effects of ALA on major lipids, there is enough support for favorable effects of ALA on triglycerides. Given the partial conversion of ALA to EPA *in vivo* (Li et al., 1999; Karvonen et al., 2002; Wilkinson et al., 2005), the well-established triglycerides lowering effects of fish oil or EPA/DHA lends additional support to possible beneficial effects of ALA on triglycerides. Whether ALA influences total and/or LDL cholesterol awaits confirmation in future studies.

VII. ALA AND BLOOD PRESSURE

Hypertension is highly prevalent in the United States and is a major cause of stroke, kidney failure, and CHD. Though the effects of ALA on CHD have been widely investigated, limited data are available on its effects on blood pressure or hypertension in humans. Existing data on the association between ALA and blood pressure remain inconclusive. In a trial of 33 normotensive men with slightly elevated cholesterol, a diet supplemented with 9.2 g/day of ALA did not influence systolic blood pressure (SBP) after 6 weeks of intervention (Kestin et al., 1990). Similarly, consumption of

a diet rich in ALA for 42 days did not influence SBP or diastolic blood pressure (DBP) among 17 male vegetarians (Li et al., 1999). In a randomized trial, a diet rich in ALA was not associated with SBP or DBP compared with linoleic-rich diet after 2 years of intervention (Bemelmans et al., 2002).

In contrast, other investigators found evidence for an effect of ALA on blood pressure. In a cross-sectional study of 399 male subjects aged 20–78 years, a 1% higher adipose tissue ALA was associated with a 5 mm Hg lower SBP and DBP (Berry and Hirsch, 1986). In the Kuopio Ischemic Heart Disease Risk Factor Study (Salonen et al., 1988), dietary intake of ALA—assessed by a 4-day food record—was inversely associated with SBP and mean blood pressure but not with DBP (Salonen et al., 1988). Rupp et al. (1996) showed that a diet supplemented with ALA lowered SBP by 6 mm Hg after 7 weeks in 28 spontaneous hypertensive rats. Similarly, data from the NHLBI Family Heart Study showed that ALA was inversely associated with SBP but not DBP (Djousse et al., 2005b).

Metabolites of ALA could favorably influence blood pressure. In particular, PGI_3—a metabolite of ALA—is a vasodilator and can influence vascular tone. It is also possible that an increased dietary intake of ALA could decrease the ratio of n-3/n-6 and thus decrease the synthesis of thromboxane A_2 from n-6 fatty acids. Thromboxane A_2 is a vasoconstrictor that could help sustain elevated blood pressure. In animal models, production of $PGF_{2\alpha}$—which stimulates vascular constriction and smooth muscle contraction—was significantly reduced in animals fed diets rich in ALA (Hoffmann et al., 1986). Alternatively, ALA could lower blood pressure through its anti-inflammatory effects (Rallidis et al., 2003; Zhao et al., 2004). Since inflammation plays an important role in the development of atherosclerosis, prevention of such process would preserve the elasticity of arterial walls and prevent an increase in vascular resistance. The anti-inflammatory hypothesis of ALA is consistent with data showing that dietary ALA is inversely related with intima-media thickness of the carotid arteries (Djousse et al., 2003a) as well as calcified atherosclerotic plaque in the coronary arteries (Djousse et al., 2005a). Although the evidence is weak, there is a strong possibility that ALA may favorably influence blood pressure. Additional controlled studies are warranted for confirmation.

VIII. ALA AND CLOTTING SYSTEM

Although an earlier report suggested antithrombotic effects of ALA (Owren, 1965), the majority of existing data provide little evidence to support such hypothesis. Epidemiologic studies have consistently reported no direct effects of ALA on hemostatic factors. In an intervention study, consumption of a diet rich in ALA for 28 days had no effects on plasma fibrinogen, factors VII, prothrombin time, activated partial thromboplastin time, antithrombin III, and plasminogen (Li et al., 1999). In a randomized trial, ALA had no significant effects on fibrinogen or von Willebrand factor after 2 years of intervention (Bemelmans et al., 2002). Others did not find beneficial effects of ALA on hemostatic factors (Freese and Mutanen, 1997; Archer et al., 1998; Allman-Farinelli et al., 1999).

Data on the effects of ALA on platelet aggregation have been inconsistent. Kwon et al. (1991) reported a reduction of collagen-induced platelet aggregation in whole-blood after a 3-week intervention with canola oil. A decreased collagen-induced platelet aggregation was also seen with an increased ratio of ALA to LA (Freese et al., 1994; Allman et al., 1995). However, other investigators could not replicate these effects of ALA on platelet aggregation (Mutanen et al., 1992; Li et al., 1999). Additional data are needed to determine whether ALA has a meaningful influence on hemostatic factors. However, the most promising hypothesis is a direct influence of ALA on platelet aggregation.

IX. ALA AND PROSTATE CANCER

Since 1975, when it was suggested that dietary fat might be associated with death from prostate cancer (Armstrong and Doll, 1975), several studies have examined the role of dietary fats and the risk of prostate cancer. However, results from those studies have been inconsistent (Graham et al., 1983;

Ohno et al., 1988; Mettlin et al., 1989; Giovannucci et al., 1993). Among polyunsaturated fats, ALA has received a lot of attention since the publication of the data from the Health Professionals' Follow-up Study in 1993 suggesting a modest 25% increased risk of prostate cancer with higher intake of ALA (95% CI: 0.82–1.92) (Giovannucci et al., 1993). Current literature on the role of ALA in the development of prostate cancer in not conclusive, as reported findings ranged from suggestive protection to a substantial increased risk of prostate cancer with ALA. Specifically, data on 58,279 subjects from the Netherlands found that higher consumption of ALA derived from food-frequency questionnaire was associated with 24% reduction in risk of prostate cancer; however, this finding was only borderline significant (95% CI: 0.66–1.04; $p = .09$) (Schuurman et al., 1999). In contrast, other cohort studies reported no increased risk of prostate cancer with ALA (Mannisto et al., 2003; Laaksonen et al., 2004; Koralek et al., 2006) or a significant increased risk of prostate cancer with ALA (Harvei et al., 1997; Leitzmann et al., 2004). Results of other observational studies assessing the effects of ALA on prostate cancer have been inconsistent (Andersson et al., 1996; Godley et al., 1996; Meyer et al., 1997; Bairati et al., 1998; Yang et al., 1999; De Stefani et al., 2000; Ramon et al., 2000; Newcomer et al., 2001; Mamalakis et al., 2002). A meta-analysis summarizing 9 of these findings reported a 70% increased risk of prostate cancer with higher ALA (RR = 1.70; 95% CI: 1.12–2.58) (Brouwer et al., 2004). Of note is that this meta-analysis does not include findings from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial that found no association between total or food-specific ALA and total, organ-confined, or advanced prostate cancer in nearly 30,000 subjects (Koralek et al., 2006).

What are possible sources of heterogeneity across studies? Is there a clear biological mechanism by which ALA may increase the risk of prostate cancer? In the absence of a large randomized and placebo-controlled trial of ALA on prostate cancer, shortcomings of noninterventional studies could influence the results and subsequent conclusions. Although many of the published studies controlled for measured confounding, there is no guarantee that unmeasured confounding does not partially or completely account for the heterogeneity across studies. In addition, several studies used food frequency questionnaires to assess ALA intake. Recall bias and/or inaccuracy in reporting consumed foods could lead to biased estimate of ALA. Similarly, studies using plasma or tissue levels of ALA are not exempted from inaccuracy in ALA estimation. ALA content in processed foods is variable, and the ALA sources could be correlated with dietary or lifestyle factors associated with prostate cancer risk. As to the biology, limited experimental studies are available. Perilla oil has been reported to decrease the rate of prostatic intraepithelial cancer in the presence of testosterone (Mori et al., 2001). In contrast, another animal model showed that linseed oil diet did not prevent prostate cancer growth in mice compared with corn oil diet (Connolly et al., 1997). Furthermore, results from *in vitro* studies using prostate cell lines have been inconsistent. Although one study reported an increased growth of the PC-3, LNCaP, and TSU cell lines (Pandalai et al., 1996), others found an inhibition of DU-1145 cells proliferation (du Toit et al., 1996) and increased cell deaths (Motaung et al., 1999) with ALA.

It is possible that reported beneficial effects of ALA on CHD might be offset by its detrimental effects on the development of prostate cancer. However, based on the current level of evidence, we do not have enough data to confirm a major role of ALA on the risk of prostate cancer or to exclude a modest increased risk of prostate cancer. Because CHD remains the leading cause of death and there are enough data to support beneficial effects of ALA on CHD, additional studies to clarify the role of ALA on prostate cancer are necessary before dismissing strategies using ALA to prevent CHD.

X. CONCLUSIONS

Plant-based omega-3 may have beneficial health effects. However, limited data are available to understand underlying biological mechanisms. Specifically, biological effects of ALA are not completely understood. Based on current evidence, there is enough data to support possible antiarrhythmic effects of ALA either directly or through its metabolite EPA. Accumulating evidence also

supports anti-inflammatory role and blood pressure lowering effect of ALA in humans. In addition, ALA may possibly exert beneficial effects on triglycerides either directly or through its metabolite EPA. Currently, there is limited support for a meaningful role of ALA on hemostatic factors. In addition, current data are insufficient to confirm an increased risk of prostate cancer with ALA. Given some of the shortcomings of fish consumption including costs, availability, and taste preference, ALA provides an alternative source of essential omega-3 fatty acids at a relatively low costs. However, because of the slow and limited conversion of ALA to EPA and the limited dada on direct benefits of ALA, it would be premature to recommend the replacement of EPA/DHA with ALA or to assign both types of n-3 fatty acids equivalent roles. Additional studies (particularly randomized placebo-controlled trials where applicable) are needed to confirm biological mechanisms of ALA in humans.

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34 Biological Effects of Conjugated Linoleic Acid

Yung-Sheng Huang, Teruyoshi Yanagita, Koji Nagao, and Kazunori Koba

CONTENTS

I. INTRODUCTION

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid with conjugated double bonds located at positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13, and these double bonds may be of *cis* and *trans* configuration (Eulitz et al., 1999) (Figure 34.1). This group of isomers is synthesized by microbial fermentation (bacteria, *Butyrinvibrio fibrisolvens*) occurring in the gut of ruminant animals (such as sheep and cattle) (Kepler et al., 1966). Thus, CLAs are predominantly found in dairy products and meat from ruminants (Chin et al., 1992). The concentration of CLA in these foods can be as high as 30 mg/g depending on the diet of the ruminant animal, and are generally higher if the animal is grazing on fresh, green pasture than feeding a grain (Dhiman et al., 1999). Interestingly, the highest concentration of CLA when compared with other foods is found in Kangaroo meat (Engelke et al., 2004). Kangaroos are not true ruminant animals, but like ruminants, they ferment feed in their foregut. Other foods including vegetable oils, eggs, seafood, poultry, and pork may also contain CLA but at very low levels. The most abundant isomer in food is *cis* 9, *trans* 11-18:2 (*c*9,*t*11-18:2), followed by *t*10, *c*12-18:2. Evidence indicates that different CLA isomers exert different health effects (Ip et al., 1999; Pariza et al., 2001; Li et al., 2006).

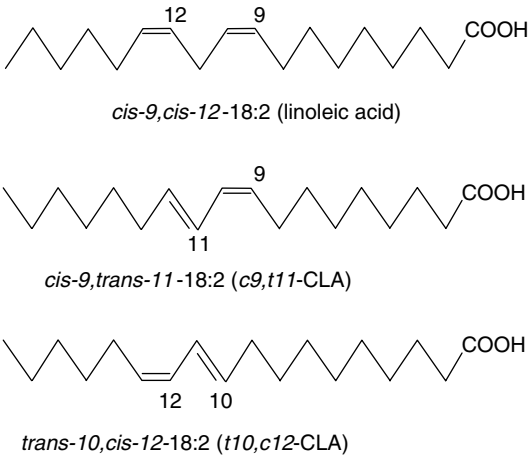

FIGURE 34.1 Chemical structure of conjugated linoleic acids.

II. CLA AND HUMAN HEALTH

Interest in the beneficial effects of CLA on human health started from the original findings by Pariza and his coworkers that red meat contains a component exhibiting the cancer-fighting properties (Ha et al., 1987). Since then, CLA has been the subject of a variety of research, and findings also suggest that CLA possesses other benefits such as increased metabolic rate, decreased abdominal fat, enhanced muscle growth, lowered cholesterol and triglycerides, lowered insulin resistance, reduced food-induced allergic reactions, and enhanced immune system.

A. CLA and Cancer

Early research with animal models demonstrated that CLA found in red meat could interfere with the growth of breast and prostate tumors (Ip et al., 1991, 1994). Since then, more studies have further shown the cancer-fighting potential of CLA. However, most of the evidence on breast as well as skin, liver, and colon cancers has come from animal and human cancer cell lines *in vitro*. Clinical studies were scarce. Nevertheless, recent results from a large French study have indicated that dietary CLA could protect against breast cancer in humans (Chajes et al., 2003).

Evidence indicates that CLA inhibits the initiation and incidence of mammary tumors in rodents. It also had a significant effect on the latency, metastasis, and pulmonary tumor burden of transplantable murine mammary tumors grown in mice (Hubbard et al., 2000). Recently, Ohtsua et al. (2005) demonstrated that CLA decreases cellular proliferation and inhibits NF-κB and activator protein-1 activation in PC3 cancerous prostate epithelial cells.

B. CLA and Weight Management

Obesity has become a major health issue in the affluent societies. In the United States, over 30% of the adult population is obese. During the past decade, the percent of obese people has doubled (Flegal et al., 2002). CLA is one of nutrients that has been shown to be able to reduce body fat and to increase lean body mass (LBM) in pigs (Dugan et al., 1997), mice (Park et al., 1997; DeLany et al., 1999; Pariza et al., 2000), and rats and chicks (Martin et al., 2000; Pariza et al., 2000; Wang et al., 2003). In humans, CLA shows a tendency to reduce body fat, particularly abdominal fat, change serum total lipids, and decrease whole body glucose uptake (Blankson et al., 2000; Smedman and Vessby, 2001; Thom et al., 2001).

On the other hand, there are studies failed to confirm this benefit (West et al., 1998; Blankson et al., 2000; Riserus et al., 2001; Smedman and Vessby, 2001; Thom et al., 2001; Kreider et al., 2002; Kamphuis et al., 2003; Larsen et al., 2003; Gaullier et al., 2004; Malpuech-Brugere et al., 2004; Wang and Jones, 2004). Atkinson (1999) did not find any differences between the CLA group (2.7 g/day) and the placebo group in a 6 months placebo-controlled, randomized, double-lind study in obese volunteers. Zambell et al. (2000) have found that CLA supplementation (3 g/day) had no effect on body composition and energy expenditure in 17 healthy, adult women for 64 days following a baseline period of 30 days.

C. CLA and Heart Health and Arteriosclerosis

One of the major risk factors for coronary heart disease is high levels of cholesterol, particularly low-density lipoproteins (LDL) cholesterol (or "bad" cholesterol); because cholesterol over time can form plaque in arteries, narrowing them, reducing heart's blood supply. Animals fed a CLA-containing diet had a significantly reduced LDL and total cholesterol and triglyceride levels in blood (Kritchevsky et al., 2000), and reduced cholesterol concentration in liver (Sakono et al., 1999). Results from studies with rabbits and hamsters have indicated that CLA can lower plasma lipoproteins and early aortic atherosclerosis in rabbits and hamsters (Lee et al., 1994; Nicolosi et al., 1997). In human, a small double-blind trial has also found that CLA could reduce blood cholesterol levels in healthy human subjects (Noone et al., 2002). Among different CLA isomers, the *t*10,*c*12-CLA as compared to linoleic acid has been shown to be more effective in lowering the concentration of total cholesterol and triglyceride, whereas raising the concentration of serum HDL-cholesterol (Akahoshi et al., 2003).

The hypotensive properties of CLA have also been reported. Feeding of CLA mixture and the *t*10,*c*12-CLA isomer has been shown to prevent the development of obesity-induced hypertension in obese, diabetic Zucker rats and obese OLETF rats (Nagao et al., 2003a,b), and prevent the development of essential hypertension in nonobese spontaneously hypertensive rats (Inoue et al., 2004).

In addition, CLAs (both *c*9,*t*11- and *t*10,*c*12-isomers) have been shown to inhibit the arachidonic acid- and collagen-induced platelet aggregation, and the formation of proaggregatory cyclooxygenase product, TXA_2 (Truitt et al., 1999).

D. CLA and Diabetes

The incidence of type II diabetes in the affluent societies is increasing dramatically. There is evidence that CLA may have the ability to normalize glucose metabolism. In studies with obese, diabetic rats, feeding of CLA diet normalized impaired glucose tolerance, and the effect of CLA was similar to that of the pharmaceutical agent troglitazone (Houseknecht et al., 1998; Belury et al., 2003). In another study, dietary supplementation with CLA also lowered body mass, levels of blood sugar, triglycerides, and insulin, and improved glucose utilization in diabetes (Belury et al., 2003).

The beneficial effect of CLA are attributable to the specific action of the *t*10,*c*12-isomer (Ryder et al., 2001). *In vitro* studies showed that the *t*10,*c*12-isomer but not the *c*9,*t*11-isomer lowered body weight but stimulated peroxisome proliferator-activated receptor-γ (PPAR-γ)-mediated reporter gene activity. The activation of PPAR-γ may play some role in the putative antidiabetic activity of CLA (Houseknecht et al., 1998).

However, there is also growing evidence that CLA may *worsen* blood sugar control. In overweight people without diabetes, CLA might promote insulin resistance and thus decrease insulin sensitivity, creating a prediabetic state (Riserus et al., 2002; Larsen et al., 2003; Moloney et al., 2004; Riserus et al., 2004).

E. CLA and Immune Function and Inflammatory Response

The immune system protects the body by fighting disease. CLA can enhance the immune system. Feeding CLA to chicks provided partial protection against the catabolic effects of endotoxin

(Cook et al., 1993). CLA also supports the production of key immune system cells and the inflammation response system. When the immune system is challenged by antigen or polyclonal T cell mitogens, such as concanavalin A and phytohemagglutinin, and their production of interleukin-2, feeding CLA could improve the age-related decrease in the ability of T cells to proliferation in mouse (Hayek et al., 1999). More, feeding CLA could significantly increase the splenic levels of immunoglobulin A (IgA), IgG, and IgM in mice (Sugano et al., 1997; Yamasaki et al., 2000).

F. CLA and Bone Health

Recently, effects of CLA on bone have been investigated (Cook et al., 1997; Watkins and Seifert, 2000; Thiel-Cooper et al., 2001; Doyle et al., 2005; Banu et al., 2006). *In vitro* studies using MC3T3-E1 osteoblast-like cells showed that CLA increased levels of osteocalcin, alkaline phosphatase activity, and calcium absorption (Fernandes et al., 1986). *In vivo* studies using young chicken, mice, and pigs have shown that in the presence of CLA there is an increase in bone mass. However, in young rats, CLA decreased bone formation rates (Ammann et al., 1992; Griffin et al., 1993), but when CLA was given along with n-6 PUFA, bone formation rates increased (Lu et al., 1994). In humans, a study that tested the effects of CLA supplementation on the biochemical markers of bone metabolism has reported that in adult men CLA supplementation did not affect markers of bone metabolism (Rahman et al., 2003). A recent study on the effects of CLA on postmenopausal women has concluded that dietary intake of CLA may benefit bone mineral density (Brownbill et al., 2005).

The mechanism by which CLA exerts its effect on bone is still not very clear, although a few theories have been reported recently. CLA has been shown to enhance bone mineralization (Kelly et al., 2003; Kelly and Cashman, 2004). In the presence of PUFA, CLA has been shown to modulate the action and expression of COX-2 enzyme, thereby alter the prostaglandin-dependent bone resorption (Li and Watkins, 1998; Li et al., 1999). Dietary CLA is also known to reduce both proinflammatory IL-1 (Thomas and Burguera, 2002) and IL-6 production (Burguera et al., 2001). Studies have reported that IL-1 and IL-6 induce bone resorption by stimulating the recruitment and formation of osteoclasts, and thereby releasing calcium from bone (Kang and Pariza, 2001; Reseland and Gordeladze, 2002).

III. POSSIBLE MECHANISMS OF CLA EFFECTS

A. Effect of CLA on Fatty Acid Oxidation Related Enzymes

The mechanism that underlines the CLA-induced changes on body fat content has been related to two aspects: (1) expedite the body's fat metabolism by increasing lipolysis in adipocyte and (2) enhance fatty acid oxidation in both adipocytes and skeletal muscle cells (Park et al., 1999; Pariza et al., 2000).

In mice, dietary CLA significantly increased total carnitine palmitoyl transferase activity in both fat pad and skeletal muscle (Park et al., 1999), and the hormone sensitive lipase activity in adipocytes (Park et al., 1997). In 3T3-L1 adipocytes, CLA inhibited the heparin-releasable lipoprotein lipase (LPL) activity and intracellular concentration on triacylglycerol and glycerol (Park et al., 1997).

B. Effect of CLA on Fatty Acid and Eicosanoid Synthesis

CLA is known to affect fatty acid and eicosanoid metabolism (Figure 34.2). As CLA can be readily incorporated into membrane phospholipids, the effect can be exerted through replacing arachidonic acid by CLA in cell membrane and reducing the availability of arachidonic acid for eicosanoid synthesis (Liu and Belury, 1998; Sugano et al., 1998; Banni et al., 1999). Belury and Kempa-Steczko (1997) have demonstrated decreased arachidonic acid levels in hepatic neutral lipids of rats fed CLA. CLA can also compete with fatty acid metabolic enzymes and thus affect the synthesis of arachidonic acid (Sebedo et al., 1997; Sugano et al., 1999). Feeding CLA has been shown to increase

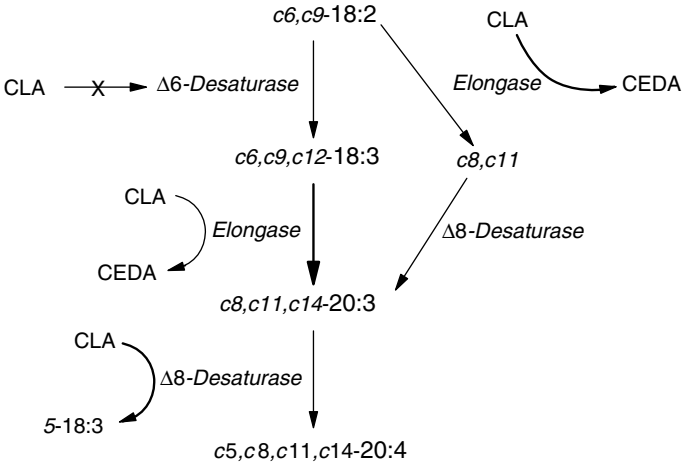

FIGURE 34.2 Effect of CLA on n-6 fatty acid metabolism. CLA: conjugated linoleic acid; CEDA: conjugated eicosadienoic acid; Δ5-18:3: *c*5,*c*9,*c*12-18:3.

the proportions of stearic, docosatetraenoic, and docosapentaenoic acids in serum lipids and thrombocytes, while proportions of palmitic, oleic, and dihomo-gamma-linolenic acids decreased, suggesting a decrease in the estimated Δ-6 and Δ-9 and an increase in the Δ-5 desaturase activities (Smedman and Vessby, 2001). Recently in a transformed yeast system, Chuang et al. (2001a,b, 2004) have demonstrated that CLA inhibited essential fatty acid metabolic enzymes such as Δ-6-desaturase, Δ-5-desaturase, and elongase. Eder et al. (2002) have demonstrated that *t*10,*c*12-CLA suppressed the Δ6-desaturase in HepG2 cell.

C. CLA as Antioxidant

It has been shown in *in vitro* and *in vivo* studies that CLA is an effective antioxidant (Ha et al., 1987; Ip et al., 1991). Indeed, comparative studies have shown that CLA is as effective as butylated hydroxytoluene (BHT), more potent than α-tocopherol, and approximately two times more powerful an antioxidant than beta-carotene (Ha et al., 1990; MacDonald, 2000). In the body CLA can be taken up by phospholipids, where CLA serves as one of the structural components of cell membranes and protect cell from the detrimental effect of peroxides. Because numerous studies have demonstrated that oxidative reactions are associated with the development of cancer and atherosclerosis, the ability of CLA to reduce the harmful effects of these oxidative reactions could be attributed to its health beneficial effect.

D. Effect of CLA on Gene Expression

CLA interacts with dozens of genes, but not all consequences are known. The ability of CLA to modulate lipid metabolism appears to be a pivotal mechanism of CLA's beneficial effects in mice and rats. The effect of CLA on body composition changes observed in animals seems to be associated mainly with the *t*10,*c*12-CLA isomer (Park et al., 1999). The *t*10,*c*12-isomer decreased the expression of stearoyl-CoA desaturase mRNA in mouse liver and adipocytes (Lee et al., 1998; Choi et al., 2000). Stearoyl-CoA desaturase activity is a key enzyme in lipogenesis, and inhibition of this enzyme activity depresses fat synthesis.

Dietary CLA induces the expression of genes dependent in part on the transcription factor, peroxisome proliferator-activated receptor (PPAR). Several CLA isomers are high-affinity ligands and activators for PPAR (Moya-Camarena and Belury, 1999).

IV. OTHER CONJUGATED FATTY ACIDS

There are also other types of conjugated fatty acids in some plant seed oils. Punicic acid (*c*9,*t*11, *c*13-conjugated linolenic acid, CLN) in pomegranate seed oil, α-eleostearic acid (*c*9,*t*11,*t*13-CLN) in bitter gourd oil and tung seed oil, catalpic acid (*t*8,*t*10,*c*12-CLN) in pot marigold seed oil (Suzuki et al., 2001; Kohno et al., 2002). It has been reported that several seaweeds contain conjugated trienoic and tetraenoic fatty acids. *Ptilota filicna* contains *t*5,*t*7,*t*9,*c*14,*c*17-conjugated eicosapentaenoic acid (CEPA) and *c*5,*t*7,*t*9,*c*14,*c*17-CEPA (Lopez and Gerwick, 1987). *Bossiella orbigniana* contains *c*5,*c*8,*t*10,*t*12,*c*14-CEPA (Burgess et al., 1991). Green seaweed *Anadyomene stellata* contains stella-heptaenoic acid, *c*4,*c*7,*t*9,*t*11,*c*13,*c*16,*c*19-conjugated docosaheptaenoic acid (Mikhailoba et al., 1995).

A. Anticarcinogenic Effect

Results from the *in vivo* study indicate that dietary CLN inhibits azoxymethane-induced colonic aberrant crypt foci in rats (Kohno et al., 2002). There are also several reports indicating the cytotoxic effects of CLN on various human cancer cell lines suggesting that conjugated trienes are stronger anticarcinogens than conjugated dienes (Igarashi and Miyazawa, 2000a; Suzuki et al., 2001). The cytotoxic actions of conjugated EPA and conjugated DHA were demonstrated in several cancer cell lines including colorectal, hepatoma, lung, breast, and stomach (Igarashi and Miyagawa, 2000b; Tsujita-Kyutoku etal., 2004; Tsuzuki et al., 2004).

B. Antiobese Effect

The antiobese effects of CLN have also been demonstrated. Dietary supplementation of CLN reduced perirenal adipose tissue weight by increasing triglyceride lipolysis, enhancing hepatic fatty acid beta-oxidation and reducing hepatic fatty acid synthesis in rats (Koba et al., 2002, 2006), in chickens, obese rats, and human liver-derived cells (Lee et al., 2002; Arao et al., 2004a,b).

V. SAFETY AND DOSAGE OF CLA

Evidence shows that for an individual with a body weight of 70 kg to reduce body fat mass is about 3.4 g (Blankson et al., 2000). Results from a randomized, double-blind, placebo-controlled trial shows that CLA supplementation (3.4 g/day in triglyceride form) for 24 months in healthy, overweight adults was well tolerated, and that CLA decreases body fat mass in overweight humans, and may help maintain initial reductions in body fat mass and weight in the long term (Gaullier et al., 2004). Thus, all evidence indicates that dietary supplementation of CLA at this dosage does not have any adverse effects. There are concerns regarding the use of CLA by nursing mothers. Results from a double-blind, placebo-controlled study indicates that use of CLA by the lactating women reduces the fat content of breast milk (Masters et al., 2002). Since infants require the fat in breast milk for energy and proper growth and development, one should be prudent to recommend CLA supplement to the nursing mothers (Bee, 2000).

CLA occurs naturally in foods such as milk, cheese, beef, and lamb as well as many processed foods (Chin et al., 1992), and whole milk usually contains less than 2 mg/g of CLA in milk fat, most people consume less than 1 g/day from these food sources (Ip et al., 1994; Parodi, 1994; Herbal et al., 1998). In order to get enough CLA from the diet one would require considerable intake of these types of foods. This approach is not only impractical, but also having a seriously negative impact on one's metabolism due to the high intake of calories. Supplemental CLA is usually derived from synthesis.

VI. CONCLUSION

CLA appears to be a generally safe nutritional substance (Whigham et al., 2004). CLA exerts many beneficial effects, such as anticarcinogenic, antiobese, antiatherogenic, anti-inflammatory, and so forth.

However, not all CLA isomers are equal; since specific CLA isomers exhibit different functions (Yotsumoto et al., 1999; Evans et al., 2002; Hargrave et al., 2002; Rodriguez et al., 2002; Nagao et al., 2003c; Yanagita et al., 2003; Jaudszus et al., 2005). The availability of different isomer-specific CLA products may provide a more efficient approach to the CLA supplementation.

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35 The Role of Omega-3 Polyunsaturated Fatty Acids in Food Intake and Energy Homeostasis

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CONTENTS

I. INTRODUCTION

Obesity, the excess accumulation of adipose tissue, is a major health problem worldwide. It is associated with more than 30 medical conditions, including type 2 diabetes and cardiovascular disease, and is a leading cause of unnecessary deaths. The control of body energy homeostasis is fundamental to mammalian life as well as to the problem of obesity. This chapter will describe the role of the omega-3 polyunsaturated fatty acids (n-3 PUFAs) in modulating some of the mechanisms that control body energy homeostasis. The chapter is divided into three major sections. The first section is dedicated to the dietary intake and metabolism of the n-3 PUFAs, as well as the interrelated n-6 PUFAs. The second section describes the major mechanisms responsible for the maintenance of body energy homeostasis. Then, in the third section, evidence regarding the role of n-3 PUFAs in these homeostatic mechanisms will be described, including evidence regarding the role of n-3 PUFAs in obesity and cancer cachexia.

II. OMEGA-3 POLYUNSATURATED FATTY ACIDS

A. Supply and Metabolism of Polyunsaturated Fatty Acids

Mammals do not produce Δ12 and Δ15 desaturase enzymes and therefore cannot produce either linoleic acid (LA) or α-linolenic acid (ALA), precursors of the n-6 and n-3 families, respectively, *de novo*. ALA is metabolized, in a series of desaturation and elongation reactions, to produce long-chain n-3 PUFA, the most prevalent of which are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This metabolism occurs primarily in the liver and cerebral microvasculature of the blood brain barrier, but also by astrocytes and the cerebral endothelium (Moore et al., 1990). EPA is further metabolized by the cyclooxygenase and lipoxygenase pathways to produce the eicosanoids (prostaglandins, thromboxanes, and leukotrienes) that are potent, short-acting, local hormones, or second messengers. The eicosanoids are derived from EPA as well as from arachidonic acid (20:4n-6). These fatty acids are liberated from the cell membrane by the phospholipase-A_2. Phospholipase-A_2 has equal affinity for both EPA and arachidonic acid, therefore, the proportion of n-3:n-6 hydrolyzed by phospholipase-A_2 is determined by the tissue n-3:n-6 profile (Calder, 1996). DHA is primarily sequestered into the phospholipid membranes of cells within the brain and central nervous system. The predominant PUFA in the brain is DHA that comprises up to 30% of the phospholipid fatty acids in the grey matter (Sinclair, 1975). DHA is a determinant in the regulation of synaptic transmission, ultrastructural changes in neural synapses and changes in dopamine and NMDA receptor function (for reviews, see Fenton et al., 2000; Kurlack and Stephenson, 1999). Furthermore, n-3 PUFA deficiency early in life may result in irreversible damage to biochemical processes in the central nervous system (Uauy et al., 2000).

The metabolism of n-3 and n-6 PUFA is interlinked as they compete for enzymes and metabolic substrates at all levels. Therefore, relative as well as absolute dietary intake is relevant in the determination of tissue n-3 and n-6 fatty acid levels. The Western diet typically contains high levels of n-6 fatty acids, as these are components of most animal and vegetable fats. Dietary sources of n-3 PUFA are varied. The most plentiful sources are fish, shellfish, and marine products, which contain large amounts of EPA and DHA. Certain plant oils, such as rapeseed (canola), soybean, and perilla contain large amounts of ALA. Although beef and lamb do contain n-3 PUFAs, both the absolute content and the n-3:n-6 ratio of PUFAs within these meats is low (Crawford and Sinclair, 1972; Sinclair, 1975).

Burr and Burr highlighted the importance of certain fats in the human diet and demonstrated that dietary deprivation of the essential fatty acids was deleterious to health (Burr and Burr, 1929). They concluded that the n-6 PUFAs were most important to function in mammals because their omission resulted in overt systemic dysfunction. Conversely, it has been shown that deprivation of n-3 PUFAs results in subtle dysfunction across a range of behavioral and physiological modalities.

The n-3 PUFAs are important components of cell membranes throughout the body, as they are incorporated into the phospholipids that form cell membranes. Each phospholipid molecule is composed of a headgroup to which fatty acid esters are bound. There are two binding positions, termed as *sn*-1 and *sn*-2. The acyl chains that bind to these headgroups interact with other chains in neighboring phospholipid molecules within the bilayer and the level of chain interaction determines the biophysical properties of the membrane. The *sn*-1 position is usually occupied by a saturated fatty acid; the *sn*-2 position is usually occupied by a relatively unsaturated fatty acid chain.

Fleischer and Rouser (1965) suggested that lipid bilayers composed mainly of saturated fat (such as cholesterol) are relatively stable due to low acyl chain interactions. These stable membranes have higher viscosity and produce rigid cell membranes. An example of cells with this type of membrane is myelinated nerve fibers. Conversely, membranes composed of phospholipids with highly unsaturated acyl chains are less rigid, owing to interactions between such bulky acyl chains. This state of membrane disorder produces a physical fluidity and it is proposed that such membranes offer minimal hindrance to the function of proteins (e.g., receptors or channels) that lie within the membrane. Thus, Fleischer and Rouser (1965) suggest that metabolically active membranes,

such as those found in neuron cell bodies, are composed of phospholipids-containing unsaturated *sn*-2 chains. Work by Brown, Dratz, and Litman supports this notion, as recombinant membranes that comprise higher levels of DHA mimic biological activity more closely when studied *in vitro* (Brown, 1994; Dratz and Holte, 1993; Littman and Mitchell, 1996).

Western diets typically contain low levels of n-3 PUFAs (Simopoulos et al., 2000). Furthermore, relative to the diet of early man, today's diet is not only higher in saturated fats but has an altered ratio of the two major groups of PUFAs. Modern-day humans are, most probably, descendants of coastal dwelling hunter-gatherers, with a primary diet of fish, shellfish, and plant matter. This omnivorous, and opportunistic, diet was varied and most probably low in saturated fat. The ratio of n-6 to n-3 fatty acids during the early part of human evolution was close to 1, whereas today the ratio is 10:1 or more (Eaton et al., 1998; Simopoulos, 1998). A recent study in Australia (A. J. Sinclair, unpublished data) indicates that DHA intake is 0.1% of fat intake or about 100 mg/day. The recommended intake is 0.5%–1% (Simopoulos et al., 2000). Furthermore, recent data from our group (A. J. Sinclair, unpublished data) has shown that reduced DHA levels in neural tissue occur in animals that are maintained on high saturated fat diets similar to that of a Western diet.

B. Omega-3 PUFA in the Brain and Central Nervous System

A significant proportion of neural development occurs postnatally, hence, early nutrition is vital to support optimal neural development (Connor et al., 1992). The large demand for DHA during pregnancy and early development is usually met via the placenta and then by maternal milk (Neuringer et al., 1986; van Houwelingen et al., 1996). Maternal dietary LA and ALA serve as precursors for n-3 and n-6 long-chain PUFAs by the maternal liver. The ability to obtain sufficient amounts of DHA to ensure normal growth and development, however, may be compromised by the decreased ability to transform precursor into DHA during pregnancy and lactation (van Houwelingen et al., 1996). There is a progressive enrichment of DHA within circulating lipids of the fetus during the third trimester, a time when fetal demands for vascular and neural growth are greatest (Clandinin et al., 1980; van Houwelingen et al., 1996). DHA is concentrated in synapses and most synaptogenesis occurs during perinatal life.

III. BODY ENERGY HOMEOSTASIS

Many factors are involved in the control of food intake. Some of the most important factors controlling the amount of food that we eat include environmental factors such as food availability, the characteristics of the food itself, for example, smell, taste, our eating habits, learned preferences and aversions, as well as other psychological and social factors including our lifestyle. Although these psychosocial factors are extremely important to the food intake patterns of humans, this review will concentrate only on the physiological factors, primarily, the role of fat in food intake.

The regulation of body energy homeostasis, the balance between energy input and expenditure, is crucial to an animal's growth and survival. Complex central nervous system mechanisms have evolved to ensure that the animal's needs and its behavioral and physiological responses are coordinated. A starving animal, motivated by hunger, seeks out and ingests food it encounters. Simultaneously, mechanisms act to conserve body energy when possible, for example, body temperature and activity may be decreased. In the long term, the interplay between the various environmental and physiological factors controlling energy input and expenditure determines the individual's body weight.

The importance of the hypothalamic brain area to the control of food intake and body weight has been known for at least 50–60 years (Anand and Brobeck, 1951; Hetherington and Ranson, 1940). Initially, evidence demonstrated the importance of the lateral hypothalamus (LH) and the ventromedial nucleus (VMN) of the hypothalamus. Lesion of the LH causes animals to lose body weight through decreased food intake and increased body temperature. Experiments suggested that the lesion of the LH reduced the animal's body weight "set point." That is, when the animal's body

weight was decreased by food deprivation prior to lesion of the LH, the normally observed under-eating did not occur after the lesion but rather the new low body weight was maintained. In contrast, lesion of the VMN causes animals to increase food intake and gain body weight. When an animal's body weight was increased prior to lesion of the VMN, the normally observed over-eating did not occur after lesion but rather the new elevated body weight was maintained. These results suggest that the "set point" is determined, at least in part, by hypothalamic mechanisms (Friedman and Stricker, 1976; Hoebel and Teitelbaum, 1966; Keesey and Hirvonen, 1997; Keesey and Powley, 1975; Keesey et al., 1979; Mrosovsky and Powley, 1977). Clearly, however, the "set point" can be influenced by various environmental and physiological factors, as evidenced by obesity. Interestingly, even "obese" humans appear to have a body weight set point, for example, following a weight loss regimen, most obese patients regain all of their lost weight within 9 years (Johnson and Drenick, 1977).

Evidence consistent with the idea of a body weight "set point" is observed in animals with normal body weight as well. For example, like others (Keesey and Hirvonen, 1997), we have observed that when animals are given free access to food after a period of food restriction, the loss in body weight is restored. Interestingly, over-eating may only be evident during the first week following the return of free food access.

Today, other brain areas are known to be important to the control of food intake and body weight. Lesion of the arcuate nucleus (ARC) or PVN result in obesity (Bray, 1993; Dallman et al., 1993; Holzwarth-McBride et al., 1976; Kirchgessner and Sclafani, 1988; Rothwell, 1990). One of the important factors contributing to obesity appears to be an increase in the activity of the hypothalamic-pituitary-adrenal axis. Adrenalectomy inhibits or prevents the development of obesity subsequent to lesion of the ARC or PVN (Dallman et al., 1993). Other brain areas, such as the amygdala, area postrema (AP), NTS, parabrachial nucleus (PBN), DMV, and frontal cortex are also involved (Bray and York, 1998; Dallman et al., 1993; Holzwarth-McBride et al., 1976; King et al., 1999; Kirchgessner and Sclafani, 1988; Ritter and Hutton, 1995; Woods et al., 1998). Disruption of the neural pathways between PVN, NTS, and DMV simulate aspects of the obesity caused by lesion of the VMN (Kirchgessner and Sclafani, 1988). Thus, these results are consistent with neural networks rather than neural centres controlling food intake and body weight. Of course, although many key brain areas have been identified, there is much to be elucidated in terms of determining the relevant pathways.

Glucose and fat are the two major sources of energy for the body. Early theories proposed that food intake was controlled by central mechanisms sensing changes in the level or utilization of these fuels. Mayer (1952, 1955) suggested that food intake was controlled by blood glucose levels or, by levels of glucose utilization. Glucostats in the hypothalamus were thought to sense the changes in glucose levels. Some evidence suggests that a small decrease in plasma glucose precedes hunger and food intake in humans (Campfield and Smith, 1986).

Another theory, the "lipostatic" theory, proposed by Kennedy (1950), suggested that food intake varied so as to maintain body fat stores, that is, the "set point." Changes in body fat stores, reflected in signals dependent on the size of those stores (e.g., blood levels of fatty acids), controlled food intake. Indeed, it has been shown that animals with lesion of the LH and VMN have pronounced disturbances in fat metabolism (Friedman and Stricker, 1976). Animals with a VMN lesion show increased fat storage such that circulating levels of the metabolic products of fat metabolism are decreased. It has been proposed (Friedman and Stricker, 1976) that this decreased availability of the metabolic products of fat metabolism stimulates food intake.

Presently, in line with the idea that fat metabolism is crucial to body energy homeostasis, evidence suggests that peptides produced in the body and correlated with body fat mass, are essential to the long-term control of food intake and body weight. Leptin is one such peptide. Leptin is the protein product of the *ob* gene. It is primarily produced in white adipose tissue and the plasma level of leptin accurately reflects total body adiposity (Friedman, 1997; Porte et al., 1998). It enters the brain from the circulation by a saturable transport mechanism (Friedman, 1997; Seeley and

Schwartz, 1999; Woods et al., 1998). Leptin expression is stimulated by cortisol, insulin, and fasting, and is attenuated by long-chain fatty acids (Houseknecht et al., 1998; Trayhurn et al., 1999). It has been shown that central administration of leptin decreases body weight, primarily by causing the loss of fat (Chen et al., 1996; Halaas et al., 1997). Interestingly, while decreased food intake normally causes a reduction in energy utilization, this does not occur during administration of leptin. Indeed, body weight decreases during administration of leptin even when food intake has returned to normal (Halaas et al., 1997).

The decrease in food intake caused by leptin is mediated by its actions on central peptidergic systems involved in food intake. For example, leptin stimulates peptidergic systems that inhibit food intake. Leptin stimulates corticotropin releasing factor (CRF) release in the PVN. CRF and urocortin, a newly discovered member of the CRF family, decrease food intake and increase energy expenditure (Richard, 1993; Rothwell, 1990; Weisinger et al., 2000). The decreased food intake caused by CRF/urocortin is mediated by CRF-R2 receptors (Smagin et al., 1998; Spina et al., 1996), found in the VMN, amygdala, and PVN (Baram et al., 1997; Eghbal-Ahmadi et al., 1997; Gray and Bingaman, 1996). It has been shown that the influence of leptin on food intake is blocked by a CRF receptor antagonist (Gardner et al., 1998). CRF is thought to decrease food intake by its influence on the release of another peptide that inhibits food intake, that is, oxytocin (Olson et al., 1991), or by its ability to decrease gastric emptying (Tache et al., 1987). The increase in energy expenditure caused by CRF/urocortin has been attributed to their activation of the sympathetic nervous system (Arase et al., 1988; Rothwell, 1990; Smagin et al., 1998; Spina et al., 1996). The influences of leptin and CRF on food intake and body weight are clearly very similar and it seems likely that the actions of leptin are, at least in part, mediated via a system involving CRF and its receptors.

In addition, leptin acts to inhibit the activity of peptidergic systems that stimulate food intake. Leptin receptors have been found on many of the peptidergic neurons thought to increase food intake and body weight. Such neurons are found throughout the brain and include the melanin concentrating hormone (MCH)-containing neurons of the LH; the neuropeptide Y (NPY)-containing neurons of the ARC and VMN, and the galanin-containing neurons of the PVN (Hakansson et al., 1999; Meister, 2000). MCH is synthesized in the LH- and MCH-containing neurons project to various brain areas involved in energy balance. Stimulation of MCH pathways causes increased food intake (Chambers et al., 1999). NPY is found in neurons in the ARC that project to the PVN, LH, and NTS. Stimulation of NPY pathways is followed by increased food intake, lipogenesis, and decreased sympathetic nervous system activity, and energy expenditure in brown fat (Billington and Levine, 1992; Grundemar and Hakanson, 1994; Levine and Billington, 1997; Richard, 1995; White, 1993). Stimulation of food intake by NPY is thought to be mediated by neurons expressing MCH, via an ARC-LH pathway (Broberger et al., 1998). NPY neurons appear to be more responsive to changes in carbohydrate metabolism than to fat metabolism (Leibowitz, 1995). Galanin-expressing neurons and neurons with galanin receptors are found in many brain areas involved in food intake such as LH, PVN, and amygdala (Merchenthaler et al., 1993). Activation of galaninergic neurons that project from the anterior PVN to the median eminence (ME) causes increased intake and accumulation of fat (Leibowitz, 1995; Leibowitz and Alexander, 1998). Interestingly, not only does leptin prevent food intake caused by NPY, MCH, and galanin but also leptin appears to block the formation of these peptides, that is, leptin decreases hypothalamic NPY, MCH, and galanin gene expression (Sahu, 1998). The decrease in MCH caused by leptin is, at least in part, mediated by α-melanocyte stimulating hormone (αMSH). Leptin increases expression of αMSH, a peptide that acts on neurons with melanocortin-4 (MC-4) receptors, and stimulation of these receptors decreases MCH (Broberger, 1999; Hanada et al., 2000). αMSH may also be involved in the decrease in food intake caused by CRF (Rothwell, 1990).

Leptin also influences body weight by interacting with peptides that control short-term food intake, that is, peptides that affect meal size and/or meal frequency. Secretion of the gut-peptide cholecystokinin (CCK) produces short-term satiety. However, while CCK can decrease meal size, meal frequency is increased such that body weight is not altered (West et al., 1984). One of the

mechanisms by which CCK decreases food intake is by decreasing gastric emptying (Moran and McHugh, 1982). Interestingly, it has been shown that when leptin (given centrally) and CCK (given peripherally) are administered simultaneously, body weight loss is greater than that observed when leptin is given alone (Matson et al., 2000). The synergy between leptin and CCK cannot be explained by decreased food intake alone. Presumably, increased energy expenditure is involved. It should be noted that most of the peptides that influence food intake also influence, inversely, the activity of the sympathetic nervous system controlling thermogenesis in the brown adipose tissue. For example, NPY increases food intake and decreases activity of the sympathetic nervous system while CRF decreases food intake and increases activity of the sympathetic nervous system (Bray, 1993).

IV. INFLUENCE OF n-3 PUFAS ON BODY ENERGY HOMEOSTASIS

A. Food Intake, Body Weight, and Body Fat (Figure 35.1)

Several studies have reported that diets containing n-3 PUFAs can decrease body weight or body weight gain. McGahon et al. (1999) reported that after 22 months on a long-chain n-3 PUFA (DHA) or short-chain n-6 diet (corn oil), male Wistar rats maintained on the DHA diet were significantly lighter. Long-chain n-3 PUFA diets were also observed to decrease body weight (but not food intake) of diabetic (streptozotocin-treated) rats. Such diets increased responsiveness of muscle glucose transport to insulin (Sohal et al., 1992). Cunnane compared a diet high in n-6 PUFA (evening primrose oil = 18:2n-6 and 18:3n-6) with a diet high in n-3 PUFA (cod liver oil, EPA, and DHA). The diets were matched for caloric content whereby fat provided 20% of energy. No differences in food intake was observed in either lean (ln/ln) or obese (ob/ob) mice. However, body weight of obese mice on the n-3 PUFA diet was less than those obese mice on the n-6 PUFA diet (Cunnane et al., 1986). After 6 weeks of treatment, body weight and body fat of Swiss albino mice maintained on a high-fat diet (40.8% of energy) that contained canola oil, a good source of ALA, was less than that of mice maintained on the high-fat diet that contained beef fat and was similar to that of mice maintained on a low-fat diet (Bell et al., 1997). In C57 mice that received 60% of their total calories from fat, body weight after 19 weeks was lowest when the fat source was fish oil while diets containing perilla or canola oil were less effective (Ikemoto et al., 1996). In a study where C57 mice

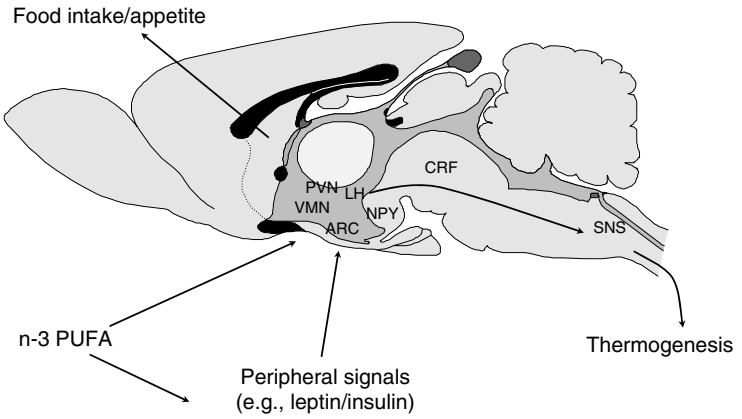

FIGURE 35.1 Model showing mechanisms controlling body energy homeostasis. Major brain nuclei and central and peripheral pathways are shown. LH, lateral hypothalamus; VMN, ventromedial nucleus of the hypothalamus; ARC, arcuate nucleus; PVN, paraventricular nucleus of the hypothalamus; SNS, sympathetic nervous system; n-3 PUFAs, omega-3 polyunsaturated fatty acids; CRF, corticotropin releasing factor; NPY, neuropeptide Y.

received 58% of calories as either saturated fat or long-chain n-3 PUFA (Wang et al., 1999), animals maintained on the high saturated fat diet gained the most body weight. In animals fed the high saturated fat diet, neural activity, as measured by an increase in fos immunoreactivity, was observed to be increased in the LH ("hunger" area) and decreased in the VMN ("satiety" area). Conversely, the activity in the VMN but not the LH was increased in the fish oil fed group. These results are consistent with the proposition that relative to diets with long-chain n-3 PUFAs, diets high in saturated fats cause obesity not only because they are less satiating but also because they act to increase activity in brain areas associated with increasing food intake and body weight.

However, not all studies have shown that the addition of n-3 PUFAs (long or short chain) to the diet of normal rats, mice, or human subjects has an influence on food/energy intake or body weight (e.g., Awad et al., 1990; Belzung et al., 1993; Chicco et al., 1996; Cunnane et al., 1986; Hill et al., 1993; Parrish et al., 1991; Raclot et al., 1997; Russell et al., 1991; Rustan et al., 1993; Sohal et al., 1992). Interestingly, Reseland et al. (2001) found that although supplementation with n-3 PUFAs did not alter either body weight or plasma leptin level in humans or rats, n-3 PUFAs did reduce leptin mRNA expression in rat epididymal fat tissue. Clearly, further work needs to be done to establish the influence on body weight of addition of long-chain n-3 PUFA to the diet. In particular, physiologically relevant amounts (e.g., 0.5%–1.0% of fat intake) of the n-3 PUFA have to be examined.

In the absence of changes in food intake and body weight, however, addition of long-chain n-3 PUFAs to the diet has been shown to significantly reduce body adiposity and increase lean body mass. For example, in a long-term study, 3–4-month-old rats were maintained on semisynthetic diets where the fat content of the diet comprised 45% of the energy. Relative to the rats maintained on a diet containing lard (high in saturated fats) or corn oil, rats maintained on a diet containing n-3 PUFA rich fish oil had less adipose tissue in epididymal, inguinal, mesenteric, and retroperitoneal stores (Hill et al., 1993). In male Wistar rats, maintained on a lard or MaxEPA (high in long-chain n-3 PUFA) diet where the fat content of the diet comprised 40% of the energy, by 5 weeks, fat in epididymal and perirenal tissues, as well as plasma triglycerides, was lower in the MaxEPA group (Parrish et al., 1991). It was suggested that n-3 PUFAs restrict adipose tissue growth by limiting the amount of triglyceride in each cell (Parrish et al., 1991). Inclusion of fish oil (7%, w/w) in the diet markedly reduced fat pad mass of sucrose fed rats (Soria et al., 2002). In KK-A^y mice, addition of fish oil to diet was shown to decrease abdominal fat (Hun et al., 1999). In humans, Couet et al. (1997) substituted 6 g of dietary fat by 6 g of fish oil in healthy adults. Body fat mass decreased by 0.88 kg ($p < .05$) in 3 weeks following substitution.

Several studies have suggested that the reduction in body fat mass is not universal but rather some adipose tissue stores are more susceptible than others. For example, Raclot offered 50-day-old male Wistar rats diets containing n-3 PUFA rich fish oil, purified DHA, purified EPA, a mixture of DHA and EPA, or a diet containing lard and olive oil (control). These semisynthetic diets were formulated so that the caloric content was matched and that 40% of caloric content was derived from fat. Diets containing n-3 fatty acids produced a site-specific antiadipogenic effect. Decreased retroperitoneal fat was noted, but subcutaneous adipose tissue remained constant (Raclot et al., 1997). Belzung obtained a similar result. In this latter study, the addition of n-3 PUFA to a diet high in saturated fat limited hypertrophy of retroperitoneal and epididymal adipose tissues, but had no effect on subcutaneous or mesenteric fat stores (Belzung et al., 1993). In humans on a diet containing 52% carbohydrates, 16% protein, and 32% fat (PUFA:SFA ratio 0.2), replacing visible fat with fish oil (6 g/day for 3 weeks) caused a reduction in body fat without altering energy intake. Although basal metabolic rate was unchanged, lipid oxidation rate was increased (Couet et al., 1997). Further work will be needed to establish the factors responsible for reduction of fat in the specific adipose tissues.

B. Fat Metabolism: Oxidation and Thermogenesis (Figure 35.2)

Given that nearly 100% of the cell volume of adipose tissue is triglycerides, factors that influence their synthesis and breakdown are crucial. Jones and Schoeller (1988) showed that when added to a

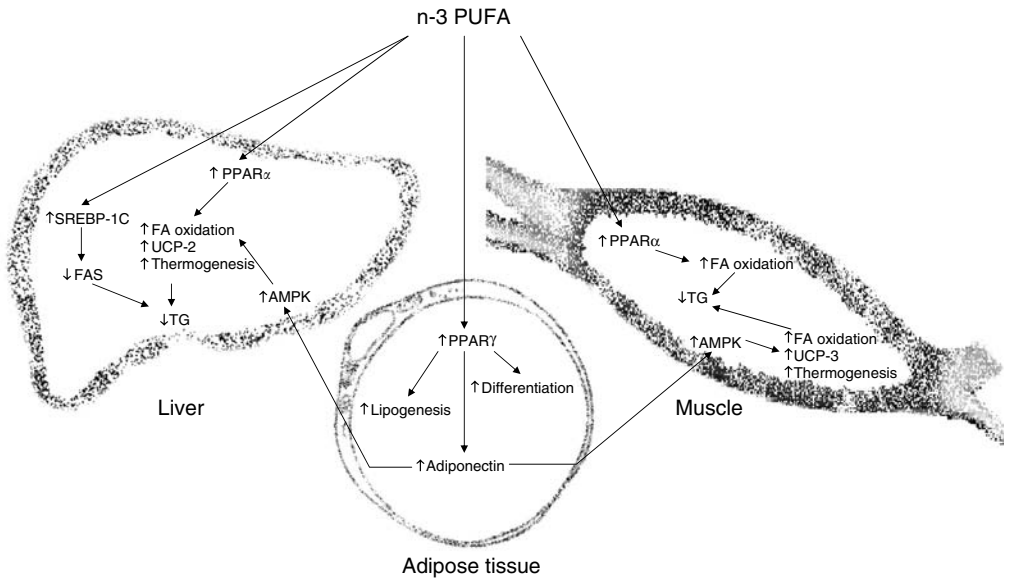

FIGURE 35.2 Model showing pathways by which n-3 PUFAs can influence body energy homeostasis. n-3 PUFAs, omega-3 polyunsaturated fatty acids; PPAR, peroxisome proliferators-activated receptor; SREBP, sterol regulatory element binding protein; FAS, fatty acid synthase; FA, fatty acid; UCP, uncoupling protein; AMPK, AMP-activated protein kinase; TG, triglycerides.

saturated fat diet, n-3 PUFA increased basal metabolic rate and total energy expenditure. Subsequent to ingestion, n-3 PUFAs rapidly cause an upregulation of genes involved in lipid oxidation and a downregulation of genes involved in lipogenesis in the liver and muscle. Hepatic oxidation of fatty acids increases within 3 days when the diet contains 12%–15% fish oil, but does not increase for several weeks when the diet contains n-6 PUFAs. Evidence suggests that unlike intake of a high-fat diet, intake of a diet rich in n-3 PUFAs may promote fat utilization rather than storage (Price et al., 2000). Dietary fish oil protected rats from visceral fat hypertrophy by increasing lipid mobilization from adipose tissue and inhibiting lipid synthesis in the liver (Peyron-Caso et al., 2003). In a study in which rats were fed diets containing 15% fat for 15 days, mitochondrial and peroxisomal fatty acid oxidation rates, many of the enzymes involved in fatty acid oxidation and carnitine palmitoyl transferase I activity were greatly increased in animals that were given perilla and fish oil compared to animals given palm or safflower oils (Ide et al., 2000; Neschen et al., 2002). Relative to rats with LA as their sole source of PUFA, animals with n-3 PUFA had lower fatty acid synthase activity (Ikeda et al., 1998). n-3 PUFA suppresses fatty acid synthesis by suppressing SREBP-1c in the liver (Jump et al., 2005). Mono- and polyunsaturated fatty acids bind directly to PPARα and PPARγ at physiological concentrations, thereby providing other mechanisms by which fatty acids modulate lipid homeostasis (Kliewer et al., 1997). Activation of PPARα increases oxidation of fatty acids in liver and muscle (Evans et al., 2004; Jump et al., 2005; Lefebvre et al., 2006; Neschen et al., 2002); activation of PPARγ, on the other hand, increases adipogenesis (differentiation of preadipocytes to adipocytes) and increases lipogenesis and lipid storage in adipose tissue (Evans et al., 2004; Forman et al., 1997). However, fish oil, via PPARγ-dependent mechanisms, also appears to increase secretion of adiponectin, a peptide produced and secreted by adipocytes, implicated in the reduction of body fat (Neschen et al., 2006). On the basis of body composition analysis and RQ, Sanz Sampelayo et al. (2006) concluded that consumption of fish oil leads to decreased oxidation of protein but increased oxidation of fat. Altered expression of genes encoding lipogenic (fatty acid synthase and lipoprotein lipase), lipolytic (hormone sensitive lipase), and glyceroneogenic enzymes due to ingestion of n-3 PUFAs appear to be important in reducing body fat stores.

Thermogenesis, the production of body heat, is one of the major components of energy expenditure and therefore a major aspect of body weight control. Uncoupling proteins (UCPs) uncouple oxidation of substrates from phosphorylation (synthesis of ATP) in mitochondria and are implicated in the regulation of thermogenesis (Ricquier, 2005). Evidence suggests that in rats fed a high-fat diet, addition of n-3 PUFA enhances expression of UCP1 mRNA in brown adipose tissue (Takahashi and Ide, 2000). Adiponectin, has been shown to reduce visceral adiposity via upregulation of uncoupling protein (UCP) genes in skeletal muscle and adipose tissue (Masaki et al., 2003). The upregulation of UCP genes is mediated by AMP-activated protein kinase (Stoppani et al., 2002). Armstrong and Towle showed that PUFAs stimulate hepatic thermogenesis by increasing hepatic UCP2 expression via a PPARα-mediated pathway (Armstrong and Towle, 2001). In addition, thermogenesis in brown adipose tissue is regulated by the sympathetic nervous system. Increased thermogenesis can be due to increased responsiveness to noradrenaline or increased release of noradrenaline. Thus, n-3 PUFAs could influence the effectiveness of the sympathetic nervous system because they increase the sensitivity of brown adipose tissue to noradrenaline (Ohno et al., 1996). In summary, the influence of n-3 PUFA on enhancing pathways involved in the metabolism of fat seems to be crucial to the changes in body composition.

C. High-Fat Diets and Obesity

Obesity is one of the major health risks for a number of diseases, particularly heart disease and diabetes. It is well known that ingestion of a diet high in saturated fats is one of the major causes of obesity. There are two explanations for this observation. First, diets high in saturated fats do not seem to be as satiating as either high-carbohydrate or high-protein diets (Doucet et al., 1998), even when the high-fat diet is less palatable (Warwick, 1996). Second, whereas increased intake of either carbohydrate or protein causes a concomitant increase in energy expenditure, for example, nonshivering thermogenesis, increased intake of saturated fat does not cause a similar increase in energy expenditure. Individuals maintained for 1 week on a high-carbohydrate or high-protein diet have increased body temperature, whereas individuals maintained on a diet high in saturated fat do not, furthermore, ingested fat is mostly sequestered to adipose tissue. That is, increased intake of saturated fat does not promote the use of fat as a metabolic fuel (Schutz et al., 1989; Thomas et al., 1992). Thus, diets high in saturated fats are more obesity producing than diets high in carbohydrate or protein because of their lesser ability to satiate appetite and to increase fat oxidation. With the ingestion of more energy than expended, storage of fat is increased and obesity results (Tremblay et al., 1989).

The peptide hormone insulin is produced in the pancreas and secreted in proportion to the degree of adiposity. Similar to leptin, insulin levels are correlated with the amount of abdominal fat (Porte et al., 1998; Woods et al., 1990, 1996, 1998). It is transported into the brain where it acts to decrease food intake and body weight (Schwartz et al., 1992; Woods et al., 1996). High insulin resistance is a characteristic of obesity, hypertension, and noninsulin dependent diabetes mellitus. There is an inverse relationship between insulin action and triglyceride content. With the ingestion of fat, insulin secretion is increased. Insulin stimulates fatty acid synthase, an enzyme that catalyzes all reactions involved in lipogenesis, and thereby results in the accumulation of triglycerides (Sul et al., 2000). Monounsaturated fatty acids (such as oleate) and saturated fatty acids (such as palmitate), have little or no influence on fatty acid synthase, are incorporated into triglycerides in adipose tissue (Parrish et al., 1990, 1991; Su and Jones, 1993), and increase insulin resistance. In contrast, n-3 PUFAs markedly inhibit fatty acid synthase, and are therefore preferentially incorporated into phospholipids for cell membrane remodeling. That is, n-3 PUFAs decrease triglyceride production, resulting in low levels of tissue and plasma triglycerides, and thereby decrease insulin resistance (Awad et al., 1990; Chicco et al., 1996; Hill et al., 1993; Parrish et al., 1991; Russell et al., 1991; Rustan et al., 1993; Sohal et al., 1992). It is clear that ingestion of diets high in n-3 PUFAs can be beneficial in the treatment of obesity and diabetes. However, this is effected by numerous factors including the

amount and type of n-3 PUFA ingested, the stage of development when n-3 PUFA is supplied and the duration that n-3 PUFA is available for. For maximal benefit, ingestion of n-3 PUFAs should begin at an early age.

D. Cancer Cachexia

In patients with cancer, weight loss indicates a poor prognosis and a shorter survival time. Cancer cachexia involves a massive loss of body weight, with extensive breakdown of both body fat and skeletal muscle, often, but not always, accompanied by anorexia (DeWys, 1985). Metabolic studies have shown that increased free fatty acid mobilization occurs prior to weight loss in cancer patients (Costa et al., 1981) and weight loss is not reversed by parenteral nutrition, thus, the weight loss associated with cancer cachexia is different from simple starvation (Brennan, 1977).

Although not fully understood, it appears that the most likely model for the development of cancer cachexia is based on increased cytokine production. Interleukin (IL)-1β, an endogenous pyrogen, is a cytokine released by activated macrophages and monocytes that mediates local and systemic responses to inflammation. Peripheral or central administration of IL-1β produces decreased food and water intake and increased body temperature. Such a decrease in food intake caused by IL-1β can be attenuated by an MC-4 receptor antagonist (Huang et al., 1999) or by depletion of neuronal histamine (Sakata et al., 1995). Activation of the MC-4 receptor (Broberger, 1999; Hanada et al., 2000) as noted earlier, and the release of histamine (Morimoto et al., 2000) contribute to the decrease in food intake caused by leptin.

In a mouse model of cancer (MAC16 mice), cachexia was reversed by a semisynthetic diet in which a high proportion of the calories were derived from n-3 PUFA rich fish oil (19% EPA; 13% DHA (Tisdale and Dhesi, 1990)), suggesting that EPA and DHA interfered with catabolic activity. Fish oil supplementation also elevated the normally reduced blood glucose levels observed in MAC16 mice suggesting a decrease in glucose utilization by the tumor. Free fatty acids were unaltered. An n-6 PUFA rich diet had no effect on weight loss or tumor growth and none of the diets influenced food intake. The weight gain by fish oil treated mice was shown to be due to reduction in loss of fat and muscle, and not due to increased water retention (Tisdale and Dhesi, 1990).

In a subsequent study, Tisdale and Beck (1991) investigated the inhibition of lipid mobilization by PUFAs in MAC16 mice. Ingestion (by gavage) of EPA, but not of DHA or ALA, prevented weight loss and minimized tumor growth. None of the n-6 PUFAs (e.g., LA, arachidonic acid) was effective in inhibiting the mobilization of lipid. EPA was also effective in inhibiting the lipolytic effect of salbutamol (β-adrenergic agonist) or adrenocorticotrophin. In combination with cytotoxic drugs used to treat cancer, it was shown that neither DHA nor EPA interfered with treatment and, in many cases, potentiated the anticancer effects of the drugs (Wynter et al., 2004). In another mouse model of cancer (C57 mice, Lewis lung carcinoma), treatment with either DHA or EPA prevented the decrease in body weight (Ohira et al., 1996), but did not alter food or water intake or tumor size.

In a rat model of cancer cachexia (Walker 256 tumor), fish oil reduced tumor growth, increased food intake and body weight, partially restored renal function and increased survival time (Fernandez et al., 2004; Togni et al., 2003). Furthermore, providing a high-fat diet containing fish oil in combination with sunflower oil, an n-6:n-3 ratio of 6:1 was able to prevent the decrease in food intake and body weight caused by the cancer (Pizato et al., 2005).

Given that weight loss in cancer patients occurs prior to a reduction in food intake, the problem appears to be due, at least initially, to disruption of the sympathetic nervous system and the regulation of energy expenditure. It is possible that some by-product of the cancer causes damage to the LH. A cachectic individual with multiple sclerosis was shown to have damage to the LH (Kamalian et al., 1975). Another possibility is that the factors that mediate the cachectic process stimulate inhibitory factors such as leptin or inhibit stimulatory factors such as NPY (Inui, 1999). Furthermore, it has been shown that patients with surgically inoperable pancreatic adenocarcinoma normally lose body weight but this is reversed by eating n-3 PUFA rich fish oil. This reversal is due to an increase

in appetite and a decrease in energy expenditure (Barber et al., 1999) and possibly reducing the demand for amino acids caused by hepatic protein synthesis (Barber et al., 2004). Weight loss is generally a potent stimulus to food intake, therefore, the failure of patients with cancer to increase their intake of food and the reversal of this by n-3 PUFA requires further investigation.

V. CONCLUSION

The evidence presented in this review shows that n-3 PUFAs can influence body energy homeostasis. With respect to the intake of food and regulation of body weight, the possibility that a critical period exists is minimal. Clearly, n-3 PUFAs can influence the accumulation and utilization of fat, both crucial to the regulation of body weight. Ingestion of n-3 PUFAs have been shown to reduce the size of adipose tissue, increase thermogenesis, and in some instances reduce body weight. In this regard, ingestion of n-3 PUFAs as a replacement for saturated fat or monounsaturated fatty acids might be important in overweight individuals. Ingestion of the n-3 PUFAs could facilitate the loss of body fat via its effect on increasing fat metabolism and thermogenesis. n-3 PUFAs could also improve glucose utilization by reducing cellular triglycerides and thus increasing insulin sensitivity. Interestingly, in animal models of cancer cachexia and in patients with cancer cachexia, n-3 PUFAs can act to increase body weight. The mechanism appears to involve interference with the catabolic influence of toxins produced by the tumor. This is clearly different to the mechanisms by which the n-3 PUFAs act to reduce body fat in individuals without cancer. Thus, the ingestion of n-3 PUFAs can influence fat mass and body weight via several different mechanisms.

Although our current understanding of the role of n-3 PUFAs in food intake and body weight maintenance requires further work, evidence suggest that these metabolites might be important in a multitude of cellular functions. Certainly, n-3 PUFAs are crucial for normal neural function and deprivation may alter cell membrane properties in crucial areas of the brain that monitor appetite. Perhaps coincidentally, there seems to be a commonality in the neural functions altered by n-3 PUFA deprivation. More often than not, affected processes are mediated by receptors belonging to the seven-transmembrane domain, G-protein-coupled receptor family. G-protein-coupled receptors are essential in the integration of feeding and drinking control mechanisms (Plata-Salaman et al., 1998). For example, with respect to feeding, NPY receptors (Blomqvist and Herzog, 1997; Du et al., 1997), galanin receptors (Kask et al., 1997), and MC-4 receptors (Huszar et al., 1997) belong to G-protein-coupled receptor superfamily. With respect to drinking, angiotensin II (ANG II) receptors (Lenkei et al., 1998), OT receptors (Barberis et al., 1998), and somatostatin receptors (Raulf et al., 1994) belong to G-protein-coupled receptor superfamily. The function of this receptor superfamily may be more susceptible to alteration by decreased n-3 PUFA because of their morphology. These large and complex membrane-bound proteins alter conformation after ligand binding and, it is possible, that a decrease in membrane fluidity would hamper conformational change such that the active conformation is no longer energetically favorable, following binding of the ligand.

Over the past 50 years, there has been an increase in diabetes, heart disease, and cancer, thought to be due, in part, to changes in environmental factors. The nutrients we eat may be the most influential environmental stimuli, and fat is a strong determinant of cell differentiation, growth, and metabolism. Although the effects of n-3 PUFAs on several behavioral indices have been widely reported, there is still much work to be done if we are to understand the mechanism by which this family of fatty acids exerts its many actions.

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36 Biological Effects of Oxidized Fatty Acids

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CONTENTS

I. INTRODUCTION

Good-quality edible fats and oils are bland, odorless, and free of impurities and are oxidatively stable. However, fats and oils may become oxidized and develop objectionable tastes and odors during processing, storage, and culinary practices. Oil processing is designed partly to remove oxidized oils and free fatty acids, as well as contaminants that may initiate oxidative reactions. However, some processing steps such as bleaching and caustic refining, if not done properly, may promote oxidation. During storage and culinary practice, fatty acid oxidation may occur in the presence of a free radical initiator. Oxidized fatty acids formed may catalyze further oxidation and/or break down to form compounds that give the rancid odor and flavor that characterize oxidized fats and oils. Hydrolysis is another main degradation process for oils during culinary practice. In deep-frying, the fatty acids are hydrolyzed off the triglycerides at high temperatures in the presence of water, yielding free fatty acids, monoglycerides, diglycerides, and glycerol. These breakdown products may speed up oxidation and degradation of the remaining triglycerides as they enhance emulsification of water from the food into the oil. In addition to polymerized fatty acids, prolonged oxidation may result in the formation of aldehydes, ketones, alcohols, short-chain fatty acids, and other compounds. Oxidized oils and their secondary products when consumed exert varying biological effects depending on the conditions of oxidation.

II. FATTY ACID OXIDATION

Oxidation is a major problem associated with storage and use of fats and oils. Mechanisms of fat and oil oxidation include enzymic oxidation, photooxidation, autoxidation, and thermal oxidation

(see Chapter 2). Lipoxygenases are primarily responsible for the increased oxidation of unsaturated fatty acids in seeds during storage (Suzuki et al., 1996). However, since enzymes are labile to heat denaturation, lipoxygenase activity is normally absent in a refined oil. Oxidation catalyzed by enzymes, therefore, does not present a problem during the storage and use of fats and oils. In the presence of oxygen and a photosensitizer, fatty acids can be peroxidized directly after exposure to light. Initial formation of fatty acid hydroperoxides by photooxidation may involve the generation of highly reactive singlet oxygen (Frankel et al., 1985). As potential photosensitizers, such as chlorophyll, dyes, heme compounds, and flavonoids, are normally removed from fats and oils during processing, photooxidation does not present a serious problem in the storage and utilization of fats and oils, either. Autoxidation and thermal oxidation reactions, on the other hand, are more directly involved in the oxidation of fatty acids during storage and use than are enzymatic oxidation and photooxidation.

Autoxidation is the process of fat/oil oxidation induced by air at room temperature. The oxidative stability of unsaturated lipids generally decreases with the increased degree of unsaturation in air. Saturated fatty acids have very low reactivity. Autoxidation behavior is different between complex lipids and simple lipids. For example, at 30°C in the dark, phospholipid is the most oxidatively stable molecular form of the fatty acids, followed by triglycerides, then fatty acid methylesters, and then free fatty acids (Ogawa et al., 1995). In addition, the position of fatty acids on triglycerides plays a role in determining the relative rate of autoxidation (Neff and El-Agimy, 1996).

Autoxidation of fatty acids occurs in a series of steps involving the generation of free radicals and can be divided into three phases: initiation, propagation, and termination. In the initiation phase, hydrogen is abstracted from the α-methylenic carbon of fatty acids to yield a free radical (Equation 36.1). Once a free radical is formed, it may form peroxyl radicals through reaction with atmospheric oxygen (Equation 36.2). In addition, free radicals can abstract hydrogen from another unsaturated molecule to form a hydroperoxide and a new free radical, thus propagating reaction (Equation 36.3). Initial formation of fatty acid hydroperoxides from fatty acids may involve the generation of singlet oxygen via photooxidation (Frankel et al., 1985) and/or free radicals formed from the breakdown of the hydroperoxides catalyzed by transition metal ions (Aust et al., 1985).

$$RH \rightarrow R^{\cdot} \tag{36.1}$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \tag{36.2}$$

$$ROO^{\cdot} + R'H \rightarrow ROOH + R^{\cdot\prime} \tag{36.3}$$

The free radical-initiated chain reaction has one critically important feature. Once initiated, the reaction is self-sustaining and is capable of oxidizing large amounts of lipids. Although hydroperoxides are the primary oxidation products formed during the propagation phase, the reaction is accelerated via chain branching or free radical multiplication (Equation 36.4 and 36.5). The free radical chain reaction may be terminated by antioxidants (AH) such as vitamin E (tocopherols) that competitively react with a peroxyl radical and remove a free radical from the system reaction (Equation 36.6). In addition, the chain reaction may be terminated by self-quenching or polymerization of free radicals (Equation 36.7). During autoxidation, the hydroperoxides formed may be split, or break down into free radicals and smaller organic compounds such as aldehydes, alcohols, and acids (Frankel et al., 1961; Schultz et al., 1962; Artman, 1969; Frankel, 1980, 1985; Weiss, 1983; Aust et al., 1985).

$$ROOH \rightarrow RO^{\cdot} + OH^{\cdot} \tag{36.4}$$

$$2ROOH \rightarrow ROO^{\cdot} + RO^{\cdot} + H_2O \tag{36.5}$$

$$ROO^{\cdot}\ (\text{or } R^{\cdot}) + AH \rightarrow ROOH\ (\text{or } RH) + A^{\cdot} \tag{36.6}$$

$$ROO^{\cdot}\ (\text{or } R^{\cdot}) + R^{\cdot\prime} \rightarrow ROOR'\ (\text{or } RR') \tag{36.7}$$

TABLE 36.1
Oxidation Products of Fatty Acids

Aliphatic hydrocarbons	Alcohols
Aldehydes	Aliphatic acids
Cyclic dimeric acids	Cyclic hydrocarbons
Cyclic ethers	Cyclic monomeric acids
Dibasic acids	Epoxides
Hydroperoxy fatty acids	Hydroxy fatty esters
Hydroxy fatty acids	Keto hydroxy fatty esters
Keto fatty esters	Ketones
Lactones	Polymeric fatty acids

Oxidation reactions are accelerated at higher temperature. The oxidation of fats and oils at high temperature is termed *thermal oxidation*. A number of free radicals, including peroxylalkoxy- and carbon-centered radicals, are formed during heating of various fatty acids or their esters in air, and the amount of free radicals generated is inversely related to the number of double bonds in the fatty acids (Vicente et al., 1995). During deep-fat cooking, baking, and frying of fats and oils, oxidation reactions are greatly accelerated. A large number of end compounds, including hydroperoxides, short-chain fatty acids, dimeric and polymeric compounds, cyclic dimmers and monomers, polar lipids, volatile hydrocarbons, and other secondary products (Table 36.1), have been detected in heated or fried oils (Frankel et al., 1961; Schultz et al., 1962; Artman, 1969; Yoshioka and Kaneda, 1972, 1974; Weiss, 1983; Hansen et al., 1994; Vicente et al., 1995; Arroyo et al., 1996; Kajimoto et al., 1996; Marquez-Ruiz and Dobarganes, 1996; Velasco et al., 2004). These compounds are generated through oxidation, hydrolysis, degradation, and/or polymerization reactions.

The type of products generated by thermal oxidation reactions is modulated by heating conditions, the lipid phase (e.g., temperature, duration, and oil type), and the presence of antioxidant(s) and prooxidant(s). For example, Fujioka and Shibamoto (2004) have shown that more glyoxal is found from salmon and cod liver oils when heated at 60°C for 3 days than for 7 days, that when oils are treated under cooking conditions at 200°C for 1 h, the aldehydes formed are comparable to those formed under accelerated storage conditions at 60°C for 3–7 day, and that fish oils produced more malondialdehyde (MDA), glyoxal, and methylglyoxal than do vegetable oils because the fish oils contained higher levels of long-chain polyunsaturated fatty acids than the vegetable oils. Prolonged heating of oils usually leads to increased formation of polymeric materials and secondary products. Used oils may contain as much as 25%–30% of polymeric materials at the end of their usable frying capability (Poling et al., 1970; Nolen, 1973). The formation of polymers darkens the color and increases foaming of the oil, and can lead to an increase in viscosity and solidification of the oil.

During deep-fat cooking, baking, and frying of fat and oil, in which oxidative reactions are greatly accelerated, a stream blanket may be formed. However, the steam blanket over the oil by water in the foodstuff does not exclude contact with air, and the presence of water increases the extent of oxidation. Since absorption of oil by the frying food is extensive, it is often necessary to replenish the fryer with fresh oil. The replenishment with fresh oil tends to dilute overall compositional changes of the oil during prolonged frying, and fairly steady-state conditions are often maintained in a normal continuous operation. As flavor and odor are very sensitive indicators of the oxidative deterioration of fats and oils, before oil has undergone a significant alteration in nutritive value, it has normally become organeleptically unacceptable.

Important parameters determining the characteristics of oils include chemical measures, such as the degree of unsaturation (iodine value), average fatty acid chain length (saponification value), and flash, smoke, and fire point, as well as physical parameters, such as melting point, refractive index, and solid fat index (Chapter 2). Methods for estimating the oxidation state of lipids include sensory

analyses, measurement of oxygen consumption, active oxygen (accelerated shelf life measurements) and free fatty acids, and analysis of hydroperoxides, peroxide value, and secondary products of lipid oxidation (Berset and Cuvelier, 1996; Kajimoto et al., 1996). These parameters are all important in determining the stability and shelf life of an oil.

III. BIOLOGICAL EFFECTS OF OXIDIZED FATTY ACIDS

Triglycerols represent over 95% of the weight of most food fats and oils. The minor components of fats and oils include monoglycerides, diglycerides, free fatty acids, sterols, phosphatides, fatty alcohols, carotenoids, and tocopherols. Although free fatty acids account for only a minute portion of the total fatty acids in fresh edible fats and oils, their content may greatly rise during processing, storage, and certain culinary practices. Free fatty acids are more labile to oxidation than the esterified ones (Schultz et al., 1962; Artman, 1969; Frankel, 1980, 1985; see Chapter 2). The oxidation reaction and products derived from fats and oils are more complicated and less well defined than those of free fatty acids. When consumed, oxidized fats/oils and their secondary products exert different biological effects depending on the levels and types of oxidation products present (Nolen, 1973; Kubow, 1990).

A number of reports dealing with the absorption and deposition of oxidized oils and their secondary products, when given at a large quantity in their diet, are available, but with inconsistent results (Kaunitz et al., 1956; Glavind and Tryding, 1960; Perkins et al., 1961; Glavind and Sylven, 1970; Glavind, 1972). As expected, the extent of oil absorption is adversely affected by heating. For example, Porsgaard et al. (1999) have shown that the transport of fatty acids is significantly lower in lymph-cannulated rats receiving heated oil (180°C for 15 min) compared to those receiving the corresponding unheated oil, that the absorption of sunflower oil is more affected by heating than the absorption of soybean or rapeseed oil, and that the largest decrease in total activity of tocopherols following heating is observed in sunflower oil. Using [U-^{13}C]-labeled hydroxy or dihydroxy triglycerides, Wilson et al. (2002) have shown that dietary hydroxy fatty acids are absorbed in humans, with plasma peak levels being reached between 4 and 6 h, and the amount of ^{13}C-labeled oxidized fat absorbed is related to that of plasma triglycerides. In addition, ^{13}C monohydroxy triglycerides are absorbed to a greater extent than those of ^{13}C dihydroxy triglycerides.

Consumption of oxidized oils is associated with an increase in lipid peroxidation products. The levels of thiobarbituric acid reactants (TBARs), for example, are increased in liver microsomes and mitochondria of male Wistar rats fed 4 weeks with a diet containing 2% oxidized corn oil (Iritani et al., 1980). Triglyceride molecules with a hydroxy, an epoxy, or a keto group attached to fatty acids, as well as triglyceride core aldehydes, are detected in pig lipoproteins after a diet rich in oxidized sunflower seed oil (Suomela et al., 2005). In addition, the lipoprotein triglycerides and total lipids are more oxidized in the pigs fed on oxidized oil than those fed on nonoxidized oil. Similarly, ingestion of oxidized sunflower oil (heated at 98°C for 48 h) that contained 262 mmol/kg of hydroperoxides, 5.7% of the oxidized esters, and 50.4% polymerized oil, results in increased levels of TBARS and *trans* fatty acid (18:1) in rat liver (Ammouche et al., 2002). As at least portions of the oxidized oils and their secondary products are absorbed and incorporated into the lipoproteins and tissues, these compounds are expected to exert various adverse effects when consumed. Studies on the toxicological and biological effects of oxidized fats and oils can be roughly classified into three types: (1) oxidized fats and oils, (2) oxidized fatty acids, and (3) degradation products of lipid hydroperoxides.

A. Oxidized Fats and Oils

Male broilers fed with 3.5% oxidized fat for 7 weeks reduce feed efficiency and growth rates as compared to those receiving normal fat (Wang et al., 1997). Broiler hens fed a diet containing oxidized oil exhibit growth depression and reduced plasma levels of fat, energy, α-tocopherol, lutein, β-carotene, and retinol than those receiving fresh oil (Enberg et al., 1996). In addition, rats fed

heated oils (175°C for 24 h) for 16 weeks have significant pathological changes in heart muscle (Rupcikova et al., 1992). In addition, hepatic injury is associated with the treatment of oxidized lipids, and intubation of oxidized lipids (0.15 mL oxidized trilinolein or 1.05 mL oxidized butter per rat) at 12 h intervals results in increased incorporation of thymidine in hepatic DNA during the early stage of intoxication and severe liver cirrhosis (Fouad et al., 1995).

As oxidized fatty acids can be absorbed and incorporated, increased consumption of oxidized oils may alter tissue lipid composition. Administration of 15% thermally oxidized sunflower oil to rats for 45 days, for example, significantly increases the concentrations of hepatic phospholipids 16:0, 16:1, 18:0, 18:1, and 18:2, whereas the concentration of 20:4 is significantly decreased (Kode et al., 2005). Similarly, feeding of oxidized oil (50°C for 16 days) reduces the milk triglyceride concentration and long-chain fatty acids (Brandsch et al., 2004). In addition, feeding of moderately heated oils (175°C for 24 h) to rats for 16 weeks results in the presence of fatty acid *trans* isomers in body fats and lung (Rupcikova et al., 1992), and feeding of the oil heated at 190°C–195°C for 28 h to broilers results in increased geometrical and positional fatty acid isomers in dark meat (Bou et al., 2005). Rats ingested oxidized soybean oil have reduced content of free thiols in the erythrocyte membrane and an increased level of reactive carbonyl group in eh proteins of the ghosts and of the muscle than the controls (Hayam et al., 1997).

Dietary fat has profound effects on gene expression, leading to changes in metabolism, growth, and cell differentiation. The effects of dietary fat on gene expression reflect an adaptive response to changes in the quantity and type of fat ingested (Jump and Clarke, 1999). In mammals, specific fatty acid-regulated transcription factors, such as peroxisome proliferator-activated receptors (PPAR-α, -β, and -γ), HNF4α, NF-κB, and SREBP1c, are regulated by (1) direct binding of fatty acids, fatty acyl-coenzyme A, or oxidized fatty acids; (2) oxidized fatty acid (eicosanoid) regulation of G-protein-linked cell surface receptors and activation of signaling cascades targeting the nucleus; or (3) oxidized fatty acid regulation of intracellular calcium levels, which affect cell signaling cascades targeting the nucleus (Jump and Clarke, 1999). The effects of fatty acids on the genome provide new insight into how dietary fat might play a role in health and disease.

The activities of hepatic aminopyrine N-demethylase, aniline hydroxylase, NADPH-cytochrome *c* reductase, UDP-glucuronyl transferase, and GSH *S*-transferase, as well as cytochrome P-450 content are significantly increased in rats fed a diet containing 15% deteriorated soybean frying oil (Huang et al., 1988). Similarly, oxidized frying oil has been shown to upregulate hepatic acyl-CoA oxidase and cytochrome P-450 4 A1 genes in rats and activates PPAR-α (Chao et al., 2001). In addition, feeding the diets with oxidized fats leads to a significant overall reduction of the relative mRNA concentrations and the activities of fatty acid synthase and glucose-6-phosphate dehydrogenase in rats (Eder et al., 2003a). The study suggests that oxidized fats contain substances that suppress gene expression of lipogenic enzymes in the liver. However, feeding male Wistar rats for 4 weeks with a diet containing 2% oxidized corn oil does not alter the levels and the activities of glucose-6-phosphate dehydrogenase, malic enzyme, and acetyl-CoA-carboxylase in the liver (Iritani et al., 1980), and feeding of thermally oxidized oil (50°C for 16 days) does not affect the activities or expression of lipogenic enzymes (fatty acid synthase and acetyl-CoA-carboxylase) in mammary glands of lactating rats (Brandsch et al., 2004).

As the levels of oxidized lipoproteins in the circulation are directly correlated to the quantity of oxidized lipid in the diet, it is suggestive that in humans dietary oxidized lipids are absorbed by the small intestine, incorporated into chylomicrons, and appear in the bloodstream where they contribute to the total body pool of oxidized lipid (Staprans et al., 1994). Thus, oxidized fats and lipid oxidation products in the diet ingested may exert deleterious cardiovascular effects and contribute to the pathogenesis of atherosclerosis (Cohn, 2002). Oxidative modification of lipoproteins, particularly low-density lipoprotein (LDL), increases its atherogenicity by altering receptor-mediated uptake by cells in the intima of blood vessels (Westhuyzen, 1997). Oxidized lipoproteins may be taken up by scavenger receptors on monocytes, smooth muscle cells, and macrophages in an uncontrolled process leading to the accumulation of lipid and the formation of foam cells, an early feature

of atherosclerotic plaque. Loading with oxidized LDL alters the endocytic and secretory activities of murine macrophages (Bolton et al., 1997), and oxidized lipoproteins, including high-density lipoprotein (HDL) and their lipid peroxidation products inhibit tumor necrosis factor α secretion by THP-1 human macrophages (Girona et al., 1997). In addition, rabbits fed a diet rich in oxidized lipid have a significant increase in fatty streak lesions in the aorta and cholesterol in the pulmonary artery (Staprans et al., 1996).

To determine the effect of dietary oxidized lipids on lipoproteins, Suomela et al. (2004) studied the effects of three sunflower seed oil diets differing in oxidation levels (PV in oils 1, 84, and 223 mEq O_2/kg) on lipoprotein lipid oxidation in growing pigs, and found that the lipoprotein lipids were more oxidized in the pigs fed on the most oxidized oil compared with those fed on nonoxidized oil, reflecting oxidation of dietary fat in the lipoprotein. In addition, rats fed oil heated at 50°C, 105°C, or 190°C have higher concentrations of HDL cholesterol and lower ratios between plasma and HDL cholesterol than rats fed the diet containing the fresh fat (Eder et al., 2003b). However, the composition of LDL apolipoproteins and uptake of LDL by macrophages are not different between rats fed the fresh fat diet and those fed the oxidized fat diet. The findings suggest that ingestion of oxidized fats does not adversely affect the lipoprotein profile in the rat model used, and does not cause modifications of apolipoproteins that would lead to enhanced uptake of LDL via macrophage scavenger receptors.

Oxidized fats and oils may promote or initiate carcinogenesis or mutagenesis. Sugai et al. (1962), for example, have shown that feeding of heated oil to rats promotes the carcinogenicity of 2-acetylaminofluorene, a substance that can cause neoplasia in a varirty of tissues. The ability of vitamin E to partially protect against experimentally induced carcinogenesis (Haber and Wissler, 1962; Wattenberg, 1972) suggests that lipid peroxidation may play a role in carcinogenesis or mutagenesis by damaging genetic materials. The percentage of embryo malformations and reabsorptions is 21.7% in pregnant Wistar rats treated *per os* (0.30 mL/day) with thermally stressed safflower oil for 12 days, compared with 5.6% in the group receiving nonheated oil (Indart et al., 2002). As vitamin E supplementation decreases malformations to 7% in the heated oil group, it is suggestive that the potent teratogenic actions of lipid peroxidation products in the heated cooking oils are partially circumventable by the vitamin. In addition, rats fed the deteriorated fat and oil with at least 107.2 mEq/kg peroxide value significantly decrease locomotor activity compared to control rats (Gotoh et al., 2006). The findings suggest that oxidized fat and oil may induce neurotoxicity, and that it is important to evaluate the toxicity of oxidized fats and oils by using other measures, in addition to using general toxicity tests.

B. Oxidized Fatty Acids

There are a large number of oxidation products of fatty acids. Autoxidation of U-^{14}C-labeled methyl linoleate, for example, results in the formation of methyl linoleate hydroperoxides, as well as their polymeric and low molecular weight fractions (Oarada et al., 1986). The polymeric fraction consists mainly of dimers of methyl linoleate hydroperoxides. 4-Hydroxy-2-nonenal (HNE), 8-hydroxy methyl octanoate, and 10-formyl methyl-9-decenoate are identified as major constituents in the low molecular weight fraction. As is shown in Table 36.2, the distribution of radioactivities in the digestive contents and feces of rats 12 h after intubation averaged 41% for methyl linoleate hydroperoxides, 53% for the polymeric fraction, and less than 3% for low molecular weight compounds. On the other hand, approximately 73% of the total radioactivity is found in the urine and as expired CO_2 of rats treated with the low molecular weight fraction compared to 25% in animals receiving methyl linoleate hydroperoxides and 23% in the polymeric fraction-treated group (Oarada et al., 1986). The findings suggest that small molecular weight compounds of oxidized fatty acids are more readily absorbable and metabolizable than the polymeric materials.

Although some fatty acid hydroperoxides are absorbed from the gut, they may not survive the transfer and exert their effects on distant organs (Glavind and Tryding, 1960; Bhalerao et al., 1963).

TABLE 36.2
Distribution of Oxidation Products of Methyl Linoleate[a]

	Polymers	Hydroperoxides	Small Compounds
Digestive contents			
Stomach	7.13	33.54	0.09
Small intestine	3.58	2.46	0.88
Cecum and large intestine	32.35	4.56	1.44
Feces	9.60	0.67	0.70
Digestive tract			
Stomach	1.17	2.50	0.22
Small intestine	0.44	0.49	0.30
Cecum and large intestine	1.24	0.21	0.25
Organs			
Brain	0.04	0.03	0.07
Lung	0.06	0.08	0.13
Heart	0.03	0.05	0.03
Liver	1.93	3.71	0.86
Kidney	0.19	0.24	0.27
CO_2	9.73	10.61	27.46
Urine	12.79	13.98	46.87

[a] Percentage (%) of radioactivity administered observed 12 h after male Wistar rats received oral administration of U-^{14}C-labeled methyl linoleate hydroperoxide, polymeric fraction, or low molecular weight fraction.

Source: Adapted from Oarada, M., et al. (1986). *Lipids 21*: 150–154.

After oral administration of methyl linoleate 1-^{14}C-hydroperoxides to rats, only labeled hydroxy acids, and not unchanged hydroperoxides of linoleate 1-^{14}C-hydroperoxides are detected in the lymph (Bergan and Draper, 1970). Kanazawa et al. (1985) have shown that 13.4% of orally administered ^{14}C-labeled linoleate hydroperoxides is detected in the feces of rats compared to 45.3% of the secondary oxidation products 90 h after treatment, and that 51.9% of the radioactivity absorbed by the rats treated with secondary oxidation products is recovered from the urine and 22.9% as CO_2 90 h after administration compared to 29.3% from urine and 23.9% as CO_2 in the linoleate hydroperoxide-treated group. The secondary product fraction employed consists of approximately 35% polymers, 25% endoperoxide-rich components, and 40% low molecular weight compounds. Approximately 2.6% of the total orally administered secondary products are found in rat liver 12–24 h after administration. The significant presence of those compounds, coupled with the observed increase in hepatic TBARS, elevation of serum transaminase activities, and slight hepatic hypertrophy suggest that the secondary products of linoleate hydroperoxides may be absorbed and deposited in distant organs. Similarly, male growing rats fed a diet containing 10% oxidized linoleic acid-rich preparation for 4 weeks result in profound alterations in membrane composition, fluidity, and function of liver microsomes, as well as higher levels of fluorescent compounds and TBARS, higher activities of aldehyde dehydrogenase (ADH) and NADPH cytochrome reductase, and enhanced cholesterol turnover than the control animals (Hochgraf et al., 1997).

Oxidative damage in biomembranes may be induced by dietary oxidized oils. When added at the initial stage or at the early logarithmic phase, linoleate hydroperoxides at 1 mM concentration completely inhibit the growth of *Escherichia coli* (Gamage et al., 1971). Linoleic acid hydroperoxides (LOOH) and/or oxidized oils with different hydroperoxide contents induce lipid peroxidation in erythrocyte ghosts and colon cells (Udilova et al., 2003). LOOH incorporated into the lipid bilayer decreases membrane fluidity and initiates lipid peroxidation in the lipid phase. In addition, exposure

of LOOH to cultured cells (IEC18 intestinal epithelial cells, SW480 and HT29/HI1 colon carcinoma cells) causes cell death both via apoptosis and necrosis. Cells with higher degree of membrane unsaturation are more susceptible and antioxidants (vitamin E and selenite) are protective indicating the involvement of oxidative stress. Similarly, vitamin E deficiency symptoms such as encephalomalacia in chicks (Nishida et al., 1960) and creatinuria and erythrocyte hemolysis in rabbits (Kokatnur et al., 1966) have been observed in animals infused with methyl linoleate hydroperoxides.

Martin et al. (2000) have shown that feeding of cyclic fatty acid monomers (0.1%–1.0%) from heated oil for 2 weeks increases some characteristic enzyme activities, such as peroxisomal acyl-CoA oxidase, and the microsomal omega- but also (omega-1)-laurate hydroxylase (CYP4A1 and CYP2E1, respectively) in rat liver. Dietary cyclic fatty acids induce a coordinated regulation between the activities of the lipogenic enzymes studied (Δ9-desaturase, phosphatidate phosphohydrolase) and peroxisomal oxidation, but not with mitochondrial β-oxidation. The dose-dependent decrease of Δ9-desaturase activity with cyclic fatty acid monomer intake is accompanied by a similar decrease of the monounsaturated fatty acid level in liver. The increase in the γ-linolenic acid level with cyclic fatty acid intake suggests an increase in Δ6-desaturase activity, and decreased liver glycogen concentration suggests an effect on the carbohydrate metabolism. The findings also suggest that the altered liver levels of eicosapentaenoic and arachidonic acids are due to the peroxisomal retroconversion process in rats fed cyclic acids.

Consumption of a small amount of linoleate hydroperoxide causes an increase in the formation of oxidatively modified LDL with a high content of conjugated dienes in plasma and liver (Machira, 1994). In addition, both acid and neutral cholesteryl esterases are suppressed in mononuclear leukocytes, liver, and aorta of the oxidized linoleate hydroperoxide-fed rats. A specific polyclonal antibody against 13-hydroperoxyoctadecadienoic acid-modified protein: formation of lipid hydroperoxide-mediated apoB-100 in oxidized LDL has been characterized (Kato et al., 1997). Oxidatively modified lipoproteins may exert an atherosclerotic effect by altering receptor-mediated uptake by cells in the intima of blood vessels (Westhuyzen, 1997). Dietary 13-hydroxylinoleic acid promotes atherosclerosis (increased aortic lesion areas, and plasma total cholesterol and LDL cholesterol, as well as oxidatively modified proteins) and plasma cholesterol concentrations in LDL receptor knockout mice in the presence of dietary cholesterol (Khan-Merchant et al., 2002). Apolipoprotein (apo) A-I, the major protein component of HDL, synthesized principally in the small intestine and liver, is increased in plasma apoA-I level in humans treated with an oxidized fat diet. Rong et al. (2002) have shown that oxidized linoleic acid causes a dose-dependent increase in the levels of apoA-I, but not apoB, protein in both differentiated and undifferentiated Caco-2 cells, that the mRNA expression for apoA-I paralleled the protein expression, and that Caco-2 cells did express PPAR-γ. As mRNA and PPAR-gamma ligand could increase apoA-I secretion in these cells, the mechanism for the induction of apoA-I might include PPAR-γ for which oxidized fatty acid is a ligand. The findings suggest that fatty acid hydroperoxides may play a role in the oxidation of lipoproteins and the development of atherosclerotic lesions.

Daily intubation of oxidized ethyl linoleate (peroxide value 700 or 1400; the major oxidized compounds are 9-hydroperoxy-*cis*, *trans*- and 13-hydroperoxy-*trans*, *cis*-octadecadienote) to rats (2.5 g/kg body weight) for 16 days results in mucosal hypertrophy of the large intestine and affected the fecal fermentation of dietary fiber (Hara et al., 1996). The data suggest that dietary oxidized fatty acids may influence cecal metabolism and play a role in the etiology of colon cancer.

C. Degradation Products of Lipid Hydroperoxides

Prolonged heating of fats and oils may result in the degradation of peroxidized fatty acids formed initially. Depending on the type of fatty acids and oxidation conditions, different secondary products of fatty acids are formed. For example, major compounds found in the low molecular weight fraction obtained from peroxidized methyl linoleate are 8-hydroxy methyl octanoate, HNE, and 10-formyl methyl-9-decanoate (Oarada et al., 1986), and azelaldehydoc acid, hexanal, azelaic acid, monocarboxylic acids (mainly hexanoic, hepatanoic, and octanoic acids), and suberladehydic

acid are found in the low molecular weight fraction of autoxidized linoleic acid (Kanazawa et al., 1985). In addition, 2-hydroxy heptabal and HNE are the major aldehydic compounds resulting from the oxidation of arachidonic acid by iron ascorbate, whereas MDA, glyoxal, and 2-hydroxy-4-decenal are the minor products (Mlakar and Spiteller, 1997). Owing to their shorter carbon chain lengths, low molecular compounds are more easily absorbed than fatty acid hydroperoxides or polymerized materials into the intestinal wall. Among a diverse array of low molecular weight compounds, MDA and HNE, the principal α,β-unsaturated aldehydic products of lipid peroxidation, have received the most research interest.

a. Malondialdehyde

Malondialdehyde (MDA), a three-carbon dialdehyde, is produced during autoxidation of polyunsaturated fatty acids. Much more MDA is produced from autoxidized linolenate than from autoxidized linoleate, whereas autoxidized oleate produces essentially no MDA (Kenaston et al., 1955). Dahle et al. (1962) proposed that only polyunsaturated fatty acids containing three or more double bonds can undergo autoxidation and form peroxyl radicals with a β,γ-unsaturation to the carbon that bears the hydroperoxide group. This peroxyl radical then cyclizes to form a five-member ring compound. Peroxyl radicals can either abstract a hydrogen atom from an alkyl group or undergo ring closure to form cyclic endoperoxide radicals (Pryor et al., 1976a,b). The resulting compounds can then generate MDA when exposed to heat or acid.

The LD_{50} levels of MDA in rats are 632 mg/kg of body weight for its enolic solium salt and 527 mg/kg for its acetate form (Crawford et al., 1965); considerably more toxic than formaldehyde or glyoxal. MDA can react with the mitochondrial membrane (Balcavage and Alvager, 1982), disrupt red blood cell membranes (Jain, 1984), and decrease erythrocyte survival (Jain et al., 1983). MDA can also form a conjugated Schiff base product by reacting with the amino group of proteins or phospholipid (Tappel, 1972). Major MDA adducts found in rat and human urine include amino acid–MDA adduct *N*-(2-propenal), lysine and its acetylated derivative (McGirr et al., 1985), and *N*-(2-propenal) serine (Hadley and Draper, 1988). Under conditions of oxidative stress (vitamin E deficiency or administration of iron nitrilotriacetate or carbon tetrachloride), increased excretion of *N*-(2-propenal) lysine, its ester, and free MDA is observed in rat urine (Mahmoodi et al., 1995). These studies suggest an accelerated rate of proteolysis by MDA. In isolated rat hepatocytes, MDA has been shown to modify proteins through covalent alkylation of lysine, histidine, and cysteine residues (Hartley et al., 1997). Increased levels of aldehyde-modified proteins are associated with oxidative stress conditions but not with cell death, suggesting that these proteins may also function to sequester electrophilic molecules during oxidative injury.

MDA is capable of reacting with DNA (Tappel, 1972; Summerfield and Tappel, 1984) and produce chromosomal aberrations in cultured fibroblasts (Bird et al., 1982). The preferential reaction sites on the DNA molecule for the MDA are the bases guanine and cytosine (Brooks and Klamerth, 1968). Increased formation of cross-link products by MDA is correlated with the loss of DNA template activity and structural changes (Reiss et al., 1972). In addition, MDA may inactive DNA polymerase and thus restrict cell proliferation in culture (Wawra et al., 1986). Increased formation of a deoxyguanosine–MDA adduct was found in the liver and kidney of aged rats (Draper et al., 1995). High-dietary polyunsaturated fat is associated with increased formation of DNA ethano–DNA base adducts of MDA in white blood cells of healthy individuals (Fang et al., 1997; Nair et al., 1997).

The amounts of MDA present in foods vary widely depending on the structure composition and conditions of processing, storage (duration and temperature), and culinary practices (Papavergou et al., 1995; Abdel-Kader, 1996; Liu et al., 1996). In addition to those originating from foods, MDA can be formed nonenzymatically in the tissues by peroxidative decomposition of polyunsaturated fatty acids and enzymatically as a by-product of the cyclooxygenase reaction in prostaglandin synthesis. Increased formation of lipid peroxidation products, including MDA, in serum and tissues of experimental animals and humans is associated with increased oxidative stress. The concentration of

MDA in the urine is a useful index of the amount of lipid peroxidation consumed in the diet (Brown et al., 1995).

The mutagenicity/carcinogenicity of MDA has been demonstrated in a variety of test systems (Shamberger et al., 1974; Mukai and Goldstein, 1976; Yau, 1979). The mutagenicity of MDA in several strains of bacteria tested is reduced by coincubation with antioxidants (Shamberger et al., 1979). As MDA may catalyze the formation of *N*-nitrosoamines from secondary amines and nitrite (Kikugawa et al., 1980; Kurechi et al., 1980), MDA may play a role in regulating tumor metastasis, host immune mechanisms, and proliferation and differentiation of tumor cells (Begin, 1987). Higher levels of MDA and other aldehydic products are found in the blood of cancer patients (Torun et al., 1995; Yazdanpanah et al., 1997) and in noninsulin-dependent diabetic patients with or without microalbuminuria (Nacitarhan et al., 1995; Ozben et al., 1995).

However, the mutagenicity of MDA may be attributable to the presence of other compounds (Marnett and Tuttle, 1980). Enzymatically synthesized MDA lacks mutagenicity in a standard *Salmonella*/microsome assay and has weak mutagenic activity in a new *Salmonella* strain that reverts by frameshift mutation in a repeating sequence of adenine residues (National Academy of Sciences, 1982). In addition, administration of MDA in drinking water fails to demonstrate the carcinogenicity of MDA in random-bred Swiss mice (Apaja, 1980). Thus, the significance of MDA to human risk of cancer remains to be determined.

b. 4-Hydroxyalkenals

4-Hydroxyalkenal is formed in relatively high quantities during lipid peroxidation (Benedetti et al., 1986; Jurgens et al., 1986, 1987; Oarada et al., 1986; Esterbauer, 1993). A range of 14–150 nmol/g of HNE is found in pork and 1–152 nmol/g in beef, and the content of HNE is linearly correlated with the content of total omega-6 fatty acids, linoleic acid, or arachidonic acid in pork (Sakai et al., 1996). HNEs are very toxic to mice and rats. The lethal dose of HNE, when given intraperitoneally, is 68 mg/kg of body weight (Schauenstein et al., 1977). HNE causes severe liver damage in rats when administered intravenously as a phospholipid emulsion at the dose of 13–18 mg/kg of body weight (Segall et al., 1985). The symptoms are very similar to those seen after CCl_4 administration.

HNE may inactivate liver microsomal P-450 cytochromes (Kuo et al., 1997); upregulate transforming growth factor β1 expression in the macrophage lineage (Leonarduzzi et al., 1997); impair signal transduction associated with muscarinic acetylcholine and metabotropic glutamate receptors (Blanc et al., 1997); mediate oxidative stress-induced neuronal apoptosis (Kruman et al., 1997); inhibit interleukin-1 β converting enzyme (Davis et al., 1997); inactivate glutathione reductase (Vander Jagt et al., 1997); interact with rhodopsin (van Kuijk, 1997); inhibit Na,K-ATPase (Siems et al., 1996); induce transcription and expression of aldose reductase in rat vascular smooth muscle cells (Spycher et al., 1996); cause cell death in murine alveolar macrophages (Li et al., 1996); modify proteins through covalent alkylation of lysine, histidine, and cysteine residues (Hartley et al., 1997); inhibit poly(ADP-ribose) formation in primary cultures of rabbit synovial fibroblasts (Ullrich et al., 1996); induce mitochondrial permeability transition (Kristal et al., 1996); inactivate ethanol-inducible cytochrome P-450 and other microsomal P-450 isozymes (Bestervelt et al., 1995); disrupt iron homeostasis and cause cell degeneration (Mark et al., 1997); inhibit hepatic DNA polymerases and O^6-methylquanine-DNA methyltransferase (Krokan et al., 1985; Wawra et al., 1986); modify glucose-6-phosphate dehydrogenase (Esterbauer et al., 1988; Grace et al., 1996); and induce coronary vasodilation in perfused rat heart (Van der Kraaij et al., 1990). In addition, HNE cross-linked protein inhibits multicatalytic proteinase (proteasome) noncompetitively and inhibits the degradation of oxidatively modified glutamine synthetase by the proteinase (Friguet and Szweda, 1997).

Oxidative stress has been implicated in the pathogenesis of many neurodegenerative conditions that involve degeneration of synapses and neurons in glutamatergic pathways, including strokes, Alzheimer's disease, and Huntington's disease. In Alzheimer's disease, the microtubule-associated protein tau is excessively phosphorylated in degenerative neurons. The ability of HNE to inhibit

dephosphorylation of the microtubule-associated protein tau suggests a role for HNE in altered tau phosphorylation and neurofibrillary degeneration (Mattson et al., 1997). Increased HNE-derived advanced lipid peroxidation endproducts, lysine-derived pyrrole adducts, are found in the neurofibrillary tangles of patients with Alzheimer's disease (Sayre et al., 1997). Removal of extracellular glutamate at synapses by specific high-affinity glutamate transporter is critical to prevent excitotoxic injury to neurons. As 4-HNE may mediate oxidation-induced impairment of glutamate transport and mitochondrial function in synapses, it is suggestive that HNE plays important roles in oxidative impairment of synaptic functions that may promote excitotoxic cascade (Keller et al., 1997). In addition, excretion of 4-HNE is increased in the bile of patients with biliary tract and pancreatic disorders (Leo et al., 1997).

4-Hydroxyalkenals can cause severe disturbance of cell functions at the biochemical and genetic levels (Esterbauer et al., 1988), and Chinese hamster ovary cells exposed to HNE show an increased frequency of sister chromatid exchange (Brambilla et al., 1986). Exposure of HNE and other 2-alkenals increases the number of revertants in bacteria (Marnett et al., 1985). Both HNE and MDA are chemotactic for neutrophils (Curzio et al., 1986, 1990), toxic to many cells (Benedetti et al., 1980, 1986), and play a role in cell-mediated oxidation of lipoproteins in cell-free systems (Andreis et al., 1981; Gavino et al., 1981). Kumagai et al. (2000) have shown that among the oxidized fatty acid metabolites tested, only HNE, a highly mutagenic and genotoxic aldehyde generated during oxidative stress, dramatically induced cyclooxygenase-2 (COX-2) gene expression in rat liver epithelial RL34 cells, and that intracellular GSH status was strictly related to HNE-induced COX-2 expression. These findings suggest the presence of a signaling pathway in the cellular response mediated by locally produced lipid peroxidation products under oxidative stress. Tumor necrosis factor α secretion by human macrophages is inhibited by 2,4-hepatadienal, hexanal, 2-nonenal, 2-octenal, and 2,4-decadienal, but not by linoleic acid hydroperoxides (Girona et al., 1997), suggesting a role of aldehydic compounds in the modulation of the inflammatory response by macrophages. Another lipid peroxidation product, 12-oxo-*cis*-9-octadecenoic acid (12-keto-oleic acid), can bind covalently to protein and amino acids and form fluorescent pigments (Kanazawa et al., 1985). In addition, the methanolic extract of cooking oil fumes contain *trans–trans*-2,4-decadienal (*t–t*-2,4-DDE), which induces genotoxicity and oxidative stress in human lung carcinoma (A-549) cells (Wu and Yen, 2004).

IV. FACTORS AFFECTING THE BIOLOGICAL EFFECTS OF OXIDIZED FATTY ACIDS

There is a general agreement that undesirable or harmful materials are generated during prolonged oxidation or high-temperature treatment. However, there is considerable disagreement regarding the biological significance of such materials formed in experimental studies. This is mainly due to differences in experimental designs and approaches, especially the extent of oxidation of oils used, and adequacy of dietary nutrients, employed for each study.

A. Extent of Fatty Acid Oxidation

As discussed previously, the types and amounts of oxidation products of fats and oils vary greatly, depending on the oxidation conditions. Studies on the biological effects of heated and oxidized oils conducted can be divided into two categories. The first involves extensively used oils and the second involves oils that have been heated or oxidized under normal-use conditions approximating current culinary practices. The first type is usually prepared form experimental oils under unrealistic conditions. The problem with this type of study is the failure to remove used oil (with a fried product) and add new (makeup) oil continuously. Since steady-state conditions are not attained, such oils continuously deteriorate, and the amount of oxidation products formed is expected to be directly proportional to heating time (Poling et al., 1960, 1962, 1970; Nolen, 1973).

The biological effects resulting from ingestion of heated oils is closely related to both the levels of oil used in the diet and the conditions of their heating treatment. Highly deteriorated frying oils are generally poorly digested and absorbed. Approximately one-half of orally administered [^{14}C] linoleate autoxidation products, for example, is found in the feces of rats (Kanazawa et al., 1985). This can be attributed to the presence of cyclic dimmers and polymeric materials in the extensively oxidized oils (Nolen et al., 1967; Kajimoto and Makai, 1970; Poling et al., 1970). Consumption of large amounts of polymeric fatty acids may result in diarrhea, and cyclic monomers, when fed at high levels (15% of the diet), cause an increase in the fat content of the liver (Poling et al., 1960; Weil, 1970). Badly abused oils also produce adverse effects such as mild enlargement of the liver, spleen, and adrenals in experimental animals (Poling et al., 1960; Weil, 1970). Consumption of oils that have undergone prolonged heating in the presence of air depresses appetite and growth and causes depression, diarrhea, histological changes in various tissues, tissue enlargement, interference with reproduction, and even death in some instances (Poling et al., 1970). Although the feed intake of male Wistar rats fed a diet containing 15% thermally oxidized sunflower oil with 19% polar materials for 27 days is similar to those receiving 15% used oil with 5.1% polar materials, final body weight, feed efficiency ratio, and protein efficiency ratio are significantly lower (Lopez-Varela et al., 1995). In addition, the liver of nine of ten animals that received high-polymeric materials exhibits moderate fibrotic degenerative areas with severe vaculization together with regenerative areas containing eosinophilic binuclear hepatocytes.

On the other hand, experimental animals are rather resistant to the effects of oils that have not been subjected to prolonged heating or abuse. The growth rate of rats maintained on a diet using heated partially hydrogenated soybean oil prepared under conditions that simulated intermittent frying in a restaurant as the sole source of dietary lipid is only somewhat lower than that of rats fed unheated oil (Nolen et al., 1967). The heated oil has a slight decrease in digestibility or absorbability, but does not adversely affect the rate of animal survival or incidence of tissue lesions during the 24-month period (Table 36.3), and the overall mortality rate and incidence of tumors and other pathological changes or disease conditions are not correlated with the extent of heating for up to 216 h. In addition, rats grow well on a diet that contains 60% ricinoleic acid-rich castor oil (Stewart and Sinclair, 1945) and are relatively insensitive to the ingestion of oxidized fats containing hydroxy or carbonyl groups (Kaunitz and Johnson, 1964). In humans, a cathartic action probably protects against absorption of significant quantities of oxidized fat. More importantly, the presence of a number of metabolic systems, such as glutathione peroxidase (GPx) and AD (Figure 36.1), in the body reduces the potentially harmful effects of oxidized fats and oils and their degradation products (Chow, 1979; Antonenkov et al., 1987).

B. Adequacy of Dietary Nutrients

As essential fatty acids and tocopherols present in fats and oils are readily oxidized during autoxidation or thermal oxidation, the levels of these nutrients remaining in the oxidized oil are important in determining its biological effect. If oxidized fats and oils are used as the only source of dietary lipid and vitamin E, deficiencies of essential fatty acids, vitamin E, or both may result. For example, using 15% autoxidized cottonseed oil as the sole source of dietary lipid, Kaunitz et al. (1956) have observed a marked inhibition of growth in growing rats, and when fresh cottonseed oil was added to the diet, normal growth resumed. These findings suggest a deficiency of essential fatty acids, vitamin E, or both in the autoxidized oil used in the diet. In a study to determine if the antioxidant status alters the adverse effects of oxidized oils, Giani et al. (1985) have shown that supplementation with vitamin E at 300 mg/kg diet neutralized the adverse effects of heated oils on vascular eicosanoid production compared to the values found in rats fed a diet containing fresh oil. The body and organ weights of animals treated with heated oil and given vitamin E supplements did not differ from those of the controls after 8 weeks. Similarly, growth depression in rats following ingestion of oxidized oils is prevented by vitamin E supplementation (Privett and Cortesi, 1972), and supplementation

TABLE 36.3
Effect of Frying Time on the Biological Effects of Frying Oils[a,b]

	Frying Time at 182°C (h)		
Observation	**0**	**60**	**216**
Polar fraction (%)	1.3	13.6	30.2
Body weight gain (g)			
Males (months)			
2	358	339	344
12	726	677	704
21	763	764	774
Females (months)			
2	204	196	201
12	392	375	377
21	518	466	463
Absorbability (%)			
Males (months)			
2	95.5	90.3	91.5
12	96.8	92.4	92.7
21	96.6	94.2	92.2
Females (months)			
2	96.4	92.1	92.7
12	96.8	92.7	94.0
21	96.4	92.5	93.6
24-Month survival (%)			
Males	53	67	53
Females	58	60	58
Respiratory disease (%)			
Males	15.6	22.2	20.0
Females	6.7	13.3	11.1
Nephritis (%)			
Males	24.0	20.0	22.0
Females	14.0	12.0	6.0
Liver pathology (%)			
Males	48.0	6.0	18.0
Females	38.0	28.0	24.0
Tumors (%)			
Males	22.2	17.8	22.2
Females	53.3	37.8	28.9

[a] Partially hydrogenated soybean oil.
[b] 50 Male and 50 female Sprague-Dawley rats in each group.

Source: Adapted from Nolen, G.A., et al. (1967). *J. Nutr. 93*: 337–348.

of α-tocopherol to chicks fed thermally oxidized sunflower oil reduces their tissue susceptibility to oxidation (Sheehy et al., 1994). In addition, dietary oxidized frying oil compromises α-tocopherol retention and enhances tissue depletion and excretion of α-tocopherol in vitamin E-deficient rats (Liu and Huang, 1995, 1996). The study suggests that chronic ingestion of oxidized lipids compromises free radical scavenging activity *in vivo*.

Besides essential fatty acids and vitamin E, dietary protein levels have been shown to play a role in the toxicity of oxidized fats and oils. Witting et al. (1957), for example, observed an almost

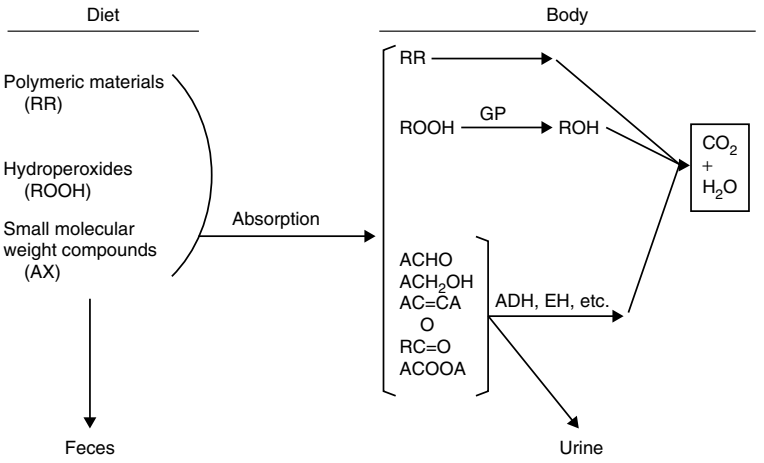

FIGURE 36.1 Possible fate of dietary oxidized fatty acids and secondary products. R, fatty acid, phospholipid, or triglyceride; RR, dimer or polymer of fatty acids; ROOH, lipid hydroperoxide; ROH, hydroxy fatty acid; A, short- or medium-chain alkyl group; X, carboxyl (COO–), hydroxy (–OH), aldehyde (–CO), ester (–COO), epoxy (–C═C–); carbonyl (–CO–), etc.; GP, glutathione peroxidases; ADH, aldehyde dehydrogenase or alcohol dehydrogenase; EH, epoxide hydrase.

total lack of growth in rats fed a diet containing laboratory-prepared heated corn oil and 10% casein. With 20% casein in the diet, severe growth depression was still seen, but at 30% or more protein in the diet, only a mild effect was observed. Similarly, long-term feeding of thermally oxidized fats induces hepatic microsomal enzymes, and the levels of hepatic microsomal enzyme induced by oxidized oil are influenced by dietary protein levels (Andia and Street, 1975). However, the extent of enzyme induction is much higher in the animal group receiving 27% protein than when dietary protein was 8%. On the other hand, animals fed the low protein diet have elevated serum glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase values, suggesting injury to the heart or liver. These findings agree with reports that the toxicity of used frying oil is much less severe when rats are fed a high protein diet (Hermans et al., 1973).

Although the mechanism of the protective effects of high-dietary protein is not yet clear, it may be linked to an enhanced detoxification capability.

V. METABOLIC FATE OF OXIDIZED FATS AND OILS

Pure fatty acid hydroperoxides are very toxic to experimental animals when administered intravenously (IV) (Horgan et al., 1957; Olcott and Dolev, 1963; Findlay et al., 1970). The 24-h lethal IV dose of a high-purity preparation of methyl linoleate hydroperoxides in adult male rats is approximately 0.07 mmol/100 g body weight (Cortesi and Privett, 1972). The major effect of injected linoleate hydroperoxides was on the lungs, which became enlarged from edema, and the animals died of lung congestion and injury similar to that observed in acute oxidant toxicity (Stockinger, 1965; Haugarard, 1968). However, lipid hydroxides are not that toxic when given orally (Dubouloz and Laurent, 1950; Dubouloz et al., 1951). In addition, only very small amounts of hydroperoxides and TBARS are detected in the lung and serum, indicating that most of the injected hydroperoxides are destroyed in the tissue. The findings suggest that the peroxidized fatty acids ingested/absorbed are metabolized. This view is supported by the observation that no deaths are seen in rabbits during a 24-h period after a single oral dose of methyl linoleate hydroperoxides of about ten times the amount that causes death by the intravenous route (Cortesi and Privett, 1972).

A metalloprotein capable of metabolizing peroxidized fatty acids has long been suggested (Dubouloz and Laurent, 1950; Dubouloz et al., 1951). The presence of an active metabolic system for lipid hydroperoxides is supported by the finding that within 24 h after intraperitoneal injection of ^{14}C-labeled linoleate hydroperoxides in rabbits, the majority of the radioactivity was collected as carbon dioxide (Findlay et al., 1970). Similarly, after giving oxidized corn oil orally to rats with thoracic lymph fistula, Iritani et al. (1980) fail to detect the presence of hydroperoxides per se in the lymph or tissues in the next 24 h. The findings suggest that peroxidized lipids absorbed are metabolized. Subsequent studies show the metalloprotein is the seleno-enzyme GPx, which utilizes the reducing equivalents of glutathione (GSH) to reduce hydrogen peroxide as well as lipid hydroperoxides (Little and O'Brien, 1968; Ursini et al., 1982, 1985) (Figure 36.1).

After feeding 7% oxidized stripped corn oil (peroxide value 1000 mEq/kg) in a diet adequate in selenium and vitamin E to weanling rats for 14 weeks, Vilas et al. (1976) observed an increase in the specific activity of GPx in stomach mucosa but not in the plasma or in perirenal and paraepididymal adipose tissues. In addition, consumption of peroxidized oils does not significantly affect GPx activity of the mucosa of the gastrointestinal tract of selenium-supplemented rats, but the enzyme activity in both the stomach and intestine declined more slowly than in other organs as a result of consuming peroxidized oil during selenium depletion (Reddy and Tappel, 1974).

Ingestion of oxidized sunflower oil (heated at 98°C for 48 h) resulted in increased levels of TBARS and activities of GPx and GSSG reductase in rat liver (Ammouche et al., 2002). As the activities of GPx and metabolically related enzymes are increased in tissues of animals fed oxidized oils and other oxidatively stressed animals (Chow and Tappel, 1972; Chow et al., 1973; Negishi et al., 1980), the enzymic system is an important mechanism protecting against the deleterious effects of hydroperoxides (Chow, 1979, 1988). Similarly, significantly higher lipase activities observed in the exocrine pancreatic juice of growing pigs fed oxidized canola oil suggests that the exocrine pancreas is able to adapt to variations in the levels and quantity of oxidized lipids in the diet (Ozimek et al., 1995). In addition, the metabolic response to the consumption of oxidized oils is supported by the findings that the activities of hepatic aminopyrine *N*-demethylase, aniline hydroxylase, NADPH-cytochrome *c* reductase, UDP-glucuronyl transferase, and GSH *S*-transferase, as well as cytochrome P-450 content are significantly increased in rats fed a diet containing 15% deteriorated soybean frying oil (Huang et al., 1988).

Although MDA is very reactive, the compound originated from foods or generated *in vivo* is normally eliminated or metabolized rapidly. Hepatic ADH is the major enzymic system responsible for MDA metabolism (Chow, 1988; Esterbauer et al., 1988; Esterbauer, 1993) (Figure 36.1). Multiple, forms of ADH isozymes exist in mitochondria, cytosol, and microsomes (Mitchell and Peterson, 1989). In addition, alcohol dehydrogenase (ALH) and GSH conjugation catalyzed by GSH *S*-transferases plays a role in the elimination of MDA (Esterbauer et al., 1991). An age-associated decline in mitochondrial detoxification of aldehydic compounds by ALH and GSH *S*-transferase may cause mitochondrial dysfunction during aging (Chen and Yu, 1996). The increased activities of microsomal ADH and NADPH cytochrome *c* reductase of rats fed a diet containing 10% oxidized linoleic acid rich preparation for 4 weeks (Hochgraf et al., 1997) also suggest a metabolic adaptation of rats to metabolize absorbed aldehydic compounds from the diet.

Although HNE is toxic to biological systems, it is also eliminated or metabolized rapidly under normal conditions (Esterbauer, 1993). For example, over 80% of infused HNE is metabolized in the perfused kidney during the first 3 min (Grune et al., 1997). GSH–HNE conjugate (35%), the corresponding alcohol 1,4-dihydroxynonene (14%), HNE–mercapturic acid conjugate (4%), 4-hydroxynonenoic acid (7%), and water (32%) are identified as primary and secondary metabolites. The formation of a HNE–mercapturic acid conjugate in the kidney and its selective excretion may be of central importance in the detoxification of a number of lipid peroxidation products. Similarly, following intravenous administration of treated HNE to rats, no unchanged HNE is detected in urine, whereas at least four mercapturic acid conjugates (HNE–mecapturic acid, 1,4-dihydroxynonene mercapturic acid, 4-hydroxynonenoic mercapturic acid, and the corresponding lactones) are

identified as HNE metabolites (Alary et al., 1995). These findings obtained suggest that two pathways are involved in the biotransformation of HNE: (1) reduction/oxidation of the aldehyde group and (2) conjugation to endogenous GSH leading to mercapturic acid conjugates in urine.

In hepatocytes, the primary metabolites of HNE are the GSH–HNE adduct, hydroxynonenoic acids, and the corresponding alcohol of HNE, 1,4-dihydroxynon-2-ene (Siems et al., 1997). The sum of these products accounts for about two-thirds of the total HNE degradation after 3 min of incubation. The fast metabolism of HNE underlines the importance of this metabolic pathway as an important part of the antioxidant defense against protein modification by aldehydic compounds. Metabolism by the alcohol/ADH pathways account for approximately 10% of the HNE elimination, whereas bioconversion by GSH *S*-transferases represent 50%–60% of the total HNE removal by hepatocytes and the pathway for the remaining 40% HNE metabolism remains to be identified (Hartley et al., 1995). Murine mGSTA4-4, a GSH *S*-transferase, possesses high activity and specificity for lipid peroxidation products, including HNE. Amino acid residue M104 is important in determining the recognition and binding of HNE to the active center of mGSTA4-4 (Nanduri et al., 1996). GSH *S*-transferase isozyme rGST8-8 in rat lens epithelium, which utilizes 4-HNE as a preferred substrate, is induced by curcumin, an antioxidant present in spice turmeric (Awasthi et al., 1996). Another GSH *S*-transferase isozyme, bGST 5.8, localized in bovine lens epithelium is also involved in the metabolism of HNE (Srivastava et al., 1996). Another enzyme, aldose reductase, which catalyzes the reduction of glucose to sorbital as part of the polyol pathway, also exhibits broad specificity for both hydrophilic and hydrophobic aldehydes, including HNE (Vander Jagt et al., 1995).

VI. SUMMARY AND CONCLUSION

Unsaturated fatty acids are susceptible to oxidation during processing, storage, and culinary use. In addition to hydroperoxides, secondary products of fatty acids, including polymerized materials and small molecular weight compounds, are formed during prolonged heating at high temperature. The adverse effects of oxidized fats and oils differ considerably depending on oxidation/heating conditions, as well as whether the heated oil is used as the sole source of dietary lipid. Feeding of oils that are extensively heated as the sole source of dietary lipids can cause diarrhea, growth retardation, organ enlargement, and even death. The adverse effects observed can be partly attributable to the loss of essential fatty acids and vitamin E. Peroxidized fatty acids and their small molecular weight products, such as MDA and HNE, are very toxic to biological systems. On the other hand, lipid hydroperoxides when ingested or absorbed can be readily metabolized by the GPx and related enzyme systems. Similarly, the aldehydic compounds, including MDA and HNE, can be rapidly metabolized by ADH or eliminated through urine following conjugation with GSH or other compounds. Thus, if oxidized or heated oils are not used as the sole source of dietary lipids, or oils are heated at conditions normally used in food preparation for periods that do not severely affect the taste of the oils of foods, they are generally considered safe and nontoxic despite the presence of many reactive oxidation products.

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37 Satiating Effects of Fat

Zoe S. Warwick, Colleen M. McGuire, and Christina Revelle

CONTENTS

I. INTRODUCTION

Consumption of a diet high in dietary fat typically promotes greater weight gain than a diet high in carbohydrates. This pattern has been revealed in experimental research in both human and animal subjects, as well as in epidemiological studies (for reviews, see Warwick and Schiffman, 1992; Weststrate, 1995; Bray et al., 2004). This greater weight gain is usually (although not invariably) attributable to greater daily kcal intake ("hyperphagia") evoked by the high-fat diet. Researchers studying high-fat diet hyperphagia have examined the attribute(s) of high-fat foods that promote overeating, as well as individual differences in response to high-fat diets. High-fat foods are often more palatable and more calorically dense than high-carbohydrate foods; however, while both taste and caloric density may contribute to high-fat diet hyperphagia, high-fat foods need not be more palatable (Lissner et al., 1987; Warwick and Weingarten, 1995) or more calorically dense (Warwick and Weingarten, 1995) than high-carbohydrate foods in order to elicit overeating.

Since 24-h intake reflects the cumulative intake over many meals, it follows that high-fat hyperphagia must reflect larger meals (in terms of kcal consumed) and/or more frequent meals. One way of conceptualizing the behavioral difference(s) elicited by high-fat and high-carbohydrate foods is in terms of their "satiating effect." The satiating effect of a food is the reduced willingness to eat associated with ingestion of the food. Satiating effects occur *during* a meal (since all meals eventually cease), and also *after* a meal (hunger/subsequent food intake is less than that would be observed

had the food not been consumed). Blundell et al. (1992) have cogently delineated the conceptual distinctions between "satiation" (processes exerting their effects during a meal that contribute to meal termination) and postprandial "satiety" (observed after the meal has ended).

This chapter will review the evidence regarding the relative satiating effects of fats vs. carbohydrates, and will also compare the satiating potency of various types of fats. The satiating effects during, and after, a meal will be considered separately, and the evidence regarding the peripheral anatomical site(s) and signals mediating these effects will be presented. Also discussed will be individual differences in response to fats, long-term body weight regulation as a function of dietary fat content, and the role of learning in the control of fat intake.

II. SATIATION DURING A MEAL

Consumption of a meal produces stimulation of oronasal receptors that produce sensations of taste, aroma, and texture; mechanical stimulation of stomach receptors by food's bulk in the stomach; and chemical stimulation of gastrointestinal receptors. Afferent signals from each of these sites contribute to a reduced willingness to eat, which will ultimately result in the cessation of eating (meal termination). This has been demonstrated in studies in which either oral (e.g., Swithers and Hall, 1992), stomach (e.g., Phillips and Powley, 1996), or duodenal (e.g., Greenberg et al., 1990) stimulation alone elicited behavioral indices of satiety. During normal feeding, of course, all of these signals are experienced and may interact; for example, oral stimulation potentiates the satiating effect of intestinally administered nutrient (Antin et al., 1977).

A. Fats vs. Carbohydrates

Meals consisting primarily of high-fat food(s) are often (but not always; see Table 37.1) larger, in terms of kcal consumed, than meals consisting of high-carbohydrate, low-fat foods. Since high-fat foods are almost always more calorically dense than high-carbohydrate foods, intake of high-fat foods on a weight basis may be equal or even less than intake of high-carbohydrate foods. Both obese (Blundell et al., 1993) and normal weight (Lawton et al., 1994) humans consumed more calories when the meal consisted of high-fat foods than when a high-carbohydrate meal was provided. Because neither the caloric density nor the palatability of the meals was equated, the larger meal size of high-fat foods in these studies cannot be specifically attributed to a unique effect of fat independent of its taste and/or density. When energy density and fat content were independently

TABLE 37.1
Comparisons of Meal Size of High-Fat (HF) and High-Carbohydrate (HC) Foods

Reference	Subjects	Gram Intake	kcal Intake	
Blundell et al. (1993)	Obese humans	HF>HC	HF>HC	Density, taste not controlled
Lawton et al. (1994)	Normal-weight adults	HF>HC	HF>HC	Density, taste not controlled
Louis-Sylvestre et al. (1989)	Teenage boys	HF<HC	HF>HC	Density not controlled
Warwick et al. (2000)	Rats	HF>HC	HF>HC	Observed across two levels of diet density (1.15; 2.3 kcal/mL)
Warwick et al. (2003)	Rats	HF>HC	HF>HC	Observed in self-regulated intragastric infusion
Synowski et al. (2005)	Rats	HF>HC	HF>HC	Observed in long-term spontaneous feeding
Sclafani et al. (1993)	Rats	HF=HC	HF=HC	Equal density (0.08 kcal/mL)
Tordoff et al. (1987)	Rats	HF=HC	HF=HC	Equal density (1.33 kcal/mL)

manipulated across separate test sessions, both lean and obese subjects consumed fewer daily kcal when offered a low-energy density menu relative to a high-density menu (Bell and Rolls, 2001).

Animal studies have also compared meal size of high-fat and high-carbohydrate foods. A larger meal (in kcal) was eaten of a high-fat (HF) liquid diet than of a high-carbohydrate (HC) liquid diet (see Table 37.2 for formulation) even when both diets had equivalent caloric density of 2.3 kcal/mL; this pattern was also observed when both diets were diluted to 1.15 kcal/mL (Warwick et al., 2000). Since the caloric density of the diets was equated, this finding demonstrates that a high-fat food need not be more dense than a high-carbohydrate food to elicit greater intake. Interestingly, the relationship between fat:carbohydrate ratio and meal size is not linear. This was determined by formulating five liquid diets representing graded ratios of HF:HC, which were 100:0 (pure HF), 75:25, 50:50, 25:75, and 0:100 (pure HC). Nondeprived rats consumed each of five liquid diets on separate test days, with counterbalanced presentation order. Consistent with the previous finding (Table 37.3), meal size of pure HF was greater than meal size of pure HC. However, the decrease in meal size associated with increased carbohydrate content of the liquid diet was only evident when the carbohydrate content reached 66% of total kcal (HF:HC 25:75) (Figure 37.1). This may reflect a threshold effect for the relatively greater satiating potency of carbohydrate, and/or a nonlinear increment in the palatability of the liquid diet as a function of HF:HC ratio. Additional tests in which oral and postingestive influences on meal size are isolated are required to discriminate among these possibilities.

Other studies have demonstrated that the relative meal size of fat and carbohydrate depends on the type and/or concentration of nutrients used. When meal size of solutions of glucose or sucrose was compared to intake of corn oil emulsion of equivalent caloric density in nondeprived rats, no difference in meal size was found (Tordoff et al., 1987; Sclafani and Ackroff, 1993). However, meal

TABLE 37.2
Liquid Diet Formulations*

	High-Fat (HF)	High-Carbohydrate (HC)
Corn oil emulsion (44.4% wt./vol.)	125 mL	—
Sucrose (g)	70	195
Evaporated milk (mL)	270	270

*Diets brought to volume of 500 mL with water.

Source: From Z.S. Warwick and H.P. Weingarten. (1995). *Am. J. Physiol. 269*: R30–R37.

TABLE 37.3
Meal Size (30-min Intake) of High-Fat (HF) and High-Carbohydrate (HC) Liquid Diets*

	Caloric Density (kcal/mL)	
Diet	**2.3**	**1.15**
HF	14.3 (1.7)*	18.9 (1.5)
HC	11.9 (1.5)	15.3 (1.1)

*Values are means (standard errors).

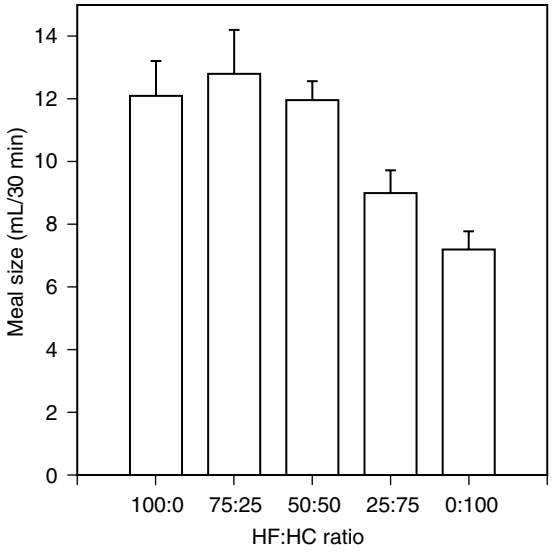

FIGURE 37.1 Meal size of liquid diets varying in HF:HC ratio. All diets 2.3 kcal/mL.

size of Polycose (a complex carbohydrate) solution was greater than intake of corn oil emulsion (Sclafani and Ackroff, 1993). Taken together, these inconsistent findings regarding relative meal size of high-fat and high-carbohydrate foods can be understood by recognizing that intake reflects the summation of stimulatory effects of the food's orosensory properties (taste, aroma, texture) and the inhibitory effects of the food's postingestive effects (stomach fill, contact with gastrointestinal chemoreceptors, postabsorptive effects). Fats differ from one another in their palatability (Naim et al., 1987) as do carbohydrates (Sclafani, 1987); within each macronutrient type there are also differences in postingestive effects under certain conditions (Maggio et al., 1987, but see Warwick and Weingarten, 1994b; Moran and McHugh, 1981; Ackroff and Sclafani, 1991). Thus meal size—the behavioral expression of the summation of food's orosensory and postingestive effects—would be expected to differ across various types of fats and carbohydrates.

In order to investigate the contribution of postingestive influences to relative meal size of high-fat and high-carbohydrate foods, it is necessary to equate or eliminate the foods' sensory properties. When high-fat and low-fat mousses were formulated so as to be equally palatable, teenage boys consumed more calories when eating the high-fat mousse compared to their intake of the low-fat mousse (Louis-Sylvestre et al., 1989). In rats, the orosensory stimulation produced by food can be eliminated by training rats to self-infuse a liquid diet directly into their stomach. Rats accustomed to infusing a high-fat liquid diet self-infused larger "meals" than did rats infusing a high-carbohydrate liquid diet of equivalent caloric density (Warwick and Weingarten, 1995; Lucas and Sclafani, 1999). Both these findings demonstrate that the postingestive effects of fats have a less potent intake-terminating (satiating) effect than carbohydrates. A recent study (Warwick et al., 2003) independently manipulated orosensory influences (highly palatable, moderately palatable) and macronutrient-specific postingestive influences (high-fat, high-carbohydrate) to investigate their independent and interactive effects on eating in a between-subjects design using self-regulated intragastric infusion. The highly palatable solution stimulated larger meals for both diet groups, but the reduced satiating effect (i.e., bigger meals) of high-fat relative to high-carbohydrate was robust across both palatability levels.

B. Hunger and Satiation

As mentioned above, nondeprived rats consumed a larger meal of a high-fat liquid diet than of a high-carbohydrate liquid diet (Table 37.3). To determine whether hunger level would differentially affect meal size, another group of rats was tested in a study in which meal size (30-min intake) of

HF and HC was measured under two conditions: 0-h food deprivation (chow removed just prior to the test session) and 24-h food deprivation (chow, but not water, removed 24 h before the test session). Order of deprivation and diet presentation were counterbalanced across rats. Consistent with the previous finding (Table 37.3), 0-h food-deprived rats consumed a bigger meal of HF than of HC. When hungry (24-h food deprived), rats consumed larger meals overall, but the intake-enhancing effect of deprivation was equivalent across HF and HC (Warwick and Synowski, 1999). However, it should be noted that the effect of deprivation on relative meal size of fat and carbohydrate apparently depends on the type of nutrients used and/or the deprivation conditions. Sclafani and Ackroff (1993) found that chronic food deprivation (body weight maintained at 85% of baseline) increased meal intake of a corn oil emulsion relative to sucrose or Polycose solution.

Another strategy for investigating the effect of deprivation on macronutrient intake is to offer two (or more) macronutrient sources simultaneously ("two-bottle" or "two-jar" test). A number of reports indicate that deprivation selectively enhances preferential intake of fat relative to other macronutrients (Piquard et al., 1978; Bligh et al., 1990; Bernardini et al., 1993; Sclafani and Ackroff, 1993; Warwick and Synowski, 1999).

C. Fatty Acid-Specific Effects

Greenberg and coworkers have utilized a novel approach to assessing the effects of fats on meal size. Instead of comparing oral intake of various fats—which would preclude isolation of the postingestive effects of the fats since their tastes would also vary—they infuse fats directly into the duodenum while allowing the rat to sham-feed. In sham-feeding, ingested food spills out of a surgically implanted hole in the stomach and thus exerts little or no postingestive activity. The type of food sham-fed is held constant across various types of infusions, and sham-feeding intake provides a measure of the satiating effect of the infusion. They observed that the reduction of sham- feeding produced by duodenal infusion of fat was dose dependent (Greenberg et al., 1990). Duodenal infusion of oleic fatty acids was more satiating than an infusion in which these fatty acids were in triglyceride form, and infusion of linoleic acid reduced sham-intake more potently than either oleic or linolenic acid (Greenberg and Smith, 1996). The satiating effect of medium-chain fatty acids differs as a function of chain length; duodenal infusion of lauric acid (C-12) produced a greater reduction of sham-feeding intake than infusion of capric acid (C-10). Caprylic acid (C-8) did not suppress sham-feeding (McCaffery et al., 1994).

D. Individual Differences

The obese Zucker rat differs from its lean counterpart by exhibiting hyperphagia when a high-fat diet is provided, in addition to other physiological differences that contribute to the obesity. Greenberg et al. (1986) found that this hyperphagia could not be explained by a blunted response to the satiating effect of fat, as lean and obese animals showed similar behavioral sensitivity to the satiating effect of duodenally infused fat. However, Greenberg et al. (1996) found that Osborne–Mendel rats, a strain of rats that is susceptible to dietary-induced obesity, were less sensitive to the satiating effect of duodenally infused fat than were S5B/PL rats, a strain that is resistant to dietary obesity. This difference was observed across two types of fat infusions: intralipid (a mixture of long-chain triglycerides [LCTs]) and linoleic acid. In addition to genetic differences in sensitivity to the satiating effect of fat, environmental influences can also play a role. Covasa and Ritter (2000) found that a high-fat maintenance diet blunted the satiating effect of intestinally infused fat.

III. SATIETY AFTER A MEAL

A frequently used strategy for evaluating the satiety produced by a food is the "preload-test meal" paradigm. In this paradigm, the subject first consumes a fixed amount of food, the "preload." Following an intermeal interval (typically <60 min), the subject consumes a "test meal," which is

a premeasured large quantity of food (or foods). The amount of test meal consumed serves as an index of satiety and is negatively correlated with the satiety value of the preload. Although preloads are most commonly consumed orally, they can also be delivered intragastricallyor intravenously, in order to eliminate sensory differences.

A. Fats vs. Carbohydrates

Overall, results from the animal literature have indicated that fat is less satiating than carbohydrate (Table 37.4). In comparisons of test meal intake following oral ingestion of either oil emulsion or an isocaloric glucose or sucrose solution (e.g., Tordoff et al., 1987; Warwick and Weingarten, 1994b; Lucas and Sclafani, 1999; Warwick et al., 2000), rats ate more following the fat preload across a variety of methodological manipulations. One study (Burggraf et al., 1997) compared intragastric and intravenous preloads. Rats were infused with either a lipid emulsion or an isocaloric glucose solution for 30 min before and during the 30-min test meal presentation. The fat infusion was less satiating under both conditions of site of infusion; site of infusion did not affect test meal intake. Although other studies have also found intragastric high-fat loads to be less satiating than isocaloric high-carbohydrate loads (e.g., Geliebter, 1979; Warwick, 1996, 2003), some (e.g., Maggio and Koopmans, 1982; Maggio et al., 1983) have found intragastric fat and carbohydrate to be equally satiating. The inconsistent results could be due to a variety of procedural differences including deprivation level, intermeal interval, rate of preload delivery, and method of preload delivery (intubation vs. catheter delivery).

Results from the human literature comparing the satiety produced by fat and carbohydrate, reviewed by Rolls and Hammer (1995), have been mixed. Although some of these studies have found a fat preload to be less satiating than a high-carbohydrate preload, others have found the preloads to be equally satiating (Table 37.5). In general, studies in which the weight or volume of the preload was held equal found no difference between the satiety produced by a high-fat and high-carbohydrate preload. However, relative responses to fat and carbohydrate differed across various subject types, as discussed below (“individual differences”).

B. Fatty Acid-Specific Effects

Studies conducted in humans have found that foods containing medium-chain triglycerides (MCTs) administered as preloads were more satiating than isocaloric foods containing LCTs (Rolls et al., 1988a). However, in rats, Maggio et al. (1983) found no difference in the relative satiating effect of corn oil and MCT oil. Another study found that relative to water-loaded controls, rats loaded with MCT showed greater satiety than did rats loaded with LCT during the first 3 h after the load. However, this effect was transient as 24-h intake did not differ as a function of fat type (Satabin et al., 1991).

Recent work has compared the satiating effects of three common dietary fats: olive oil, corn oil, and safflower oil in rat. Emulsions at 15% wt./vol. of each fat were prepared using the emulsifier sodium steroyl lactylate (Emplex, American Ingredients, Inc.) as described previously (Warwick and Weingarten, 1995). Fifteen rats consumed a 6 mL preload of emulsion, followed 20 min later by a 10% sucrose test meal. Each rat was tested with all emulsions. Both corn and safflower oil emulsions produced a significant suppression of test meal intake; the suppression of intake produced by olive oil just failed ($p = .08$) to attain statistical significance (Figure 37.2).

The within-subjects design of this study allowed investigation of whether the satiating effects of corn, olive, and safflower oils are mediated by similar or different mechanisms. Correlation coefficients were calculated among test meal intakes following these three fats: the rationale behind this strategy being that if two oils elicit satiety through the same physiological mechanism (e.g., receptor site), then the satiating effects of these two oils (as indexed by test meal intake) should be correlated. However, if the oils elicit satiety through different mechanisms (e.g., different receptor sites), then the satiating effects of these oils would not correlate. The satiating effects of corn and olive

TABLE 37.4
Comparisons of the Satiety Produced by Preloads High in Fat or Carbohydrate: Animal Studies*

Reference	Preload Volume	Nutrient Sources	Finding	Notes
Burggraf et al. (1997)	1.6 mL for 30 min before and during 30-min intake test	*Fat:* microlipid emulsion *CHO:* glucose	Fat less satiating	Loads delivered IV or IG between groups design
Geliebter (1979)	Two daily loads of 10 mL	*Fat:* corn oil *CHO:* corn starch or sucrose	Fat less satiating	Loads delivered IG
Maggio et al. (1983)	8 mL	*Fat:* corn oil emulsion *CHO:* glucose	*Obese rats:* fat less satiating at 10, 30 min. *Lean rats:* equal satiety	Loads delivered IG
Tordoff et al. (1987)	10 mL	*Fat:* corn oil emulsion *CHO:* glucose solution	Fat less satiating at 1 and 24 h, not 23 h	Loads consumed orally. Entire preload not always consumed
Warwick and Weingarten (1994b)	3 mL	*Fat:* corn oil emulsion *CHO:* sucrose solution	Fat less satiating	Loads consumed orally
McGuire and Warwick (1997)	2–8 mL	*Fat:* corn oil emulsion *CHO:* sucrose solution	*Sucrose testmeal:* oil less satiating *Oil testmeal:* oil and sucrose equally satiating. No compensation for increasing preload size	Loads consumed orally
Maggio and Koopmans (1982)	8 mL	*Fat:* emulsions of either SCT, MCT, or LCT *CHO:* glucose solution	Equal satiety produced by triglycerides and glucose	Loads delivered IG
Warwick (1996)	5 mL	*Fat:* corn oil *CHO:* sucrose	Fat less satiating	Loads delivered IG
Warwick et al. (2000)	2–8 mL	*Fat:* HF mixed diet *CHO:* HC mixed diet	Fat less satiating	Loads consumed orally
Warwick (2000)	4 × 7.2 mL overnight	Five isocaloric preloads ranging from 17% to 60% fat kcal	Percentage of fat in preload inversely related to satiating effect	Loads delivered IG

*CHO, carbohydrate; IV, intravenous; IG, intragastric; LCT, long-chain triglycerides; MCT, medium-chain triglycerides. All preloads isocaloric except as noted.

TABLE 37.5
Comparisons of the Satiety Produced by Preloads High in Fat or Carbohydrate: Human Studies

Reference	Weight or Volume Equal (HF vs. HC)?	Nutrient Sources	Finding	Notes
Rolls et al. (1988)	Yes	*Fat:* either LCT or MCT + 6% LCT	MCT more satiating at all preload levels for nondieters	Female dieters vs. nondieters
Shide et al. (1995)	Yes	*Fat:* intralipid *CHO:* dextrose	No difference in satiety	15- or 210-min nasogastric or IV infusion Males
Blundell et al. (1993)	No	Mixed meals	At some (not all) time points fat less satiating than CHO	Males
Caputo and Mattes (1992)	No	Mixed meals	Mean daily intake higher with high fat meal	Males and females. Fat preload contained 14% or 22.5% more kcal than the CHO preload
Van Amelsvoort et al. (1990)	No	Mixed meals	CHO more satiating	Males
Van Amelsvoort et al. (1989)	No	Mixed meals	Reduced hunger ratings 4 h after CHO compared to fat preload	Males
Driver (1988)	Yes	Skim milk, Skim milk + lactose, or full cream milk powder	No difference in satiety	Males and females. Information on calories not provided
Rolls et al. (1991)	Yes	Yogurts with either *CHO:* maltose or *Fat:* corn oil and cream emulsion	No difference in satiety	Males and females
Rolls et al. (1994)	Yes	Yogurts with either *CHO:* maltose or *Fat:* corn oil and cream emulsion	No difference for nondieting males; all others fat less satiating	Subjects varied in dieting status and body weight

de Graaf et al. (1992)	Yes	Drink with either *CHO:* maltodextrine maltose or glucose *Fat:* cream	No difference in satiety	Females
Foltin et al. (1992)	No	Range of fat levels	Effect of fat on satiety depends on fat level in preloads	Males. Protein content higher in fat meals. Fat preload contained 1%–5% more kcal than CHO preload
Foltin et al. (1990)	No	Mixed meals	No difference in satiety	Males
Geliebter (1979)	Yes	*Fat:* corn oil *CHO:* corn starch	No difference in satiety	Males
Warwick et al. (1993)	Yes	Drink with either *Fat:* corn oil or *CHO:* glucose polymer	No difference in satiety	Males and females
Rolls et al. (1988b)	No	Various foods	CHO more satiating	Females
Johnson and Vickers (1993)	No	*CHO:* pasta *Fat:* whipped cream	CHO more satiating	Females and males. Protein content higher in CHO
Westerterp-Plantenga et al. (1997)	No	High fat and high CHO versions of the same lunch	HF less satiating than HC lunch	Males and females
Cotton et al. (1994)	No	Mixed breakfasts	CHO more satiating	Males
Woodend and Anderson (2001)	Yes	*CHO:* sucrose in water *Fat:* safflower oil emulsion	CHO more satiating	Males
Robinson et al. (2005)	Yes	*CHO:* high-maltodextrin soup *Fat:* high-olive oil soup	Fat more satiating	Males; soup delivered IG

* CHO, carbohydrate; IG, intragastric; LCT, long-chain triglycerides; MCT, medium-chain triglycerides. All preloads isocaloric except as noted. All subjects normal weight, nonrestrained eaters except as noted.

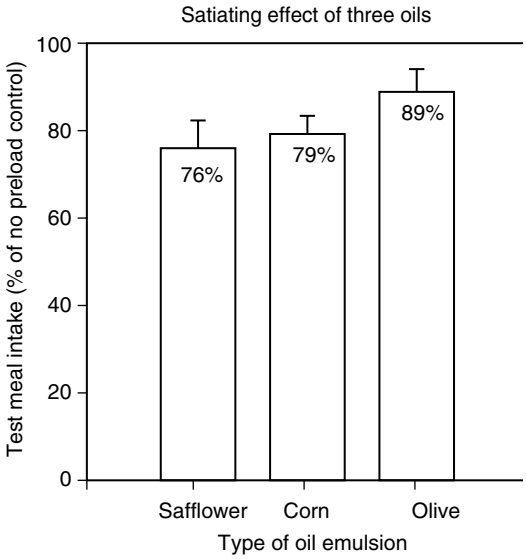

FIGURE 37.2 Test meal intake following preloads of three types of dietary fat.

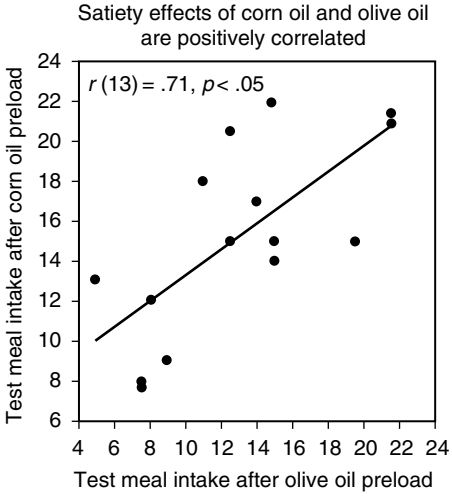

FIGURE 37.3 Correlation of test meal intake following preloads of corn and olive oil.

oils were significantly correlated $r(13) = .71$, $p < .05$ (Figure 37.3), but no other correlations were significant. This suggests that corn and olive oils produce satiety through a similar mechanism, whereas safflower oil exerts its satiating effect though a different mechanism.

Woltman et al. (1995) compared effects of intraduodenal infusions of triolein, oleic acid, and oleic acid + monoolein (2:1 ratio) on subsequent 4-h intake. The three infusions were equivalent with regard to oleic acid content. Infusions of oliec acid and 2:1 oleic:monoolein were equally satiating, and the satiating effect was dose dependent. In contrast, triolein infusion was markedly less satiating. These findings demonstrate that the breakdown products of triglyceride (monoglyceride and free fatty acids) are more satiating than the intact triglyceride, and are consistent with the finding cited previously (Greenberg and Smith, 1996) in which infusion of fatty acids into the duodenum reduced sham-feeding intake more potently than infusion of triglyceride. However, Maggio

and Koopmans (1982) found intragastric triolein and oleic acid + glycerol to be equally satiating. Methodological differences between these studies, which in addition to infusion site included the animals' degree of deprivation and the type of food consumed during the test meal, may explain these inconsistent findings.

C. Individual Differences

The satiety produced by intragastric nutrient infusion has been compared in lean and genetically obese rats. In lean rats, corn oil, MCT, and glucose were equally satiating as measured by test meal intake given 20 min after the preload, whereas in obese rats corn oil was the less satiating nutrient (Maggio et al., 1983). In humans, Rolls et al. (1994) compared the satiating effects of a fat preload and a carbohydrate preload in subjects who differed in their degree of concern about food intake and body weight ("eating restraint"). In normal-weight unrestrained males, fat and carbohydrate were equally satiating. In normal-weight restrained males and in females (lean and obese, restrained and unrestrained) fat was less satiating than carbohydrate. Thus, there are individual differences, in both humans and animals, in the potency of the satiety produced by fat.

To investigate the relationship between individual differences in sensitivity to the postprandial satiety effect of fat and spontaneous daily intake, outbred Long–Evans rats consumed chow ad lib. for 3 baseline days, and then for further 3 days ate a supplement of 48 kcal/day of fat (corn oil emulsion) plus ad lib. chow. Each rat's sensitivity to the satiating effect of fat was indexed by chow intake during the fat supplement phase as a percent of baseline chow intake (thus a lower percent indicated greater sensitivity). Individual differences in sensitivity were reliable across two replications and predicted spontaneous daily kcal intake when animals were allowed to consume a high-fat food ad lib. for 16 days: animals with the least sensitivity to the satiating effect of fat had the highest ad lib. intake (Warwick and Rice, 2003). In humans, Blundell et al. (2005) found that individuals who habitually consumed a high-fat diet and were overweight (termed susceptible) showed a weaker satiety response (suppression of hunger) to a high-fat preload than did individuals who also consumed a high-fat diet but remained lean (termed resistant).

IV. PHYSIOLOGICAL MECHANISM(S) MEDIATING THE SATIATING EFFECT OF FAT

A preabsorptive locus for the anatomical site mediating the satiety produced by LCT (corn oil, intralipid) in rat is indicated by a series of studies by Greenberg and colleagues. In these studies, the satiety produced by fat infusion was indexed by sham-feeding intake. Duodenal infusion of intralipid (LCT) suppressed sham-feeding, whereas intravenous (i.v.) infusion had no effect on sham-feeding (Greenberg et al., 1990, 1993). Although this earlier work used an infusion rate that may have exceeded the physiological rate of delivery of fat into the duodenum, recent data (Greenberg and Strohmeyer, 1997) confirm that a slower rate of intraduodenal infusion of intralipid is satiating. This is consistent with findings from a recent study (Burggraf et al., 1997) in which rats received lipid infusions intragastrically or intravenously continuously during a 17-h period of food access. Intragastric infusion reduced spontaneous food intake to a greater extent than isocaloric infusion of lipid given intravenously.

Researchers have also compared different intestinal sites for their sensitivity to fat's satiating effect. Welch et al. (1988) found that corn oil emulsion infused directly into the jejunum reduced food intake in healthy human volunteers; a less pronounced reduction of food intake was also noted when lipid was infused into the ileum. In rat, duodenal infusion of oleic acid was more inhibitory of food intake than was ileal infusion, whereas glucose infusion showed the opposite pattern (Woltman and Reidelberger, 1995).

Observations (Rolls et al., 1988; Satabin et al., 1991) that MCTs and carbohydrate were more satiating than LCTs implicate the liver as mediating the satiety effects of nutrients. MCTs, similar

to carbohydrates, are absorbed from the intestine into the portal vein and thus flow through the liver, whereas long-chain fats are secreted into the lacteals and bypass the liver. The liver, as a source of satiety signals, has also been indicated by other findings (Friedman, 1991). Afferent signals mediating the satiety produced by at least one fat, oleic acid, are transmitted vagally, since total subdiaphragmatic vagotomy produces insensitivity to the satiating effect of intraduodenal oleic acid infusion (Yox et al., 1991).

The role of the duodenal peptide cholecystokinin (CCK) in the satiating effect of fat is demonstrated by multiple lines of evidence. Administration of the CCK-A antagonist lorglumide attenuated the satiating effect of intraduodenal intralipid (Greenberg et al., 1989) and intraduodenal oleic acid (Yox et al., 1992). Another CCK-A antagonist, devazepide, reversed the satiating effect elicited by intraduodenal oleic acid, but only when relatively small loads of oleic acid were given (Woltman et al., 1995). This suggests that additional, CCK-independent mechanisms mediate the satiating effect of oleic acid, and that these additional mechanisms were more potently stimulated by the higher doses of oleic acid. Measurement of endogenous systemic CCK levels associated with infusion of various nutrients revealed no correlation between the nutrient's satiating effect and plasma CCK levels, suggesting that CCK receptors mediating satiety are not stimulated by bloodborne CCK (Brenner et al., 1993).

V. LONG-TERM BODY WEIGHT REGULATION

A. Fats vs. Carbohydrates

As stated at the beginning of this chapter, a high-fat diet often elicits hyperphagia, a caloric intake that exceeds energy expenditure. If this hyperphagia is sustained, as it typically (although not inevitably, see Boozer, 1997) is, weight gain will result. A dose–response relationship between dietary fat content and spontaneous daily kcal intake has been observed (Warwick, 2003). It is notable that high-fat foods need not be the sole nutrient source in order to promote greater weight gain than high-carbohydrate foods. Rats consuming 30 mL (69 kcal) per day of high-fat liquid diet (HF, Table 37.2) in addition to ad lib. chow gained more weight over 16 days than rats consuming an identical number of calories from a high-carbohydrate liquid diet (HC). A similar finding was noted when the daily ration was smaller (32 kcal) and consisted of pure fat (corn oil emulsion) or pure carbohydrate (sucrose solution) (Z. S. Warwick, unpublished data). Previous studies in which intake of dietary supplements of fat or carbohydrate were not equated had similar findings (Sclafani et al., 1993). Earlier studies compared the effects of fixed daily loads of various macronutrients delivered intragastrically on spontaneous food intake and weight gain. Geliebter et al. (1984) gave rats daily intragastric loads of either fat (corn oil) or carbohydrate (sucrose) for 6 weeks, while Liu and Yin (1974) gave intragastric loads of either fat (shortening) or carbohydrate (glucose) for 5 weeks. In both studies the fat- and carbohydrate-loaded groups had similar ad lib. kcal intake and weight gain. The inconsistent findings from studies in which dietary supplements were consumed orally (Sclafani et al., 1993; Warwick et al., 2000) compared with studies in which supplements were intubated may reflect the different route of nutrient delivery and/or other methodological differences.

B. Fatty Acid-Specific Effects

Medium-chain fatty acids are less obesogenic than long-chain fatty acids. Rats fed diets rich in MCT gained less weight than animals consuming a diet containing LCT (Bray et al., 1980; Geliebter et al., 1983). The lesser weight gain associated with MCT can be due to lower daily kcal intake (Edens and Friedman, 1984), greater energy expenditure via postprandial thermogenesis (Seaton et al., 1986), or a combination of these factors.

A diet high in saturated fat (from beef tallow) promoted greater daily kcal intake than a diet high in polyunsaturated fat (corn oil), an effect observed across two levels (5% and 34%) of dietary fat

(Mullen and Martin, 1990). Weight gain was greatest in rats fed a 34% tallow diet. In one study, a diet high (45% of kcal) in safflower oil produced less weight gain than a diet containing the same proportion of kcal from tallow (Shimomura, cited in Hill et al., 1993), while another comparison of these two fat sources, at slightly lower levels (32%) of dietary kcal failed to reveal differential weight gain (Awad, cited in Hill et al., 1993). A comparison of diets formulated with either safflower or flaxseed oil found no difference in energy intake over 34 days, at two levels of fat content (Rice and Corwin, 2002). Hill et al. (1993) fed rats with diets containing fish oil, corn oil, lard, or MCT oil as 45% of kcal for 6 months; no differences in body weight gains were noted, although across the entire study, average kcal intake by fish-oil fed rats tended to be lower than lard- and MCT-fed rats.

Lucas et al. (1989) gave rats ad lib. access to either pure corn oil or pure vegetable shortening in addition to ad lib. chow for 36 days. The shortening group ate more total kcal (fat + chow) and gained more weight than the corn oil group. However, when the diet options were presented as emulsified gels, no differences in intake or body weight were observed. Taken together, these findings indicate that differential responding to various fats is attributable not only to differences in fatty acid composition, but also to other factors such as physical form.

C. Individual Differences

In rats, differences in the obesity-promoting effects of high-fat diets have been found both across (Schemmel et al., 1970) and within (Levin et al., 1997) strains. Rats susceptible to diet-induced obesity differ metabolically from rats that do not gain excess weight when put on a high-fat diet (Boozer, 1997; Levin et al., 1997). In a group of ten rats fed a high-fat liquid diet (Table 37.2), a significant correlation was found between kcal intake and weight gain (Figure 37.4). However, the proportion of variance in weight gain accounted for (r^2) by kcal intake was .37, indicating that 63% of the variance in weight gain was attributable not only to kcal intake but also to other factors such as individual differences in metabolic rate and/or activity.

VI. LEARNED CONTROLS OF SATIETY: FATS VS. CARBOHYDRATE

Rats and humans can learn to associate a food's flavor (the composite of its taste/smell/texture) with its postingestive effects (e.g., satiety, relief from nutritional deficiency), a process called

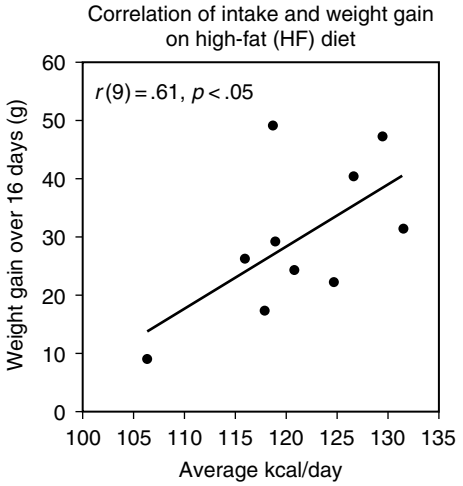

FIGURE 37.4 Relationship between caloric intake and weight gain on a high-fat diet.

flavor-postingestive consequence (or calorie-based learning). The behavioral evidence of such learning is modification of intake in response to the flavor, independent of the unique postingestive effects of the food. This is illustrated by the following example (from Warwick et al., 1997): rats repeatedly consumed a fixed amount (e.g., 30 mL) of a concentrated nutrient solution (1.6 kcal/mL) containing a distinctive flavor such as grape, and had an equal number of trials consuming 30 mL of dilute nutrient solution (0.2 kcal/mL) containing a different flavor such as cherry. Then a "two-bottle test" was conducted, in which cherry- and grape-flavored solutions were offered simultaneously. Importantly, in this test both solutions had identical nutrient density (0.9 kcal/mL), which was midway between the densities used in the initial training. When the nutrient used was carbohydrate (sucrose, glucose, fructose, maltodextrine), in two-bottle test rats consumed *less* of the flavor previously paired with the more concentrated nutrient solution (grape in the example given). This is interpreted as indicating a learned control of satiety: rats had learned during training that grape was associated with strong satiety signals elicited by the concentrated nutrient, while cherry was associated with weak or no satiety signals. When given a choice in the two-bottle test, rats consumed less of the grape-flavored solution since they anticipated its greater satiating effect (which was, however, not realized since in the two-bottle test the nutrient density of grape and cherry solutions were equated).

In contrast, when fat (corn oil) was used as the nutrient, rats did not exhibit learned satiety in the two-bottle test. However, when these same corn oil emulsions that failed to entrain learned satiety were sweetened with noncaloric saccharin during training, rats did exhibit learned satiety by consuming less of the flavor previously paired with the more concentrated fat emulsion (Figure 37.5).

This is consistent with earlier work by Booth (1972), who reported that the acquisition of conditioned satiety to solid foods differing in fat content depend on the particular taste–odor cues used. Lucas and Sclafani (1989) found that saccharin facilitated acquisition for a flavor paired with intragastrically administered fat, probably because it increased the quantity of fat infused. Differential intake can be ruled out as an explanation of saccharin's facilitation of learned satiety in the Warwick et al. (1997) study, since intake was equated at 30 mL per trial during training. There are a number of

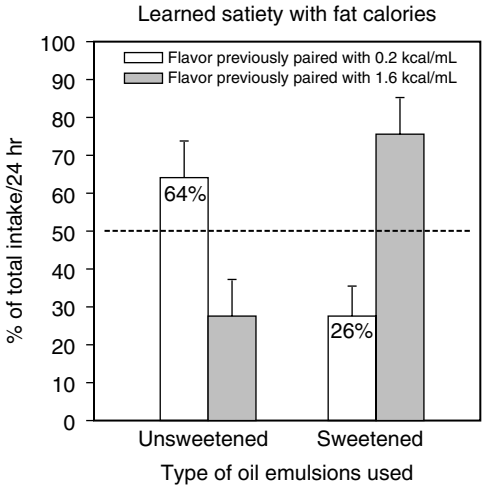

FIGURE 37.5 Two-bottle test intake in rats trained with unsweetened (left bars) or sweetened (right bars) distinctively flavored corn oil emulsions at 0.2 kcal/mL and 1.6 kcal/mL. Both flavors presented in 0.9 kcal/mL emulsion in two-bottle test. Dashed line indicates 50% intake (no preference). Percentages within bars indicate intake of the 0.2 kcal/mL-paired flavor as a percent of total two-bottle test intake.

possible explanations, not mutually exclusive, for the finding that saccharin enhanced conditioned satiety entrained by fat. Tasting saccharin alters the digestive profile of intragastrically administered corn oil (Ramirez, 1985), which may under certain conditions enhance its satiating effect. A human study comparing nutritionally identical preloads differing in sweetness found the sweeter preload to be more satiating (Warwick et al., 1993). Another possibility relates to the observation that rats trained with sweetened oil emulsions appeared to consume the 30 mL of training solution more rapidly than rats trained with unsweetened emulsions, although consumption time was not directly measured. More rapid consumption would produce a bigger calorie bolus that would presumably increase satiety. Finally, "flavor–flavor" conditioning may also have been a factor; although both concentrations of emulsion contained equal amounts of saccharin (0.4%), the more concentrated oil emulsion may have partially masked saccharin's sweetness with the result that the less-concentrated emulsion had greater sweetness intensity. Since association of a flavor with sweetness is sufficient to condition a preference (Warwick and Weingarten, 1994a), preferential intake of the flavor previously paired with the dilute fat emulsion in two-bottle testing may have been due to this flavor's previous association with greater sweetness.

Additional evidence that fat-entrained flavor-cued conditioned satiety is less readily acquired comes from recent work in which the number of training trials was varied. Separate groups of rats were trained with two distinctively flavored concentrations (0.2 and 3.6 kcal/mL) of either sucrose solution or corn oil emulsion. Learned satiety (i.e., lesser intake of the flavor previously paired with the higher concentration) was evident after only two training trials with each concentration for sucrose-trained animals, but required at least twice as many trials for fat-trained animals (C. Revelle, unpublished data).

Although flavor-cued learned satiety represents an inhibitory effect on meal size (negative feedback); calorie-based learning can also exert a stimulatory effect on meal size (positive feedback) by increasing the acceptance and/or preference of an associated flavor. "Flavor preference learning" is observed when a flavor previously paired with calories is preferred, in two-bottle testing, to a flavor paired with fewer or no calories. All energy-yielding substances (fat, carbohydrate, protein, alcohol) can reinforce such flavor preference learning (e.g., Mehiel and Bolles, 1988; Perez et al., 1995); the energy must be metabolically available since diabetic rats did not learn to acquire a preference for a flavor paired with carbohydrate, although they did acquire a fat-based preference (Tordoff et al., 1987). In a recent study comparing the reinforcing effect of various fat sources in flavor preference learning (Ackroff et al., 2005), safflower and corn oils tend to condition stronger preferences (relative to a water-paired flavor) than did beef tallow and vegetable shortening. (For discussion of the interaction of the behaviorally opposing effects of learned flavor preference and learned satiety on the control of meal size, see Warwick, 1996; Lucas et al., 1998; Ackroff and Sclafani, 2006.)

VII. SUMMARY AND CONCLUSIONS

When compared to an isocaloric quantity of carbohydrate, dietary fat is often observed to exert a less potent satiating effect during a meal and also after a meal. The higher caloric density of fat plays a role: per kcal, fat produces less stomach distention, which is known to generate potent satiety signals. However, even when the caloric density of high-fat and high-carbohydrate foods is equated, fat is often less satiating. Individual differences in sensitivity to the fat-induced satiety have been found, and various fats and their constituent fatty acids differ in their satiating effects. Learned orosensory control of intake is less readily acquired when the calorie source is fat, although experimental conditions such as the composition of the maintenance diet and whether or not the fat is associated with a sweet taste moderate this effect. Further research is needed to clarify the variables—such as taste, density, subject characteristics—that mediate the relative satiating effects of fat and carbohydrate, and the mechanisms by which these variables exert their effect.

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38 Fatty Acids and Growth and Development

Margit Hamosh

CONTENTS

I. INTRODUCTION

This chapter will address several aspects of fatty acid requirements specific to the perinatal and neonatal period. It is not intended to cover all aspects of fatty acid needs and metabolism during development, since these topics have been reviewed extensively (Hamosh, 1987, 1988, 1991a; Innis, 1991, 1993; Giovannini et al., 1995; Koletzko, 1997). The supply of fatty acids to the newborn is in great measure dependent on the digestive and absorptive maturity of the gastrointestinal system (Hamosh, 1996; Hamosh and Hamosh, 1999); therefore, this aspect of neonatal function will be briefly reviewed.

II. FETAL PERIOD

During fetal development, fatty acids are transferred from mother to fetus through the placenta (Soderhjelm, 1953; Koren and Shafrir, 1964). The magnitude of the transfer, depending on species (Koren and Shafrir, 1964; Hershfield and Nemeth, 1968), is a function of maternal–fetal gradient (Nobel et al., 1979; Knopp, 1986), fetal blood albumin concentration (Hershfield and Nemeth, 1968; Dancis et al., 1977; Elphick and Hull, 1977b; Thomas and Lowy, 1983; Hendrickse et al., 1985), occurs rapidly (within minutes) (Portman et al., 1969), and is subject to maternal metabolic regulation (higher in fasted animals or after heparin administration to the mother) (Muller et al., 1964; Edison et al., 1975).

TABLE 38.1
Fatty Acids in the Fetus

Placental transfer from mother; magnitude depends upon species and is a function of
Maternal–fetal gradient
Fetal blood albumin concentration
Possibly fatty acid chain length subject to maternal metabolic regulation
De novo synthesis
High fetal hepatic lipogenesis in late gestation precursors are
Glucose, glycogen, lactic acid, ketone bodies

The controversy whether fatty acid chain length affects the placental free fatty acid (FFA) transfer rate is still not settled (Dancis et al., 1974; Elphick and Hull, 1977b). Uptake of FFAs by the placenta and synthesis of triglycerides suggest that the placenta may protect the fetus from excessive fatty acid transfer (Freinkel, 1965; Diamant et al., 1980). The significance of FFA transport to the fetus is supported by a relationship between maternal fatty acid levels and infant birth weight (Knopp et al., 1991). Triglyceride fatty acids are transferred to the fetus only after release of the fatty acids by placental lipoprotein lipase (Mallov and Alousi, 1965; Elphick and Hull, 1977a; Clegg, 1981; Thomas et al., 1984). Estimates of the significance of placental transfer of lipids vary from 20% of the amount required for the accumulation of fetal adipose tissue during the last trimester of pregnancy (Dancis et al., 1973) to 50%–100% of fetal needs (Elphick et al., 1977; Coleman, 1986) and vary according to species (Kimura, 1991). Because placental transfer of fatty acids cannot account for all fetal needs, endogenous synthesis of fatty acids by the fetus may be important (Kimura, 1991). Indeed, fetal hepatic lipogenesis is high in late gestation at a time of rapid adipose tissue accumulation and is higher than in the adult (Villee and Hagerman, 1958; Roux, 1966; Ballard and Hanson, 1967; Taylor et al., 1967). Precursors of fatty acid synthesis in the fetus are glycogen, glucose, lactate (especially in the lung), and ketone bodies (mainly in liver and brain) (Ville and Hagerman, 1958; Roux, 1966; Ballard and Hanson, 1967; Taylor et al., 1967; Edwards, 1974; Seccombe et al., 1977; Maniscalco et al., 1982; Farrell and Bourbon, 1986). Increased lipogenesis in hyperglycemic fetuses suggests that availability of glucose controls fetal lipogenesis (Ktorza et al., 1983; Williamson, 1988) (Table 38.1).

III. NEONATAL PERIOD

At birth, there is a sudden change from a high-carbohydrate to a high-fat diet. Fatty acids in milk or formula are, however, present almost exclusively as triglycerides rather than as fetal FFAs transferred through the placenta to the fetus. Although classic (colipase-dependent) pancreatic lipase is poorly developed at birth, the newborn is able to digest milk and formula triglyceride with the aid of gastric lipase and probably also pancreatic carboxyl ester lipase and, in the case of the breast-fed infant, the bile salt-dependent lipase of human milk (Hamosh et al., 1985; Hamosh, 1994a,b, 1996; Hamosh and Hamosh, 1999). Fatty acids of animal origin are usually unbranched, monocarboxylic acids containing an even number of carbon atoms varying from 2 to 24 in chain length.

The fatty acid chains may be either saturated or unsaturated (Table 38.2). Most biologically important fatty acids are esterified with glycerol, whereas a small portion are linked with other compounds or are free. Fatty acids are generally classified according to chain length (short C_2C_6; medium C_8–C_{10}; intermediate C_{12}–C_{I4}, and long chain = C_{16}) and degree of desaturation; that is, saturated (no double bond), monounsaturated (one double bond), and polyunsaturated (two or more double bonds). The double bonds occur in two isomeric forms: *cis* and *trans.* Most plants and mammals contain fatty acids of the *cis* configuration, which is more flexible and has greater fluidity than

TABLE 38.2
Structure of Fatty Acids

Descriptive Name	Systematic Name	Carbon Atoms (Chain Length)	Double Bonds	Position of Double Bonds[a]	Position and Terminal Double Bond	Formula
Short-Chain FA						
Acetic		2	0			C_2:0
Butyric		4	0			C_4:0
Caproic	Hexanoic	6	0			C_6:0
Medium-Chain FA						
Caprilic	Octanoic	8	0			C_8:0
Capric	Decanoic	10	0			C_{10}:0
Intermediate-Chain FA						
Lauric	Dodecanoic	12	0			C_{12}:0
Myristic	Tetradecanoic	14	0			C_{14}:0
Long-Chain FA						
Palmitic	Hexadecanoic	16	0			C_{16}:0
Palmitoleic	Hexadecaenoic	16	1	9	n-7	C_{16}:1n7
Stearic	Octadecanoic	18	0			C_{18}:0
Oleic	Octadecaenoic	18	1	9	n-9	C_{18}:1n9
Linoleic[b]	Octadecadienoic	18	2	9,12	n-6	C_{18}:2n6
alpha-Linolenic[b]	Octadecatrienoic	18	3	9,12,15	n-3	C_{18}:3n3
gamma-Linolenic[b]	Octadecatrienoic	18	3	6,9,12	n-6	C_{18}:3n6
Homolinolenic[b]	Eicosatrienoic	20	3	8,11,14	n-6	C_{20}:3n6
Arachidonic[b,c]	Eicosatetraenoic	20	4	5,8,11,14	n-6	C_{20}:4n6
	Eicosapentaenoic	20	5	5,8,11,14,17	n-3	C_{20}:5n3
Cervonic[b,d]	Docosahexaenoic	22	6	4,7,10,12,15,19	n-3	C_{22}:6n3

[a] Position of the one or more double bonds listed according to the D numbering system. In this numbering system, only the first carbon of the pair is listed; that is, 9 means position 9, 10, starting from the carboxyl end.
[b] Essential fatty acids.
[c] No commonly used descriptive name.
[d] In the n numbering system, only the first double bond from the methyl end is listed and, as above, only the first carbon of the pair is written.

Source: From Hamosh, M. (1998b). *Biochem. Soc. Trans.* 26:96–103.

the *trans* isomeric form formed in ruminants' stomach by bacteria or by the technical hydrogenation of fat. *Trans* fatty acids have lower melting points, are less fluid than their *cis* isomers, and are structurally and functionally similar to saturated fatty acids. Polyunsaturated fatty acids (PUFAs) of the n-3 and n-6 families are not synthesized by humans and higher animal species and have to be added to the diet. Because of their important physiological functions, they are considered essential fatty acids (EFA). In general, stored lipid contains higher amounts of saturated fatty acids than do structural lipids, which contain a higher percentage of PUFAs (Figure 38.1). The function of lipids in mammals is listed in Table 38.3.

Over 95% of the fat in human milk is present in eight major fatty acids (C_{10}–C_{22}:6n-3) (Table 38.4). The fatty acids in human milk (and in the milk of other species) are present mainly (98%–99%) as triglycerides (Bitman et al., 1983b) (Table 38.5). FFAs in human milk are an indicator of poor collection and storage techniques and are a sign of lipolysis after collection (Bitman et al., 1983a).

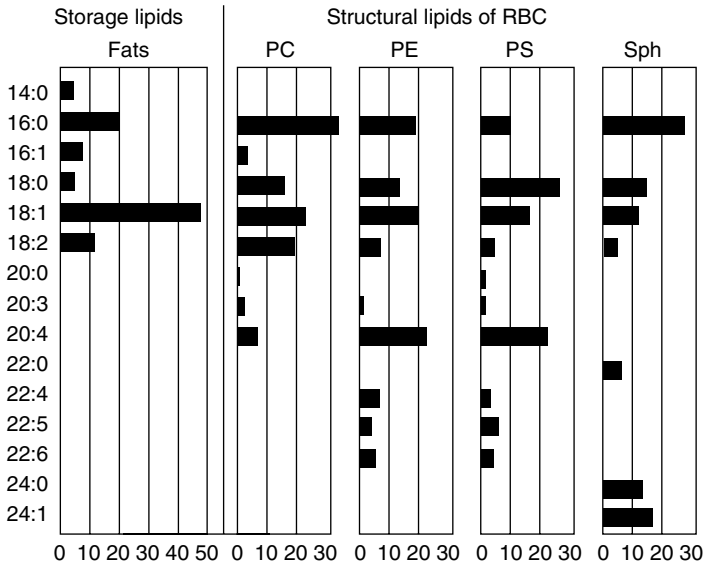

FIGURE 38.1 Fatty acid composition of storage and structural lipids. Storage lipid is triglyceride, whereas structural red blood cells (RBC) lipids are phospholipids and sphingomyelin: PC, PE, PS are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, respectively, Sph is sphingomyelin. *(*From R.W. McGilvery and G.W. Goldstein (1983). *Biochemistry: A Functional Approach*, Philadelphia, Saunders.)

TABLE 38.3
Function of Lipids in Mammals

Lipid Class	Function
Glycerides	Fatty acid storage, metabolic intermediates
Phospholipids	Membrane structure, lung surfactant
Sterols	
Cholesterol	Membranes and lipoprotein structure; precursor of steroid hormones; degradation products are bile salts important in fat digestion and absorption
Cholesteryl ester	Storage and transport
Fatty acids	Major energy source, components of most lipids, precursors of prostaglandins, essential for normal brain development (long-chain polyunsaturated fatty acids)

Source: From Hamosh, M. (1998b). *Biochem. Soc. Trans.* 26:96–103.

Medium- and intermediate-chain fatty acids amount to 10% of total fatty acids in mature milk of mothers of term infants but contribute 17% of total fatty acids in milk produced by mothers of preterm infants (Bitman et al., 1983b).

Saturated fatty acids constitute 42% and unsaturated fatty acids account for 57% of total lipid in human milk. Linoleic acid concentrations are higher in recent studies (Bitman et al., 1983b) than in earlier reports (Jensen et al., 1980) and reflect the higher intake of polyunsaturated fats by the American population. EFA contents are higher in colostrum and transitional milk than in mature milk (Bitman et al., 1983b). Long-chain PUFAs (LCPUFAs) derived from linoleic acid (20:2n-6, 20:3, 20:4, 22:5n-6) and from linolenic acid (20:5, 22:5n-3, 22:6n-3) show a similar decrease throughout lactation. The level of these fatty acids is significantly higher in colostrum and milk of mothers of preterm infants than mothers of full-term infants (Bitman et al., 1983b). Thus, human milk provides the essential long-chain unsaturated fatty acids linoleic (C18:2n-6) and linolenic (C18:3n-3), as well

TABLE 38.4
Fatty Acid Composition (%) of Human Milk (Comparison of Milk[a] from Mothers Who Delivered at 26–30 Weeks [VPT], 31–36 Weeks [PT], and 37–40 Weeks [T] of Pregnancy)

	Fatty Acid	VPT[b] 26–30 Weeks	PT 31–36 Weeks	T 37–40 Weeks
Capric	10:0	1.37 ± 0.17	1.27 ± 0.18	0.97 ± 0.28
Lauric	12:0	7.47 ± 0.72	6.55 ± 0.77	4.46 ± 1.17
Myristic	14:0	8.41 ± 0.83	7.55 ± 0.89	5.68 ± 1.36
	15:0	0.23 ± 0.04	0.27 ± 0.05	0.31 ± 0.07
Palmitic	16:0	20.13 ± 1.40	23.16 ± 1.49	22.20 ± 2.28
	16:1	2.56 ± 0.24	2.92 ± 0.26	3.83 ± 0.39
	17:0	0.34 ± 0.22	0.60 ± 0.24	0.49 ± 0.36
Stearic	18:0	7.24 ± 1.13	7.25 ± 1.21	7.68 ± 1.85
Oleic	18:1	33.41 ± 1.67	33.74 ± 1.79	35.51 ± 2.73
Linoleic	18:2	15.75 ± 1.22	13.83 ± 1.30	15.58 ± 1.99
Linolenic	18:3	0.76 ± 0.13	0.76 ± 0.14	1.03 ± 0.21
	20:0	0.17 ± 0.07	0.09 ± 0.08	0.32 ± 0.11
	20:2	0.35 ± 0.13	0.33 ± 0.13	0.18 ± 0.20
	20:3	0.51 ± 0.09	0.43 ± 0.10	0.53 ± 0.15
Arachidonic	20:4n-6	0.55 ± 0.18	0.58 ± 0.19	0.60 ± 0.29
	20:5	0.04 ± 0.05		
	21:0	0.05 ± 0.07	0.07 ± 0.08	0.17 ± 0.12
	22:4	0.13 ± 0.10	0.24 ± 0.11	0.07 ± 0.16
	22:5n-6	0.11 ± 0.05	0.04 ± 0.05	0.03 ± 0.08
	22:5n-3	0.42 ± 0.09	0.12 ± 0.10	0.11 ± 0.15
Docosahexaenoic	22:6n-3	0.24 ± 0.09	0.21 ± 0.09	0.23 ± 0.14

[a] Milk was collected at 6 weeks of lactation.
[b] Means ± SE.

Source: Data from Bitman, J., et al. (1983b). *Am. J. Clin. Nutr.* 38:300–312.

TABLE 38.5
Composition of Human Milk Fat[a]

	%	Concentration	Location
Glycerides		3.0–4.5 g/dL	Major components of the core of milk fat globules
Triglycerides	98.70[b]		
Diglycerides	0.01		
Monoglycerides	0		
Free fatty acids	0.08		
Cholesterol		10–15 mg/dL	
Phospholipids		15–20 mg/dL	Major components of milk fat globule membrane
Sphingomyelin	37[b]		
Phosphatidylcholine	27		
Phosphatidylserine	9		
Phosphatidylinositol	6		
Phosphatidylethanolamine	19		

[a] Mature milk from mothers of term infants.
[b] Percentage in lipid class (glycerides and phospholipids, respectively).

Source: Data from Hamosh, M., et al. (1985). *Pediatrics* 75(Suppl):146–150.

as their longer chain elongation and desaturation derivatives, such as arachidonic acid (AA, C20: 4n-6) and docoshexaenoic acid (DHA, C22:6n-3).

Although EFA deficiency is rare, it might occur (Holman et al., 1972). EFA deficiency is assessed by the triene:tetraene ratio. The n-9, n-6, and n-3 unsaturated FAs compete for the same desaturase enzymes in their metabolic pathways. Desaturation of n-9 FAs leads to the formation of trienes, whereas desaturation of n-6 FAs leads to formation of tetraenes. The n-6 and n-3 families have greater affinity for these desaturases than does the n-9 structure (oleic acid), resulting in competitive inhibition of the production of trienes. Deficiency in linoleic acid (18: 2n 6) leads to lower production of tetraenes, whereas the production of trienes is increased. A triene:tetraene ratio of >0.4 was considered to be indicative of EFA deficiency (Holman, 1979); however, currently a ratio of 0.2 is considered a mere accurate indicator of the presence of mead acid (eicosatrienoic acid, C20: 3n-9). The above ratio is, however, not an indicator of deficiency of only n-6 or only n-3 fatty acids or of their supply to the infant (Innis, 1991). Furthermore, this ratio is not an indicator of deficiency or sufficiency of DHA or AA.

Infants fed parenterally have to be given lipid, since EFA deficiency interferes with normal lung surfactant synthesis (essential for pulmonary function) (Friedman and Rosenberg, 1979) and platelet function (Friedman et al., 1977).

The fatty acid profile of human milk is generally used as the "gold standard" for the composition of the fat in infant formulas. It has to be taken into account, however, that the fatty acid composition of milk fat changes in the course of lactation and that it is highly dependent on maternal diet. Thus, although the amount of fat in milk seems to be constant throughout lactation and is typical of each woman (Bitman et al., 1983b), the composition of fat (i.e., the fatty acid profile of milk triglyceride) is highly variable and dependent on diet (Insull et al., 1959; Craig-Smith et al., 1984; Harris et al., 1984; Michaelsen et al., 1994). Indeed, dietary effects on milk fatty acid composition are detectable within several hours after consuming a meal (i.e., the time necessary for fat absorption and digestion) and, after incorporation of the circulating triglyceride fatty acids into mammary gland triglyceride (Hamosh et al., 1970; Hamosh and Hamosh, 1983), the time necessary for the formation of milk fat globules and their secretion into milk (Stemberger and Patton, 1981; Hachey et al., 1987). Table 38.6 lists the factors that affect milk fat content and composition. Length of gestation and length of lactation affect especially the content of the lipids that constitute the milk fat globule membrane, phospholipids, and cholesterol (Bitman et al., 1983b). The latter are higher in the early stages of lactation (colostrum and transitional milk), because the milk fat globules are much smaller than in mature human milk. It has long been known that the milk fat content changes drastically during each feed (Macy et al., 1931; Hytten, 1954). More recent studies have shown that the mechanism for endogenous synthesis of fatty acids seems to be exhausted in women of very high parity (Prentice et al., 1989), that infants who receive low-fat containing milk tend to nurse more frequently and for longer periods, thereby causing an increase in milk volume (Tyson et al., 1992), and that there is a strong positive relationship between weight gain during pregnancy and milk fat content (Michaelsen et al., 1994).

IV. *TRANS* FATTY ACIDS

The relationship between increased maternal consumption of industrially hydrogenated unsaturated vegetable oils (margarine) (Senti, 1985), which is high in *trans* fatty acids and an increase in these fatty acids in mother's milk has led to an evaluation of these fatty acids during development. *Trans* fatty acids are not natural components (most unsaturated fatty acids have the *cis* configuration) and are formed during hydrogenation in the process of the manufacture of margarine. The common *trans* fatty acids are the monounsaturated elaidic (*cis*: 1n-9t) and *trans* vaccenic (*cis*: 1n-7t) acids and the *trans* polyunsaturated linolelaidic (*cis*: 2n-6t) acid (Putnam et al., 1982; Dotson et al., 1992; Koletzko, 1997). Recent studies suggest that *trans* fatty acids impair the conversion of linoleic acid to its long-chain metabolites *in vivo* in animals and *in vitro* in animal cells and human fibroblasts,

TABLE 38.6
Factors That Affect Milk Fat Content and Composition

Variable	Change	Reference
Gestation	LCPUFAs higher in preterm[a] and transitional[b] milk	Bitman et al. (1983b)
Lactation stage	Phospholipid, cholesterol higher in colostrums (preterm[a] > term[a])	Bitman et al. (1983b)
Parity	P 10+: lower endogenous synthesis of FAs (C_6–C_{16})	Prentice et al. (1989)
Volume	Low milk fat concentration associated with high volume	Tyson et al. (1992)
Feed	Fat: fore < mid < hind milk	Macy et al. (1931); Hytten (1954)
Diet		
High-CHO intake	Increase in endogenous synthesis of FA (C_6–C_{16})	Insull et al. (1959)
Low-caloric intake	Increase in palmitic acid (C_{16})	Insull et al. (1959)
Hydrogenated fat	Increase in *trans* fatty acids	Chappell et al. (1985); Craig-Smith et al. (1984)
High LCPUFAs	Increase in LCPUFAs	Harris et al. (1984)
Pregnancy weight gain	Positively associated with milk fat content	Michaelsen et al. (1994)

[a] FA, fatty acids; CHO, carbohydrate; LCPUFAs, long-chain polyunsaturated fatty acids. *Preterm, term* refer to milk or colostrums of women who deliver prematurely (26–37 weeks gestation) or at term (37–40 weeks gestation).
[b] *Transitional* refers to the period between secretion of colostrums and mature milk (week 2–3 of lactation).

apparently caused by competitive inhibition of desaturation enzymes (Mahfouz et al., 1980, 1981; Koletzko, 1992, 1997). Indeed, inverse correlations between *trans* fatty acids and LCPUFAs have been reported in human premature infants and in healthy children (Koletzko, 1992, 1997; Decsi and Koletzko, 1995b). Koletzko (l997) suggests that these findings are compatible with impairment of LCPUFA synthesis in humans, which is a potentially serious side effect of *trans* fatty acids in view of the importance of LCPUFA availability for infant growth and development. An impairment of growth has also been attributed to *trans* fatty acids as seen in pre- and postnatal studies in animals (Koletzko, 1991) and in premature infants studied at birth (Koletzko, 1992).

The potential sources of *trans* fatty acids in infant formulas are partially hydrogenated vegetable oils or *trans* fatty acids formed during the formula manufacturing process (Carrol, 1989).

Current regulatory bodies restrict the upper limit of *trans* fatty acid content of infant formulas to 4% of total fat (Scientific Committee for Food, 1993) or to none (Life Sciences Research Office, 1998).

Since the human milk fat composition reflects the composition of the fat consumed by the mother, general consumption of fatty acids as well as transfer through milk to the infant can be assessed. In general, consumption of Western diets that contain higher levels of *trans* fatty acids leads to higher *trans* fatty acids in maternal milk. Table 38.7 summarizes some of the data on *trans* fatty acids in human milk and compares them with the concentrations in bovine milk. Consumption of a diet low in animal fat and high in carbohydrate and fiber leads to lower levels of *trans* fatty acids in the milk of Nigerian women.

In Western countries where consumption of olive oil is high (France, Spain, and Italy), the human milk *trans* fatty acid concentration tends to be lower (Billeaud et al., 1997). Data on *trans* fatty acid consumption in the United States across ages 2–70 years have recently been published for a cohort of 11,258 subjects (Allison et al., 1999). It is interesting that identical levels of *trans* fatty acids (2.6% of total energy, 7.4% of fat energy) are consumed irrespective of age (Allison et al., 1999). Furthermore, consumption of *trans* fatty acids in the United States has not changed markedly in the past decade (Hunter and Applewhite, 1991).

TABLE 38.7
***Trans* Fatty Acids in Human and Bovine Milk**

	Human Milk				
Country	**United States[a]**	**United States (1)**	**Germany (2)**	**Nigeria (3)**	**Bovine Milk (4)**
Fatty Acid (%)					
14:1n-5t	0.07	—	0.19	0.04	0.9
16:1n-7t	0.36	—	0.46	0.27	1.8
18:1n-9t	2.67	4.72	3.12	0.86	—
18:2tt	—	—	0.14	0.12	0.47

[a]Normalized compilation from 15 papers (Tomarelli, 1988) as cited in Jensen (1995).

Source: (1) From Dotson, K.D., et al. (1992). *Lipids* 27:933–939; (2) From Koletzko, B., et al. (1988). *Am. J. Clin. Nutr.* 47:954–959; (3) From Koletzko, B., et al. (1991a). *Z. Ernahrungswiss. Eur. J. Nutr*. 30:289–297; (4) From Jensen, R.G. (1995). *Handbook of Milk Composition*, Academic Press, San Diego, CA, p. 566.

In view of the recent (October 2006) voluntary elimination of *trans* fatty acids by several fast food chains in the United States, it can be assumed that the food industry will follow suit and the general content of *trans* fatty acids will be lowered in the United States. This will also affect the newborn, since maternal milk FA content reflects maternal intake, and thus also the level of *trans* fatty acids.

About 20%–25% of *trans* fatty acids in the American diet are naturally occurring and that value, 1.1–1.34 g/day (total *trans* fatty acid consumption of 5.3 g/day), has remained quite constant during the past decade (Hunter and Applewhite, 1991; Allison et al., 1999).

Recent studies show that *trans* fatty acids have decreased in human milk in Canada, where their amount in food has to be listed. The relatively low content of *trans* fatty acids in bovine milk suggests that *cow* milk-based formulas might provide lower *trans* fatty acid amounts to infants than does mother's milk (Tomarelli, 1988; Jensen, 1995; Life Sciences Research Office, 1998).

V. MEDIUM-CHAIN FATTY ACIDS

Medium-chain triglyceride (MCT) oil is normally prepared from coconut oil and contains a *mixture of* fatty acids from C6 to C12 (C6:0-1-2%; C8:0-65-75%; C10:0-25-35%; C12:0-1-2%) (Bach and Babayan, 1982). Although human milk, usually contains less than 10% medium-chain fatty acids (MCFAs) (Bitman et al., 1983b) except for certain maternal diseases (Hamosh and Bitman, 1992), such as hypobetalipoproteinemia, diabetes, cystic fibrosis (Bitman et al., 1987; Hamosh and Bitman 1992), or consumption of a high-carbohydrate diet (Insull et al., 1959), some infant formulas for premature infants contain up to 50% MCFAs. Standard formulas for healthy term infants contain only little MCFAs. Because of the frequent use of MCT formula for preterm infants, we will discuss aspects of their synthesis and levels in human milk as well as their digestion and absorption in infants.

The human mammary gland is able to synthesize de novo MCFAs as well as to elongate and desaturate *some* long-chain fatty acids (LCFAs) shortly after parturition. Prepartum mammary secretions (collected between 1 and 70 days before full-term delivery) contain lower amounts of fat than colostrum and mature milk (1.15 and 1.28 g/dL at an average of 42 and 9 days before delivery, respectively), and have a markedly different lipid composition than postpartum secretion (Bitman et al., 1986). The data presented in Table 38.8 show that prepartum secretions contain considerably lower concentrations of C10, C12, and C14 than postpartum colostrum or mature milk. As prepartum secretions collected at an average of 42–70 days before term delivery correspond to secretions collected at 6–10 weeks prepartum, the MCFA composition of total lipids in these secretions

TABLE 38.8
Medium-Chain Fatty Acids in Human Mammary Prepartum Secretion, in Colostrum, and in Milk Secreted after Premature of Full-Term Delivery

		Fatty Acid (%)		
Specimen	***n***	**10:0**	**12:0**	**14:0**
Prepartum secretion[a]	10	0.10 ± 0	1.7 ± 0.4	4.9 ± 0.6
Postpartum colostrum[b]				
Premature delivery				
26–30 weeks	15	0.26 ± 0.14	3.09 ± 0.59	5.52 ± 0.68
31–37 weeks	23	0.31 ± 0.10	3.14 ± 0.43	5.87 ± 0.50
Full-term delivery	6	0.27 ± 0.22	3.10 ± 0.91	6.81 ± 1.06
Mature milk[c]				
Premature delivery				
26–30 weeks	15	1.37 ± 0.17	7.47 ± 0.72	8.41 ± 0.83
31–37 weeks	23	1.27 ± 0.18	6.56 ± 0.77	7.55 ± 0.89
Full-term delivery	6	0.97 ± 0.28	4.46 ± 1.17	5.68 ± 1.36

[a] Prepartum secretion collected from women 1–70 days before full-term delivery (Bitman et al., 1986).
[b] Data for colostrums and milk fatty acid composition after preterm and full-term delivery (Bitman et al., 1983b).
[c] Mature milk collected at 6 weeks lactation (Bitman et al., 1983b).

were compared with those in the colostrum secreted by women who delivered at similar periods of prematurity. As can be seen from the data presented in Table 38.8, there was no difference in the MCFA level between the colostrum of women who delivered prematurely and colostrum of women who delivered after a full-term pregnancy (Bitman et al., 1983b, 1986). Thus, parturition, irrespective of length of pregnancy, seems to be the trigger for the increase in de novo fatty acid synthesis within the mammary gland. It is also possible that milk removal, whether by the newborn's suckling or by milk expression, after full-term or premature delivery, respectively, is an important factor in the increase of lipogenesis within the human mammary gland. Animal studies have shown that a marked increase in the lipogenic enzymes acetyl-coenzyme A (acetyl-CoA) carboxylase, ATP citrate lyase and fatty acid synthetase occurs immediately after parturition and that milk removal is essential for this increase (Martyn and Hansen, 1981). Whether the presence of long-chain fatty acyl-CoA, which is a potent inhibitor of the two former enzymes in the mammary gland (Romsos et al., 1978; Grigor and Warren, 1980), or other factors (Wilde et al., 1988) that might contribute to the lower concentration of MCFAs in prepartum secretions is not known. The absence of lipoprotein lipase from prepartum secretions (Bitman et al., 1986) suggests low activity in the mammary gland before term delivery, as previously shown in the rat (Hamosh et al., 1970). This would indicate that the LCFAs present in these secretions might originate mainly from the albumin-bound FFAs in the circulation. The absence of tight junctions between mammary epithelial cells before parturition would facilitate this transfer of fatty acids into the mammary gland.

Synthesis of MCFAs is specific to the mammary gland, because only this tissue contains a specific enzyme, thioesterase II, which terminates chain elongation at 6–14 carbon atoms (Chivers et al., 1977; Libertini and Smith, 1978). In all other tissues, lipogenesis results in LCFAs associated with the enzyme thioesterase I, which terminates chain elongation at or above 16-carbon atoms (Lin and Smith, 1978). One could, therefore, use the milk/serum fatty acid ratio to assess the origin of milk fatty acids (Spear et al., 1992b). Indeed, the serum concentration of fatty acids shorter than C14 was extremely low. Concentrations were, however, considerably higher in colostrum, resulting in very high-milk/serum fatty acid ratios for C10:0 and C12:0 at 16.23 and 17.11, respectively (Table 38.9) as compared to milk/serum fatty acid ratios of 0.18–2.0 for all other major long-chain saturated, monounsaturated, and polyunsaturated fatty acids (Spear et al., 1992b).

TABLE 38.9
Medium-Chain Fatty Acid Composition of Colostrum and Serum after Full-Term Delivery[a]

Medium-Chain Fatty Acid	Colostrum[b]	Serum[b]	Milk/Serum Ratio
8:0	0.05 ± 0.01	0.02 ± 0.01	3:18
10:0	0.32 ± 0.04	0.02 ± 0.00	16:23
12:0	2.53 ± 0.20	0.15 ± 0.02	17:11
14:0	5.68 ± 0.25	1.31 ± 0.06	4:35

[a]Data are from 31 healthy women who delivered full-term infants. There were no statistically significant differences in colostrum fatty acid composition as function of weight gain during pregnancy or parity except that myristic acid (14:0) concentration was significantly higher in primipara than in multipara (6.60 ± 0.38 vs. 5.19 ± 0.46% $p < .05$).
[b]Percentage of total fatty acids.

Fatty acid synthetase has been characterized (Thompson et al., 1981; Thompson and Smith, 1982) in a permanent human mammary epithelial cell line, SkBr 3 (Weber and Osborn, 1969), and the production of MCFAs in *in vitro* studies has been reported (Thompson and Smith, 1985). The onset of fatty acid synthetase and thioesterase I activity in the human mammary gland is, however, unknown. Because the human lactating mammary gland cannot be studied for ethical reasons, one depends on indirect observations such as those described above. Based on these studies, which include colostrum (see Table 38.8), milk, and serum as well as prepartum mammary secretion, one can conclude that, in the human, as in other species, parturition, irrespective of length of pregnancy, is the trigger for the synthesis of MCFAs (Spear et al., 1992b).

Whether this is dependent on hormonal changes associated with delivery and the onset of lactation or with the removal of milk from the mammary gland (by suckling or pumping), or a combination of both, remains to be determined. As in other species, in the human, the rate of synthesis of MCFAs increases with length of lactation (see Table 38.3) (Roy et al., 1975; Tantibhendyangkul, 1975; Bitman et al., 1983b).

At birth, the digestive function of premature infants is not fully developed (Hamosh, 1994b, 1996; Hamosh and Hamosh, 1999; Hamosh, 2006). The physiological immaturity of pancreatic and hepatic function results in reduced levels of pancreatic lipase (Zoppy et al., 1972; Sbarra et al., 1996) and bile salts (Watkins, 1975), both necessary for the digestion of dietary fat (Hamosh, 1996). To reduce the fat malabsorption of premature infants, fat blends have been adjusted to contain relatively high levels of MCFAs; that is, chain length of 6–10 carbons (Roy et al., 1975; Tantibhendyangkul and Hashim, 1975; Shenai et al., 1980). Although MCFAs have certain advantages, such as absorption through the gastric mucosa (Aw and Grigor, 1980; Fernando-Warnakulasuriya et al., 1981; Bitman et al., 1985), direct transport from the intestine to the liver via the portal vein (Patton, 1981; Bach and Babayan, 1982; Carey et al., 1983), and efficient oxidation without the need for carnitine-mediated transport through mitochondrial membranes (Innis et al., 1996b), they also have certain drawbacks (Watkins, 1975), such as ketosis and acidosis, the production of dicarboxylic acids (Henderson and Dear, 1986; Whyte, 1986; DuPont et al., 1987), and the fact that they are not used as building blocks for cell membranes and are only minimally incorporated into adipose tissue (Sarda et al., 1987).

However, several studies have shown that fat blends containing almost exclusively long-chain triglycerides (LCTs) (86%–100%) or 40%–50% MCTs are absorbed to the same extent by preterm infants (Okamoto et al., 1982; Whyte et al., 1986a; Hamosh et al., 1989, 1991b) even in crossover studies in which each infant was randomly fed for 1 week the LCT and for 1 week the MCT formula

(Hamosh et al., 1989, 1991b). The data for fat absorption and infant growth on these different types of formulas are shown in Tables 38.10 through 38.12 and Figures 38.2 and 38.3. Thus, the only advantage of MCTs in formula fat might be that octanoic acid (C8:O) is absorbed through the gastric mucosa (Hamosh et al., 1989) and might provide a readily available energy source. Figures 38.2 and 38.3 present the individual data for each infant while being fed either MCT- or LCT-containing formula as well as for a group of nine preterm infants fed a full-term infant formula (S26). It is clear that only one infant in the SMA formula (Wyeth) and one in the Enfamil formula (Mead-Johnson) groups benefited from MCT, which was absorbed at a better rate. It seems, therefore, that with the exception of a small number of very premature infants with poorly developed digestive ability who might benefit from MCT, fat absorption in most preterm infants is a function of development of digestive potential rather than the fat blend (MCT or LCT) of the formula (Hamosh et al., 1989, 1991a). It is, therefore, advisable not to include MCT in fat blends of formulas for full-term infants, whereas in formulas for preterm infants, MCT should not exceed the level in the milk of women who deliver prematurely (15% fatty acids C6:O-C:14).

TABLE 38.10
Fat Absorption and Growth Rate in Preterm Infants Fed Medium-Chain Triglyceride or Long-Chain Triglyceride Formula

Formula	Dietary Fat (g/kg/day)	Fat Excretion (g/kg/day)	Fat Absorption (%)	Wt Gain (g/day)
Medium-chain triglycerides	6.04 ± 0.54	1.04 ± 0.24	84.63 ± 3.14	23.0 ± 1.50
(n = 12)	(4.18–8.38)	(0.15–1.87)	(60.21–97.05)	(15.71–34.28)
Long-chain triglycerides (n = 12)	5.88 ± 0.48	1.02 ± 0.26	82.81 ± 4.01	20.85 ± 1.80
	(3.63–7.60)	(0.18–2.29)	(49.34–97.50)	(22.42–32.85)

Data are mean ± SEM and ranges (in parentheses). Fat excretion and absorption were determined during a 3-day balance study. Weight gain was determined during 1 week when infants were fed either medium-chain or long-chain triglyceride formula. Each infant was fed each formula for 1 week. Infants were randomly assigned to start with either formula. The formulas were Enfamil (Mead-Johnson).

Source: From Hamosh, M., et al. (1989). *Pediatrics* 83:83–92.

TABLE 38.11
Fat Absorption in Infants Fed SMA or S-26 Formula

	Study 1 SMA Preterm Formulas		Study 2 S-26 Formula
Fat Type	**MCT**	**LCT**	**LCT**
Dietary fat (g/kg/day)	6.38 ± 0.14 (5.53–6.87)	6.19 ± 0.28 (4.66–7.26)	6.08 ± 0.39 (4.72–9.02)
Fat excretion (g/kg/day)	0.69 ± 0.15 (0.35–1.73)	0.83 ± 0.26 (0.16–2.53)	1.00 ± 0.15 (0.36–1.72)
Fat absorption (%)	89.08 ± 2.37 (73.9–94.6)	87.0 ± 3.81 (63.1–97.4)	83.00 ± 2.89 (69.7–92.3)

Abbreviations: MCT, medium-chain triglycerides; LCT, long-chain triglycerides, S-26 is an LCT formula for full term infants of lower caloric density than formula for preterm infants.

Data are mean ± SEM and ranges (in parentheses). Fat excretion and absorption were determined during a 3-day balance study. Weight gain was determined during 1 week when infants were fed either MCT or LCT formula. Each infant was fed each formula for 1 week. Infants were randomly assigned to start with either formula.

Source: From Hamosh, M., et al. (1991a). *J. Pediatr. Gastroenterol. Nutr.* 13:143–149.

TABLE 38.12
Effect of SMA Preterm (MCT or LCT) and S-26 Formulas on Infant Growth

	Study 1 SMA Formula		Study 2 S-26 Formula
	MCT	LCT	LCT
Weight gain (g/day)	23.2 ± 2.3 (12.8–35.7)	21.20 ± 1.7 (12.8–28.5)	14.28 ± 25[a] (0.66–30.0)
Length (cm/day)	0.17 ± 0.06 (0–0.42)	0.16 ± 0.04 (0–0.33)	0.22 ± 0.07 (0–0.7)
Head circumference (cm/day)	0.13 ± 0.014 (0.08–0.17)	0.14 ± 0.017 (0.07–0.18)	0.12 ± 0.03 (0–0.37)

MCT, medium-chain triglycerides; LCT, long-chain triglycerides.

[a] Statistically significant difference between studies 1 and 2 ($p < .04$).

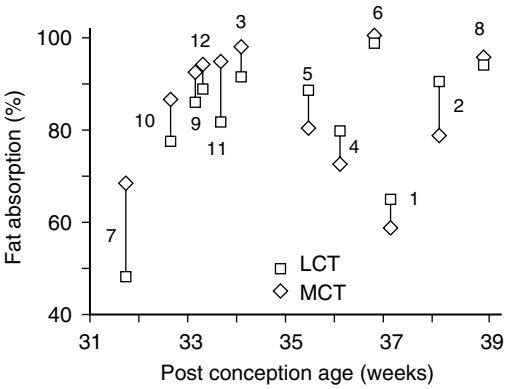

FIGURE 38.2 Comparison of fat absorption in premature infants fed alternately medium-chain (MCT) and long-chain triglyceride (LCT) formula. Numbers next to symbols designate individual infant numbers. *(*From Hamosh, M., et al. (1989). *Pediatrics* 83:83–92.)

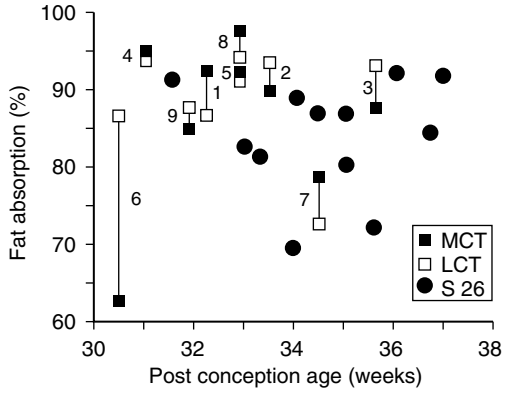

FIGURE 38.3 Comparison of fat absorption in premature infants fed SMA or S-26 formulas. The SMA formula contained two different fat blends: 50% medium-chain triglyceride (MCT) or 86% long-chain triglyceride (LCT). Each formula was fed alternately for 1 week to a group of nine infants. A second group of 11 infants was fed S-26 formula containing 98% LCT for 1 week. Fat absorption studies were conducted during a 3-day balance study. Numbers next to symbols designate individual infant numbers. (From Hamosh, M., et al. (1991a). *J. Pediatr. Gastroenterol. Nutr*. 13:143–149.)

It is interesting that within formulas fat blends containing up to 40% MCT, the latter do not adhere to feeding tubes (Mehta et al., 1991). The same is true of human milk fat with a content of 5% C8:0 and C10:0 (Mehta et al., 1988). However, the addition of MCT oil and feeding through gastric gavage tubes after mixing with formula or human milk leads to separation of the MCT oil and preferential adherence of C8:0 and C10:0 to the tubing (Figures 38.4 and 38.5). Given the tendency of MCT to adhere to feeding tubes of gastric gavage-fed infants (Mehta et al., 1988, 1991), caution should be exercised in the addition of MCT oil to either formula or human milk with the aim to increase the caloric content of feeds for low-birth weight infants.

Because increasing the proportion of MCT in formulas might lower the availability of long-chain mono- and polyunsaturated fatty acids essential for the infant's normal growth and development, it might not be advisable to prepare infant formulas with a high content of MCT.

VI. LONG-CHAIN POLYUNSATURATED FATTY ACIDS

LCPUFAs are essential for normal growth and development. There are two families of LCPUFAs, one derived from linoleic acid (C18:2n-6) and the other from linolenic acid (C18:3n-3) (Figure 38.6).

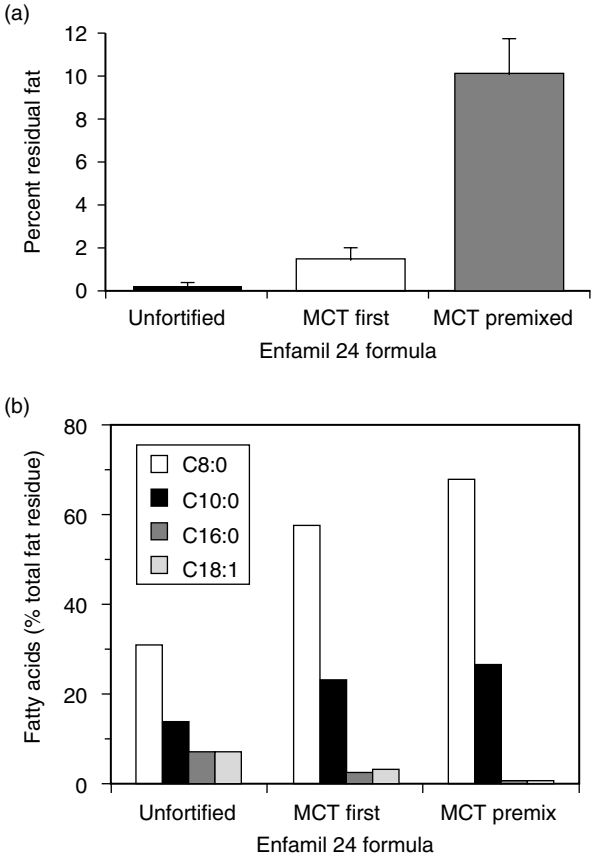

FIGURE 38.4 (a) Amount of fat adhering to feeding sets during intermittent feeding to Enfamil formula fortified with MCT oil. Data are percentage (means ± SEM) of total fat adhering to feeding sets during three feeding regimens. (b) Fatty acid composition of fat adhering to feeding sets during gavage feeding of Enfamil formula with MCT fortified with MCT oil. Enfamil premature formula contains 18% C8:0, 11.3% C10:0, 7% C16:0, and 11.7% C18:1. (From Mehta, N.R., et al. (1991). *J. Pediatr. Gastroenterol. Nutr*. 13:267–269.)

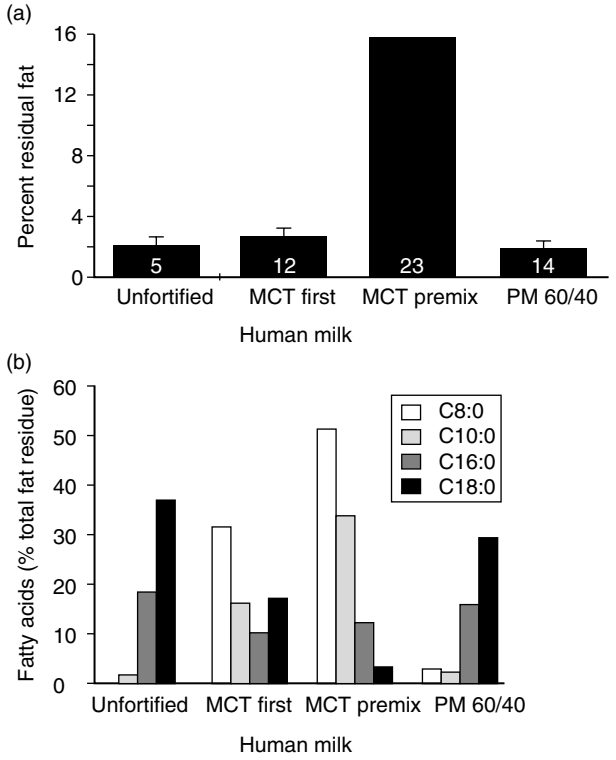

FIGURE 38.5 (a) Amount of fat adhering to feeding sets during intermittent feeding of fresh human milk fortified with MCT oil. Numbers in each bar represent number of experiments. Data are mean ± SEM. (b) Fatty acid composition of fat adhering to feeding sets during gavage feeding of human milk fortified with MCT oil in four feeding regimens: unfortified human milk, MCT oil delivered first, followed by milk, MCT oil premixed with milk before gavage feeding and milk fortified with PM 60/40. C8:0 and C10:0 are major components of MCT oil, C16:0 and C18:1 are major components of human milk fat. (From Mehta, N.R., et al. (1988). *J. Pediatr.* 112:474–476.)

n6				n3	
Linoleic acid	18:2n6			18:3n3	α-Linolenic acid
		−2H	Δ6 Desaturation	−2H	
	18:3n6			18:4n3	
		+2C	Elongation	+2C	
	20:3n6			20:4n3	
		−2H	Δ5 Desaturation	−2H	
	20:4n6			20:5n3	EPA
		+2C	Elongation	+2C	
	22:4n6			22:5n3	
		+2C	Elongation	+2C	
	24:4n6			24:5n3	
		−2H	Δ6 Desaturation	−2H	
	24:5n6			24:6n3	
		−2C	Chain shortening	−2C	
	22:5n6			22:6n3	DHA

FIGURE 38.6 Synthesis of LCPUFA from 18 carbon precursors. (From Hamosh, M., and Salem, N., Jr. (1998). *Biol. Neonate* 74:106–120.)

Among the derivatives of linolenic acid, DHA (C22:6n-3) is essential for normal brain development and visual function, whereas the elongation and desaturation product of linoleic acid, AA (C20:4n-6) is the precursor of prostaglandins and leukotrienes and is essential for neonatal growth. These highly unsaturated fatty acids affect many aspects of membrane function such as membrane order, permeability, lipid–lipid, as well as lipid–protein interaction. These specific interactions may in turn affect receptor function, enzyme activities, signal transduction, and membrane excitability (Uauy et al., 1996). For the developing infant, DHA is of major importance, because it amounts to a major part of the total fatty acids in cerebral cortex and retina phospholipids. The photoreceptor outer segments of the retina contain very high concentrations of DHA, which is important for normal photochemical activity of the visual pigment rhodopsin (Litman and Mitchell, 1996; Uauy et al., 1996; Innis, 2004). We and others have reviewed the topic recently and present here several aspects from these reviews (Hamosh, 1998; Hamosh and Salem, 1998; Innis, 2004; Heird and Lapillone, 2005; Uauy and Dangour, 2006).

Fatty acids with double bonds at the n6 and n3 positions (i.e., linoleic and linolenic acids) have to be provided by the diet because humans cannot synthesize them. Both types of fatty acids are elongated and further desaturated by the same enzymes (desaturases and elongases) to produce AA and DHA. Figure 38.6 shows the steps involved in this process (Voss et al., 2001), which occurs in adults (Hoffman et al., 2001) as well as in infants (Sauerwald et al., 1997).

LCPUFA requirements of the newborn have been a major focus of research for the past two decades. The reason for this intense interest is based on several observations: (1) accretion of LCPUFAs occurs primarily during the last trimester of pregnancy (Kneebone et al., 1985), making the preterm infant especially vulnerable to LCPUFA deficiency; (2) plasma levels of LCPUFAs decrease markedly after birth in formula-fed preterm infants as compared to almost constant levels in breast-fed infants of comparable gestational age; (3) maternal LCPUFA status does not seem optimal during both pregnancy and lactation in well-nourished women in the United States (Koletzko, 1997) and The Netherlands (Koletzko et al., 1988; Koletzko, 1992); (4) the level of LCPUFAs in human milk is not constant as a function of length of lactation or geographical distribution (i.e., nutrition) (Hamosh, 1998; Hamosh and Salem, 1998), and therefore to what extend can human milk be the "gold standard?"; and (5) studies that evaluate the effect of formula supplementation with LCPUFAs provide only partial answers as to benefit of such supplementation to preterm or full-term infants (Hamosh, 1998; Hamosh and Salem, 1998).

Although accretion occurs prenatally through preferential transfer of LCPUFAs from mother to fetus, possibly as a result of the high-LCPUFA affinity of α-fetoprotein (Koletzko et al., 1991a,b), postnatal accretion of these fatty acids is also important, because myelination and synaptogenesis occur during the postnatal brain growth spurt.

The above points assume that LCPUFAs are conditionally essential nutrients in the newborn; that is, although adults have the capacity to synthesize these fatty acids by elongation and desaturation from their 18-carbon EFA precursors, infants are unable or may have only a limited capacity to accomplish this and therefore have to receive LCPUFAs, especially DHA and possibly AA, in their diet. This assumption is currently questioned as a result of several studies that show that stable isotope-enriched 18-carbon precursors can be incorporated into LCPUFAs by newborn infants (Carnielli et al., 1996; Salem et al., 1996; Sauerwald et al., 1997). Furthermore, these studies (Ktorza et al., 1983) also show that newborn premature and full-term infants use the recently described Δ4 desaturase independent pathway (Voss et al., 1991) in the endogenous synthesis of DHA (Sauerwald et al., 1997) and that this ability is similar in preterm and full term infants (Uauy et al., 2000). The question, therefore, now is to what extent does endogenous LCPUFA synthesis cover the DHA and AA needs of the newborn? Salem et al. (1996) made an order of magnitude estimate and concluded that AA might be produced in adequate amounts, whereas the amount of DHA produced is inadequate.

A. LCPUFAs in Human Milk: Effect of Diet and Length of Lactation

The sufficiency of breast milk LCPUFAs for the full-term infant is worth examination. Although milk AA concentrations are relatively uniform and do not seem to vary with maternal nutrition, DHA levels in milk show great variability among populations, being lowest in countries with a relatively high-meat intake (e.g., United States) and highest in populations where most animal foods are provided as fish (Far East, Nigeria, Canadian Inuits) (Table 38.13) (Sanders et al., 1978; Hall, 1979; Jensen et al., 1981; Putnam et al., 1981; Villacampa et al., 1982; Bitman et al., 1983b; Harzer et al., 1983; Kneebone et al., 1985; Sas et al., 1986; Muskiet et al., 1987; de Lucchi et al., 1988; Innis and Kuhnlein, 1988; Koletzko et al., 1988, 1991b; Tomarelli, 1988; Van der Westhuizen et al., 1988; Prentice et al., 1989; Boersma et al., 1991; Dotson et al., 1992; Jackson et al., 1994; de la Presa-Owens et al., 1996; Hamosh, 1998; Hamosh and Salem, 1998). Furthermore, milk LCPUFA levels change as a function of length of lactation and decrease markedly after the first 3 months postpartum (Nobel et al., 1979; Bitman et al., 1983b; Boersma et al., 1991; Spear et al., 1992a,b; Luukkainen et al., 1994; Henderson et al., 1996; Hamosh, 1998). Again, as discussed above, AA levels seem relatively constant, whereas DHA shows the greatest change (Table 38.14). The decline

TABLE 38.13
Docosahexaenoic Acid (22:6n-3) and Arachidonic Acid (20:4n-6) in Mature Human Milk: Geographical Variations

Country	Reference	DHA	AA
United States	Putman et al. (1982)	0.10	0.60
	Dotson et al. (1992)	0.16	0.53
	Bitman et al. (1983b)	0.18	0.57
	Tomarelli et al. (1988)	0.25	0.46
	Jackson et al. (1994)	0.21	0.71
Hungary	Sas et al. (1986)	0.10	0.50
Germany	Koletzko et al. (1988)	0.22	0.36
	Harzer et al. (1983)	0.16	0.39
Sweden	Jansson et al. (1981)	0.30	0.40
England	Hall (1979)	0.29	0.19
	Sanders et al. (1978)	0.59	0.54
Spain	DeLucchi et al. (1988)	0.40	0.80
	Villacampa et al. (1982)	0.30	0.57
	dela Presa-Owens et al. (1996)	0.34	0.50
South Africa	van der Westhuizen et al. (1988)	0.20	0.60
Tanzania	Muskiet et al. (1987)	0.27	0.60
Gambia	Prentice et al. (1989)	0.39	0.31
Nigeria	Koletzko et al. (1991b)	0.93	0.82
St. Lucia	Boersma et al. (1991)	0.56	0.58
China	Kneebone et al. (1985)	0.71	0.64
Malay	Kneebone et al. (1985)	0.90	0.47
India	Kneebone et al. (1985)	0.90	0.57
Canadian Inuit	Innis et al. (1988)	1.40	0.60

AA, arachidonic acid; DHA, docosahexaenoic acid. Data on 3–40 individuals per study. Milk collected at 0.5–8.0 months after delivery.

Source: From Hamosh, M. (1998). *Biochem. Soc. Trans.* 26:96–103.

TABLE 38.14
Effect of Length of Lactation on LCPUFAs: Docosahexaenoic Acid (DHA) (22:6n-3) and Arachidonic Acid (AA) (20:4n-6) in Human Milk

Lactation	Bitman et al. (1983b)	Spear et al. (1992b)	Spear et al. (1992a)	Boersma et al. (1991)	Luukkainen et al. (1994)	Henderson et al. (1996)
DHA						
Colostrum	0.31	0.34	0.32	1.10	0.44	—
1–3 weeks	—	0.20	—	0.88	0.38	—
1–3 months	0.18	0.15	—	0.56	0.25	0.20
6 months	—	—	—	—	0.18	0.08
12 months	—	—	—	—	—	0.08
AA						
Colostrum	0.78	0.84	1.20	1.60	0.60	—
1–3 weeks	—	0.76	—	0.84	0.46	—
1–3 months	0.57	0.52	—	0.54	0.40	0.54
6 months	—	—	—	—	0.28	0.43
12 months	—	—	—	—	—	0.39

LCPUFAs, long-chain polyunsaturated fatty acids. The number of subjects in each study varied from 2 to 31.

Source: From Hamosh, M. (1998). *Biochem. Soc. Trans*. 26:96–103.

in DHA levels is more pronounced in the milk of mothers of full-term infants (Bitman et al., 1983b; Luukkainen et al., 1994; Henderson et al., 1996), possibly because LCPUFA reserves are greater in women who deliver prematurely. However, infants who are breast-fed for 1 year and receive milk with only traces of DHA have adequate plasma DHA levels (Henderson et al., 1996), suggesting that at 6 and 12 months of age, endogenous synthesis of LCPUFAs is well developed. Cell culture studies indicate that astrocytes convert C20:5n3 and C22:5n3 to DHA that is then transferred to neurons (Bernoud et al., 1988; Moore et al., 1991; Moore, 1993).

B. Effect of LCPUFA Supplementation on Blood Levels of DHA and AA of Formula-Fed Infants

The effect of low n-3 fatty acid diets on neural functions of rodents (mice and rats) and primates has been reviewed (Salem et al., 1986; Salem, 1989; Hamosh and Salem, 1998). Earlier reports of irreversible changes in *Rhesus* monkey brain function associated with temporary DHA deficiency during pregnancy and lactation (Neuringer et al., 1984, 1986) were the trigger for studies on the LCPUFA status of the newborn infant with special emphasis on the premature infant. Indeed, a number of studies have evaluated the LCPUFA status of the newborn by measurement of DHA and AA in cord blood vessels, a potentially more sensitive indicator of EFA status than cord blood composition. These studies have shown a close relationship between the maternal and fetal LCPUFA status and the lower neonatal EFA status in successive pregnancies and in multiple pregnancies (Muller et al., 1964). A marked decrease in plasma and red blood cell (RBC), DHA and AA in formula as compared to breast-fed infants (Putnam et al., 1982) was taken as further indication of rapid postnatal deterioration of the LCPUFA status in the absence of dietary supplementation. LCPUFA supplementation of formula-fed infants has received much attention lately. Supplementing pregnant women with fish oil (that provides DHA and EPA) during the last trimester of pregnancy (Van Houwelingen et al., 1995) has improved fetal DHA reserves.

Improvement in the DHA status was achieved without impairing the AA status, although the supplementation was with n-3 fatty acids only (Van Houwelingen et al., 1995). However, the preterm infant cannot be helped by maternal supplementation, because birth often occurs before the LCPUFA accretion of the last trimester of pregnancy. Postnatal supplementation seems therefore the correct approach to solve this problem. The initial studies carried out by Carlson et al. (1991) supplemented preterm formula with marine oil, which provides a mixture of DHA and EPA. This supplementation led to higher EPA and DHA levels in plasma and RBCs, but concomitantly there was a decrease in AA (Carlson et al., 1991). Supplementation with marine oil also led to impaired growth, resulting in lower weight, length, and head circumference in the supplemented compared to the regular formula-fed group. Subsequent studies by Carlson et al. (1992, 1993) showed that the depressed growth was evident at 1 year of age, that it was related to the lower AA levels, and that it was due to the EPA present in marine oil. Subsequent studies in the United States, Canada, Europe, and South America reported similar findings (Clandinin et al., 1992; Hoffman and Uauy, 1992; Hoffman et al., 1993; Uauy-Dagach et al., 1994; Carnielli et al., 1995; Koletzko et al., 1995). In order to avoid the growth-depressing effects of EPA in fish oil preparations, studies were carried out with other sources of DHA and AA. LCPUFA derived from egg yolk phospholipids, evening primrose oil, and single-cell organisms (algae and fungi) have been used successfully to increase plasma and RBC concentrations of DHA and AA in preterm infants. The rationale for quantifying LCPUFA levels in RBC membranes is the assumption that they reflect tissue (including brain) DHA and AA concentrations. Although this has been reported to be true in the rat (Carlson et al., 1986), studies in *Rhesus* monkeys show that diet-induced differences in RBC DHA can greatly overestimate differences in retinal and cerebral cortex DHA (Riesbich et al., 1986).

Brain lipid (Farquhrson et al., 1992; Makrides et al., 1994) and phospholipid (Farquhrson et al., 1995a), liver (Farquhrson et al., 1995b), and subcutaneous adipose tissue (Farquhrson et al., 1993) DHA and AA were lower in formula-fed than in breast-fed full-term infants who died of SIDS (sudden infant death syndrome), suggesting that the full-term infant might also benefit from DHA supplementation. Within the past few years, studies have been carried out to assess whether LCPUFA supplementation changes the DHA and AA profile of blood (plasma and RBC lipids) in full-term infants. Most of these studies have used varied sources of DHA and AA and have shown that supplementation results in plasma and DHA levels that are similar to those of breast-fed infants and higher than in unsupplemented formula-fed infants (Agostoni et al., 1994; Decsi and Koletzko, 1995b; Makrides et al., 1995; Carlson et al., 1996; Hoffman et al., 1996; Innis et al., 1996a; Auestad et al., 1997).

C. Effect of LCPUFA Supplementation on Visual and Cognitive Functions of Formula-Fed Infants

Studies have also been carried out to assess whether improving LCPUFA status, as measured by plasma and RBC-phospholipid concentrations of DHA and AA, affects visual and cognitive function in preterm and full-term infants. These studies have used a number of different tests such as forced-choice preferential looking (FPL), which evaluates the infant's tendency to gaze at a pattern rather than a blank field (Dobson and Teller, 1978; McDonald et al., 1985); visual evoked potential (VEP), which measures the electrical potential at the occipital visual cortex in response to patterns of contrast and is expressed as $1\log_{10}$ of minimum angle of resolution (MAR): the smaller log MAR the better visual acuity (Sokol et al., 1983; Norcia and Tyler, 1985); electroretinogram (ERG), which measures the summed activity of the retina in response to a flash of light (Naka and Rushton, 1966; Hood and Birch, 1990); the novel preference test (NPT), also known as the Fagan test of infant intelligence (FTII), which tests the preference of infants to look at new images (Fagan and McGrath, 1981; Rose and Wallace, 1985), or the Brunet–Lezine test (a modification of the Gesell test), which evaluates psychomotor development by testing posture and gross motor function, adaptation, and fine motor function, social reaction, and language. Results of studies in preterm infants

showed improved visual and cognitive function associated with LCPUFA supplementation, but in most cases the effect was of short duration and, generally, after 2–6 months of age there was no difference in performance among formula-fed, supplemented formula-fed, or breast-fed infants (Uauy et al., 1990; Birch et al., 1992a,b, 1993; Carlson et al., 1993, 1994; Hoffman et al., 1993; Uauy-Dagach et al., 1994; Carlson and Werkman, 1996). Only in one study was the functional benefit of supplementation still evident at the age of 1 year (Werkman and Carlson, 1996).

Functional studies of the effect of DHA and AA supplementation have also been carried out in full-term infants. Although some of these studies clearly show improvement of visual and cognitive function, other studies fail to show effects (Makrides et al., 1993; Innis et al., 1994, 1996b; Agostoni et al., 1995; Carlson et al., 1996; Jorgensen et al., 1996; Hamosh, 1998; Hamosh and Salem, 1998). The reason for the great variation in the results obtained in the functional studies in preterm and full-term infants is not clear. Potential causes of variability could be sample size, infrequent testing, and differences in infant age at testing as well as an insufficiently long follow-up for meaningful evaluation. More recent studies on large numbers of preterm and full-term infants show benefits of supplementation in the former, but not in the latter (Auestad et al., 2001; O'Connor et al., 2001; Makrides et al., 2005).

Studies to change DHA concentrations of blood lipids, and thereby to affect the functional development of infants, by changing the ratio of the precursor α-linolenic and linoleic acids have also been carried out. Although low linoleic to α-linolenic acid ratios improve DHA status, they tend to decrease AA levels (Clark et al., 1992; Innis et al., 1997; Jensen et al., 1997) as well as growth (Jensen et al., 1997; Hamosh, 1998; Hamosh and Salem, 1998).

It seems, therefore, that maternal supplementation during pregnancy (Van Hauwelingen et al., 1995), or during lactation (Makrides et al., 1996), or eventually during both pregnancy and lactation might be an acceptable way of ensuring optimal LCPUFA supply to the newborn. The former form of supplementation would, of course, benefit only full-term breast-fed infants and the latter preterm infants. As far as formula-fed infants are concerned, the need for supplementation of the full-term infant is not clearly evident, whereas supplementation of the preterm infant might have temporary beneficial effects.

In view of the fact that humans evolved on and are likely to be best adapted to a diet much richer in LCPUFAs than the modem American diet (Sinclair and O'Dea, 1990), it would be prudent to increase the dietary intake of AA and DHA, particularly during times of dietary stress such as pregnancy and lactation.

Although much attention has been given to maternal nutrition as the determinant of milk LCPUFAs, especially DHA levels, very little or no consideration has been given to the possible regulation of milk LCPUFAs at the mammary gland level. To gain insight into this aspect, we have quantified tissue and milk LCPUFAs in the lactating ferret, a species with an adequate and constant intake of LCPUFAs (Pereira et al., 1994; Henderson et al., 1998). Concomitantly, we have quantified LCPUFA accretion in the late fetal and suckling ferret offspring (kits). Figure 38.7 summarizes these studies.

Ferrets were studied at 5 weeks of pregnancy and throughout 4 weeks of lactation. Duration of pregnancy and lactation is 6 weeks each in this species. Because the kits start to nibble on the mother's food after the first 4 weeks after birth, no studies was conducted beyond the first 4 weeks of age. Data from pregnant or lactating females were compared to those from virgin animals who served as control (Henderson et al., 1998).

Maternal adipose tissue LCPUFAs (total n-3 and n-6 fatty acids as well as DHA and AA) were virtually constant during pregnancy and lactation and were identical to those of the virgin control females (see Figure 38.7a). Four different adipose tissues were sampled for analysis—omentum, perirenal, periovarian, and subcutaneous at all study times. LCPUFAs as well as total (saturated, monoene, polyene, DHA, and AA) fatty acid profiles were identical at the different anatomical sites (Hamosh, 1998) (Table 38.15). We have, therefore, presented the mean values of the different fat depots at each study point in Figure 38.7b. Although the dietary intake of DHA and AA remained

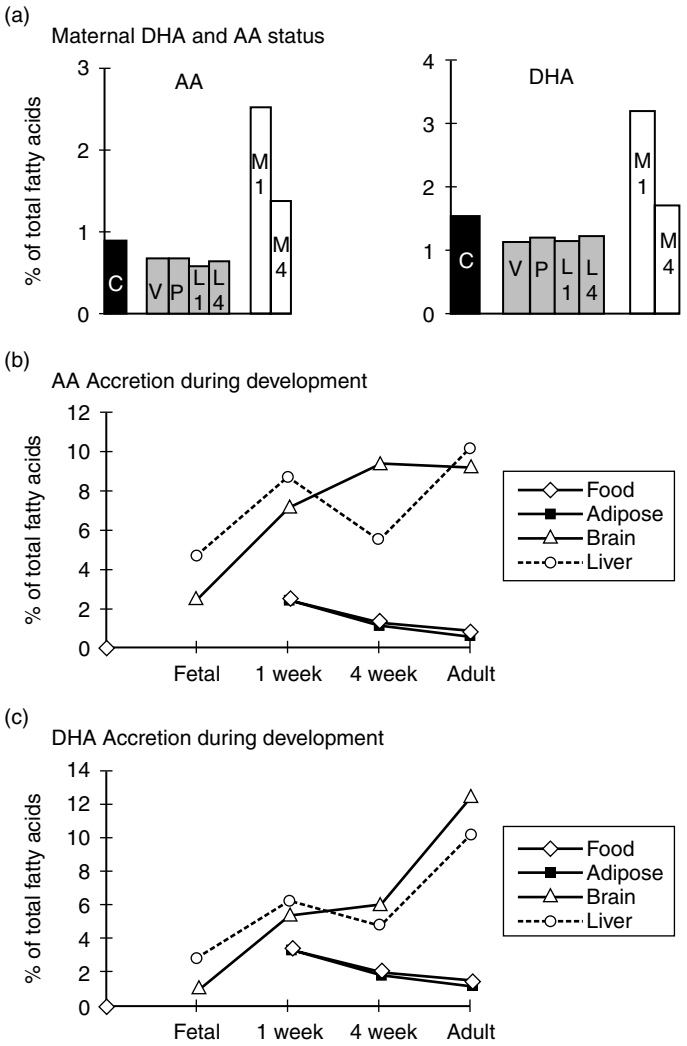

FIGURE 38.7 Maternal arachidonic acid (AA) and docosahexaenoic acid (DHA) status during pregnancy and lactation. (a) Adipose tissue AA and DHA levels of virgin (V), pregnant (P), and lactating 1 weeks (L1) and 4 weeks (L4) ferrets are compared to ferret chow levels (C) and to milk levels in early (M1) and advanced (M4) lactation. (b) Arachidonic acid (AA) and (c) docosahexaenoic acid (DHA) accretion during late fetal and postnatal development in ferret kit brain, liver and adipose tissue. Values are compared to food (i.e., milk or chow) values. (From Hamosh, M., and Salem, N., Jr. (1998). *Biol. Neonate* 74:106–120.)

constant during pregnancy and lactation, as did the concentration of these fatty acids in maternal adipose tissues, milk DHA and AA levels decreased throughout lactation. The data suggest that milk LCPUFA levels are intrinsically regulated within the lactating mammary gland, the concentration of DHA and AA in milk secreted during early lactation being twofold and threefold higher than dietary content and threefold and fourfold higher than adipose tissue content. The pattern of decrease of milk DHA and AA in advanced lactation is very similar in the ferret and in the human (Luukkainen et al., 1994; Henderson et al., 1996), although absolute values are higher in the ferret.

D. LCPUFA Accretion in the Newborn

Although we have quantified the individual fatty acids in all classes (saturates, monoenes, polyenes, and LCPUFAs), because of space constraints, we present only the data for DHA and AA here. No

TABLE 38.15
Effect of Formula Supplementation with DHA or DHA and AA on Blood Lipid Levels of DHA and AA in Preterm Infants

Reference	Outcome Tested Plasma and RBC DHA and AA	Results	Age
Carlson et al. (1991)	Marine oil suppl. (DHA + EPA)	DHA suppl. lowers AA levels	
Carlson et al. (1992)	Marine oil suppl. (DHA + EPA)	DHA > 0.5% suppl. lowers AA levels	
Carlson et al. (1992)	Marine oil suppl. (DHA + EPA)	Lower growth rate	1 year
Clandinin et al. (1992)	Plasma DHA, AA	SF > F, SF = BF	1 month
Carlson et al. (1993)	AA suppl.	Increases growth rate	
Hoffman et al. (1990, 1992, 1993)	Plasma DHA, AA	SF > F, SF = BF	1,6 months
Koletzko et al. (1995)	Plasma DHA, AA	SF > F, SF = BF	3 weeks
Carnielli et al. (1996)	Plasma DHA, AA	SF > F, SF = BF	1 month

DHA, docosahexaenoic acid, 22:6n-3; AA, arachidonic acid, 20:4n-6. Comparison made between supplemented formula (SF) and regular formula (F) and, in most studies, also with breast milk (BF). (From Hamosh, M., and Salem, N., Jr. (1998). *Biol. Neonate* 74:106–120.)

adipose tissues were detectable in the fetus at the start of the last week of gestation; however, 1 week after birth (i.e., within an interval of 2 weeks) the newborn had well-developed adipose deposits at all sites examined. As in the adult, there was no difference in LCPUFA profiles among various adipose tissues examined. DHA and AA were highest in adipose tissue in the first week after birth (see Figure 38.7b and 38.7c). Kit adipose tissue DHA and AA were identical to milk DHA and AA levels, indicating rapid deposition of milk LCPUFAs into adipose depots during early postnatal development. The rapid postnatal development of adipose tissue and the rapid accretion of DHA and AA in fat depots suggests that these might serve as reservoir for the LCPUFA accretion in other tissues during later periods when milk supply of DHA and AA decreases. Analysis of fatty acid profiles in brain and liver showed a very different developmental pattern from that in adipose tissue. Brain DHA levels increased sixfold from the late fetus to the first week after birth, and at 4 weeks after birth, they had only reached 50% of the DHA level of the adult brain. The percentage of DHA was much higher in brain than in adipose tissue. AA levels were higher than DHA levels in fetal brain. AA increased threefold from the late fetal period to 1 week after birth and reached adult levels 4 weeks after birth. Accretion of DHA and AA in the liver closely parallels that of the brain. DHA levels increased more than twofold from the late fetal period to the early neonatal period, but amounted to only 50% of adult levels 4 weeks after birth, whereas AA levels were much higher in fetal liver than DHA levels, and by 1 week after birth, they had reached adult levels (see Figure 38.7). The study shows marked differences in LCPUFA accretion rates among different organs and suggests that tissues with rapid growth and LCPUFA accretion such as adipose tissue might act as subsequent reservoirs of LCPUFA when the dietary supply (milk LCPUFAs) decreases. An additional conclusion that can be reached from the data presented for brain and liver LCPUFA accretion is that more rapid accretion of n-6 LCPUFAs might be due to earlier development of endogenous synthesis of the n-6 than the n-3 LCPUFA series, rendering the latter more dependent on dietary supplementation than the former. Similar conclusions have recently been reached in studies of conversion of stable isotope-labeled precursors to DHA and AA in premature and full-term infants (Salem et al., 1996). Accretion of DHA and AA in brain and liver occurs mainly postnatally, emphasizing the need of adequate levels of LCPUFAs during neonatal development. In the ferret, this might be achieved by a combination of dietary (milk) and reserve (adipose tissue) sources as well as endogenous synthesis. Whether the human infant uses similar resources is yet to be established.

Because LCPUFAs contain a large number of double bonds, they might be especially vulnerable to oxidation. Recently, studies were conducted to assess whether pasteurization of banked human milk (heating at 62.5°C for 30 min, without precautions to prevent oxidation) might cause a measurable decrease in milk LCPUFAs. Pasteurization is mandatory to prevent passage of microorganisms through donor milk to the recipient infant. The data of this study show that all fatty acids in milk, including LCPUFA (DHA and AA), are not affected by pasteurization (Henderson et al., 1998).

Another interesting aspect of the role of lipid in the nutrition of preterm infants was recently explored in preterm infants with bronchopulmonary displasia (BPD). These infants have significant pulmonary disease combined with higher metabolic rates. Feeding a high-fat diet rather than a high-carbohydrate diet resulted in significantly lower CO_2 production (6.6 ± 0.3 vs. 7.4 ± 0.4 mL/kg/min) and, therefore, lower respiratory quotients (0.80 ± 0.02 vs. 0.94 ± 0.01 mL/kg/min, $p < .005$) whi1e maintaining adequate growth and nutritional status (Pereira et al., 1994).

VII. PROTECTIVE FUNCTIONS OF FATTY ACIDS

Fatty acids (especially lauric acid, Cl2:0) and monoglycerides have antiprotozoan, antibacterial, and antiviral activity, specifically against lipid-enveloped viruses (Kabara, 1980; Thormar et al., 1987; Isaacs and Thormar, 1991). These fatty acids and monoglycerides are produced during the intragastric digestion of either human milk or formula fat (Hamosh, 1991; Armand et al., 1996). The fatty acids with highest anti-infective activity (Cl2:0, C18:1, C18:2) are also preferentially released during gastric lipolysis, suggesting that release of these fatty acids in the stomach might provide maximal protection along the gastrointestinal tract.

Conjugated linoleic acid (CLA, C18:9c, 11t) was recently shown to have anticarcinogenic activity (Pariza, 1997; Parodi, 1997). This fatty acid is found in grilled ground beef and dairy products and was also shown to have antioxidant activity and to be antiatherogenic. Its presence in human milk in amounts varying from 3.64 to 11.8 mg/g fat, depending on geographical location and maternal nutrition, was reported by several investigators (Fogarty, 1988; McGuire et al., 1997; Jensen et al., 1998). Although studies in animals have shown that CLA prevents endotoxin-induced growth suppression, improves weight gain, and feed eficiency, and enhances select immune functions, there is no information about its potential function in the newborn infant.

A. LCPUFA and Atopic Disease

In addition to their role in development, EFAs, among them LCPUFAs may also play a role in atopic disease. It is interesting that the observation of a disturbance in EFA metabolism in atopic disease (Hansen, 1937) is almost as old as the discovery of EFAs (Burr and Burr, 1930). It is quite amazing that by the rather primitive analytical techniques available 60 years ago, Hansen was able to find lower AA levels in the blood of atopic disease patients (Brown and Hansen, 1937) and to ascertain that a high supply of EFAs will ameliorate this condition (Hansen et al., 1947). Evidence of disturbances of EFA metabolism, specifically of impaired conversion of 18-carbon precursors to their LCPUFA products, is present in cord blood and may be predictive of atopic disease (Stranngard et al., 1987; Galli et al., 1994; Yu et al., 1996).

Similar changes in EFA and LCPUFA profiles in the milk of mothers of infants with atopic eczema, indicative of a defect in the conversion of linoleic acid to its long-chain products (Wright and Bolton, 1989), might explain why breast-feeding may not alleviate the infant's symptoms. A significant difference in the ratio of linoleic acid to the sum of its metabolites, γ-linolenic, dihomogammalinolenic, and arachidonic acids, in the milk of mothers of infants with newly developed atopic disease as compared to mothers of healthy infants (11.78 vs. 9.02, respectively) has been reported (Businco et al., 1993). The recent study of Yu et al. (1998) advances our understanding of this condition further by reporting that not only are the levels of the products of linoleic acid lower in the milk of atopic mothers but also that, in addition, the ratio of n-6 to n-3 LCPUFAs is higher than

in healthy women. Furthermore, the decline in milk n-3 LCPUFAs during lactation occurred more rapidly in the milk of atopic mothers. This imbalance could result in higher levels of mediators of inflammatory responses and lower anti-inflammatory mediators.

The fatty acid profiles in atopic disease suggest a lower activity of Δ6 desaturase (Manku et al., 1982; Harrobin, 1993; Yu et al., 1998). The recent identification of two candidate clones for mammalian Δ6 desaturase (an 820-bp mouse and 1500-bp human cDNA) with 75% nucleotide and 58% amino acid homologies between the two species (Cho et al., 1998) suggests the possibility for direct measurement of Δ6 desaturase expression and activity rather than inference on activity potential by means of LCPUFA metabolites. Such studies will provide the needed evidence for the link between Δ6 desaturase and atopic disease and might clarify the basis for the genetic predisposition to this condition.

B. Breast-Feeding and Development of Cognitive Function

The effect of breast-feeding, that is, providing the infant mother's milk that contains the LCPUFA's DHA and AA is based on numerous studies in infants as well as studies that have shown that 80% of the FAs in retinal photoreceptors is DHA. Furthermore, recent studies have shown that DHA may affect photoreceptor signal transduction (Litman et al., 2001). The beneficial effect of breast-feeding on visual function has led to supplementation of infant formulas with both DHA and AA. The role of DHA in brain function is much less understood. It is known that DHA is present in high concentration in neural gray matter, that is, it regulates genes involved in synaptic plasticity and the functions derived from the latter (Innis, 2004). Low brain DHA is associated with lower cytochrome *c* oxidase activity and glucose uptake (Ximenes da Silva et al., 2002), the latter could have important functional consequences because of the high-metabolic activity of the developing brain (Innis, 2004). Low-DHA in the developing brain has also been shown to affect dopamine and serotonin metabolism (de la Presa Owens and Innis, 1999; Zimmer et al., 2002), which in turn can affect cognitive function during early development.

TABLE 38.16
Effect of Breast-Feeding or DHA Supplementation on Cognitive Function

Author	Year	Breast-Feeding Months[a]	Effect (IQ Points)	Age of Assessment
Hoefer and Hardy[b]	1929	4–9	Higher IQ	7–13 years
Menkes	1977	Less breast-feeding in children with learning disability	Higher IQ (small difference)	
Fergusson et al.	1982		Higher IQ (small difference)	5–7 years
Rodgers	1978		Higher IQ (small difference)	8–15 years
Morrow-Tlucak and Hande	1988	4 months	Higher IQ (small difference)	1–2 years
Florey et al.	1995		Higher IQ (small difference)	18 months
Rogan and Gladen	1993		Higher IQ (small difference)	
Lucas et al.[c]	1992		Higher IQ (7–8 point diff.)	7.5–8 years
Lanting et al.	1994		Higher IQ	9 years
Harwood and Ferguson[d]	1998		Higher IQ	18 years
Gale and Martyn[d]	1996		Higher IQ	
Mortensen et al.[a]	2002	1–9 months	Higher IQ (7 points)	Adults
Helland et al.	2003		Higher IQ (4 points)	4 years

[a] Where breast-feeding duration is not indicated, it lasted for several months.

[b] Breast-feeding longer than 9 months was associated with the poorest cognitive development.

[c] Preterm infants, gavage fed expressed human milk.

[d] Increase in IQ related to length of breast-feeding attributed to exposure to higher n3 LCP level during pregnancy.

The effect of mode of infant feeding on subsequent cognitive function has been investigated for the past 80 years. IQ and motor development measured either during infancy, early or later childhood, or even in adults showed that feeding breast milk rather than formula has beneficial effects on the infant. The effects were, however, small, especially when correcting for confounding factors such as maternal age and education, socioeconomic status, and birth order. More recent studies indicate a relationship between duration of breast-feeding and IQ in teenagers and adults. It is interesting that breast-feeding for more than 9 months is associated with a decrease in IQ levels (Table 38.16). Whether this is due to lack of proper nutritional complementation during prolonged breast-feeding is not known.

While cognitive benefits might be associated with DHA in human milk, the latter might affect the former by additional factors such as growth factors, especially nerve growth factor (NGF) in human milk and with other components. Furthermore, breast-feeding might also be an indicator of maternal involvement in the infant's well-being and development not only during infancy, but also well beyond it. Well-designed, prospective studies are needed to ascertain the exact role of human milk and its components on cognitive development and function.

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39 Fatty Acids, Cognition, Behavior, Brain Development, and Mood Diseases

Jean-Marie Edouard Bourre

CONTENTS

I. INTRODUCTION

Fatty acids control the structure and function of biological membranes, including membranes in the nervous system. The brain has higher lipid content than any other of the body's organs, except adipose tissue itself. All its lipids, which are mostly phospholipids, are found in cell membranes, and they are almost never sources of energy. Position 2 of the glycerol molecules in phospholipids generally bears a polyunsaturated fatty acid such as docosahexanoic acid (DHA; 22:6(n-3), 22:6ω3, cervonic acid), or arachidonic acid (ARA; 20:4(n-6), 20:4ω6). There may well be smaller amounts of adrenic acid (22:4ω6) and eicosapentanoic acid (EPA; 20:5(n-3), 20:5ω3) or docosapentaenoic acid (22:5ω3). The brain contains very little alpha-linolenic acid (ALA; 18:3(n-3), 18:3ω3) although this fatty acid is the precursor of all the other omega-3 fatty acids. The families of fatty acids are shown in Figure 39.1. A major component of brain membrane phospholipids is the omega-3 fatty acid DHA, and high concentrations of this fatty acid are found in the more metabolically active area of the brain, including the frontal cerebral cortex, which is involved in cognition, mitochondria, nerve endings, and synaptic vesicles. Most studies on cognition have focused on omega-3 fatty acids, or the

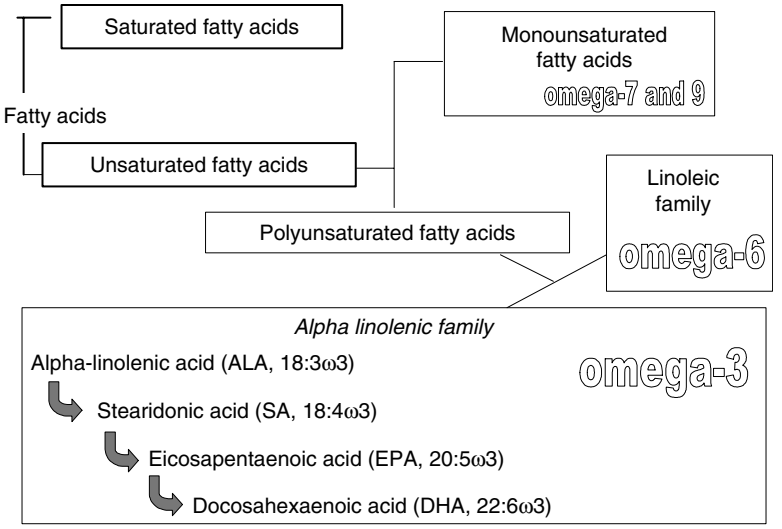

FIGURE 39.1 Omega-3 and other fatty acids. Quite possibly, at least part of the body's oleic acid requirement must come from the diet (Bourre et al., 1997), including that for the peripheral nerves (but not the brain) (Bourre and Dumont, 2003). Saturated fatty acids are synthesized in the cell cytosol and endoplasmic reticulum and in the process of myelinization (Bourre et al., 1976). Most lignoceric and nervonic acids are synthesized in the brain, but some may come from the diet (Bourre et al., 1977).

balance between these fatty acids and omega-6 fatty acids. However, one review has examined the role of the ARA cascade in affective disorders (Sublette and Trappler, 2000).

The importance of the omega-3 fatty acids (ω3) for brain development is based on three findings. First, the brain is exceptionally rich in polyunsaturated fatty acids, including omega-3 fatty acids. They account for 15%–20% of cerebral fatty acids, and for as much as 40% in the neurons and nerve terminals (Bourre et al., 1984). Second, a lack of dietary ALA results in a reduced cerebral DHA content, which is offset by increase in 22:6 omega-6 (Bourre et al., 1984; Galli et al., 1972). Finally, human mother's milk is particularly rich in ALA, but the cow's milk used to prepare baby formula in the early 1980s is not. This was because many formula producers used sunflower oil (without any omega-3 fatty acids) in the 1980s rather than rapeseed oil (9% ALA). The effect of such a deficiency on brain structure and functions, including cognition was first examined in animal studies and later in human newborns. Human fetuses and newborns accumulate considerable quantities of omega-3 fatty acids, mainly DHA (Cunnane et al., 2000).

Experimental studies showed that the differentiation (Bourre et al., 1983) of brain cells in culture requires omega-3 fatty acids as well as omega-6 fatty acids. A lack of dietary ALA disturbs the composition of brain cell membranes (Bourre et al., 1984). Chemical, physicochemical, biochemical, and enzymological analyses, plus toxicological, physiological, electrophysiological, and behavioral studies on ALA-deficient animals provided the first experimental evidence that a dietary component could influence the structure and function of the brain (Bourre et al., 1989b). There is a dose–effect relationship between the amount of ALA in a nursing rat's diet and its accumulation in the pup's brain, a minimum must be added to the diet: 0.4% of the dietary calories in a rat, thus 0.8% in humans, taking into account the brain/body weight ratio and the energy need per unit of surface area (Bourre et al., 1989b). It was subsequently shown that the omega-3 fatty acids in modified baby formula influence the visual and cerebral (including intellectual) capacities of the child, as measured by its neurological development, intellectual quotient, and motor index (Uauy et al., 2003). Hence, all baby formulas now contain at least ALA, in quantities equivalent to those in mother's milk.

Omega-3 fatty acids have both long-term and short-term actions in the brain. Long-term, they influence the production and maintenance of brain structures, and hence their function. Many studies

have shown their effects on the development of the brain. Their short-term effects are on the physiology of signal transduction. These are biochemical pathways that have no direct link with the diet, except that the molecules involved are of dietary origin. Several reports have claimed that these reactions underlie such disorders as autism, dyslexia, or even schizophrenia. And of course, both long-term and short-term influences can be present at the same time, such as when membrane alterations are accompanied by neuroinflammation (Bourre, 2005a).

Several studies have examined the effects of omega-3 fatty acid on brain biochemistry, membrane physical chemistry, enzyme activities and carrier function, neuromediators, electrophysiological parameters, behavior, and cognition. Others have looked at how these and other fatty acids are implicated in the nervous systems of animals and humans during life, particularly during development and aging. They have been covered in recent reviews (Bourre, 2004a,b); there have even been popular books written about them (Bourre, 1990, 2004c), including a recent one on omega-3 fatty acids (Bourre, 2004c).

Psychiatrists are interested in omega-3 fatty acids for several reasons. The high content of these fatty acids in the brain is one reason. Another reason is the experimental demonstration that a lack of dietary ALA results in behavioral and cognitive defects, particularly problems of learning, memory and habits, and reactions to toxins such as morphine. A lack of ALA also results in the abnormal metabolism of neuromediators.

The main feature of the omega-3 and omega-6 fatty acids is that they are both essential and strictly complementary, but they compete for the desaturase enzymes. The human diet usually contains enough omega-6 fatty acid, but the diets of people in occidental countries may contain insufficient ALA and DHA for pregnant women, mainly because of an insufficient intake of rapeseed oil (canola oil), walnut oil, and oily fish.

II. BRAIN DEVELOPMENT: EVIDENCE FROM ANIMAL MODELS AND STUDIES ON HUMANS

A. Experimental Evidence

Animal studies have provided convincing and consistent evidence linking a decrease in brain concentrations of DHA to altered performance in cognitive and behavioral tests. ALA deficiency affects the cognition/behavior of animals. For example, a lack of dietary ALA does not severely alter the neuromuscular function of rats (Frances et al., 1995), but it does affect learning (Bourre et al., 1989b) and behavior in humans (Frances et al., 1996a; Wainwright et al., 1994). Those activities that are associated with memory and habituation are particularly affected (Frances et al., 1996b, 2000), as has been confirmed in studies (Carlson, 2000; Coscina et al., 1986; Heird and Lapillonne, 2005; McCann and Ames, 2005; Salem et al., 2001; Wainwright, 2002; Yamamoto et al., 1988) using other tests. The effects on rodent cognition were shown in tests that involved sensory pathways other than the visual pathway.

The suggested relationship between the changes in learning performance and the composition of the microsomal membrane (Yoshida et al., 1997) seems to correlate well with the cognitive and behavioral deficits seen in rats fed a low-ALA diet and the defects in their monoaminergic nerve transmission, particularly in the frontal cortex (Chalon et al., 2001; Levant et al., 2004). A lack of dietary omega-3 influences specific neurotransmitter systems, particularly the dopamine systems of the frontal cortex, which is involved in cognition, but restoring dietary ALA does not completely restore dopaminergic neurotransmission in the frontal cortex and other areas (Kodas et al., 2002). Thus, the slow incorporation of fatty acids into the brain (Bourre et al., 1989a) is correlated with a slow improvement in learning (Lim and Suzuki, 2001). There is a clear relationship between polyunsaturated fatty acids and neurotransmission, and so between them and behavior (Chalon et al., 2001). Cholinergic and serotoninergic neurotransmission (Aid et al., 2003; Kodas et al., 2004) are also influenced by a lack of dietary ALA.

The most commonly used developmental outcomes examined in human infants are visual function and general neurodevelopment. Neurodevelopment is assessed by the Bayley Scale of Infant Development (BSID), first or second edition. It includes both the mental developmental index (MDI) and psychomotor developmental index (PDI). Other developmental assessments include the clinical Adaptative test (CAT); clinical Linguistic and Auditory Mileston Scale (CLAMS) and Gross Motor Scale (GMS) of the revised Gesell Developmental Inventory. Developmental quotients (DQ) are determined for language development (CLAMS DQ), visual problem solving ability (CAT DQ) in overall cognition (the mean of CLAMS and CAT DQ). A meta-analysis showed that infants fed ALA-supplemented formula had significantly higher concentrations of DHA in plasma and erythrocyte phospholipids, but further studies are needed to provide convincing evidence of the effect of ALA itself on growth and development (Udell et al., 2005).

ALA alone does not seem to be sufficient to ensure that the brain has an optimal concentration of DHA (Bowen and Clandinin, 2000), although an adequate equilibrium between linoleic acid (LA, 18:2(n-6), 18:2ω6) and ALA could ensure the optimal synthesis of DHA (Cheon et al., 2000). The brain seems to use preformed very long-chain omega-3 polyunsaturated (and omega-6) fatty acids most effectively under normal circumstances (Cunnane et al., 2001), which implies that they are synthesized in the liver from ALA (and LA, respectively) or are not obtained directly from the diet. DHA injected into the blood stream is taken up by the brain (Bazan and Scott, 1990). Supplementing the diet of animals with DHA (and EPA) improves certain aspects of their cognitive performance (Carlson, 2000). This has led to a comparison of diets without ALA and those containing either ALA (rapeseed and linseed oils), or DHA (seaweed extracts, fish oils, and purified products), or ALA plus DHA (algae, brain or egg extracts, and mixtures of vegetable and fish oils). There is a clear relationship between omega long-chain polyunsaturated fatty acid status during infancy and the neurodevelopmental status of babies at 1 year of age (Table 39.1) (Voigt et al., 2002).

Diets containing only very long-chain omega-3 fatty acids (EPA and DHA) are not satisfactory: a diet very rich in fish oil alters the composition of brain membranes by increasing the DHA content and considerably reducing the ARA content (Bourre et al., 1990). This favors arousal and learning in young mice, but reduces motor activity and learning in older mice (Carrie et al., 2000). The omega-6 fatty acids must therefore be in balance and accompanied by omega-3 fatty acids. Consequently, a diet supplemented with DHA and ARA prevents the decrease in dopaminergic and serotoninergic neurotransmitters in the frontal cortex of piglets caused by a lack of ALA (de la Presa and Innis, 1999).

Nearly all studies on cognition have concentrated on mammals; we have found no studies on birds and fish.

TABLE 39.1
Effect of ALA Concentration on Blood Parameters and the Mental Performance of Newborn Infants

ALA in milk	0.40	0.95	1.70	3.20
Plasma phospholipid ALA	0.10	0.16	0.23	0.46
Erythrocyte phospholipid ALA	0.05	0.09	0.12	0.23
Erythrocyte phospholipid DHA	0.83	1.55	1.72	2.52
Bayley PDI	88.80	95.50	93.70	96.40
Bayley MDI	94.80	96.30	101.00	100.50

MDI = mental developmental index; PDI = psychomotor developmental index.

Source: From Voigt, R. G., et al. (2002). *J. Hum. Nutr. Diet*. 15: 111–120.

B. EPA and DHA in Human Baby Formulas

DHA is clearly required by babies, including full-term infants (Gibson et al., 1996). Analyses of the frontal cortex of dead infants have shown that babies fed formula lacking DHA have a lower-than-normal DHA content (Byard et al., 1995). The brain of a child takes up 48% of the 10 mg of DHA incorporated each day. The brains of breast-fed babies (mother's milk provides about 60 mg/day) contain about 1 g DHA, while the brains of bottle-fed babies contain only 0.6 g DHA (Cunnane et al., 2000). Fetal accretion of omega-3 fatty acids was estimated at 67 mg/day during the third trimester (Clandinin et al., 1980a,b). In practice, formulas for premature babies should contain both ALA and DHA (Rodriguez et al., 2003), although there is yet to be a clear demonstration of the absolute need of DHA for cognition (Simmer and Patole, 2004). The negative effect of a lack of omega-3 fatty acids on the development of the nervous system (and vision) initially led to the addition of omega-3 fatty acids alone (both EPA and DHA, no omega-6 fatty acids), generally as fish oil. This improved visual acuity, but it does not favor the overall development of the infant, in terms of body weight, height, and head circumference. Finally, almost all experiments have provided evidence of causal links between altered brain concentrations of DHA during the perinatal period and subsequent cognitive or behavioral performance (McCann and Ames, 2005).

C. Importance of the Balance between Omega-6 and Omega-3 Fatty Acids

Consequently, baby formula must contain both DHA and ARA to maintain the omega-3/omega-6 fatty acid ratio. Although the biochemical and physicochemical results are quite conclusive, the behavioral results are somewhat less clear. Most studies on the cognitive/behavioral development of infants fed formula containing long-chain (omega-6 and omega-3) fatty acids have used the BSID, which is considered to be the best standard for assessing the overall ability of infants from birth to 42 months of age. They provide standardized indices of both psychomotor development (PDI) and mental development (MDI). The Fagan Test of Infant Intelligence (FTII) has also been used. An analysis of eight trials carried out in 2001 (Simmer, 2001) was inconclusive, but a second analysis, 3 years later, of 14 published studies comparing baby formulas with and without long-chain polyunsaturated fatty acids was positive. The 14 studies included 7 on the effects on cognitive development; the results take into account the fact that brain DHA is derived from both ALA (with a 10% conversion rate) and preformed dietary DHA (Uauy et al., 2003).

One study (Agostoni et al., 1995) showed a benefit at 4 months, as assessed by the DQ (Brunet–Lezine test), but there was no longer any difference when these children were examined later, at age 2–4 years (Agostoni et al., 1997) (Table 39.2). Another study found that adding ALA and DHA to the formula improved cognitive performance at 4 months and that the improvement persisted (Willatts et al., 1998; Willats, 2002). Thus, an effect at 4 months does not predict an effect at 24 months. However, there is still a close correlation between the long-chain fatty acids in erythrocytes and neurological development (Agostoni et al., 1997). A review of the literature showed a benefit for preterm infants somewhat more convincingly than for term infants (Heird and Lapillone, 2005).

The feeding habits of families undoubtedly vary considerably, so that the DHA status of an infant will be more influenced by the dietary pattern than by the nature of the baby formula/breast milk consumed during its initial months of life. This is probably why no difference was found in infants aged 39 months (Auestad et al., 2003), or any association between the status at birth and performance at age 7 years (Bakker et al., 2003). Another study found a statistically insignificant improvement in children fed formula containing DHA and ARA (Wezel-Meijler et al., 2002). In a more detailed study, a restricted number of children were monitored from before birth, showed that very long-chain polyunsaturated fatty acids are closely associated with an increased IQ at age 6.5 years (Gustafsson et al., 2004). The children of women who take cod liver oil during pregnancy and

TABLE 39.2
DHA in Milk, and Phospholipids in the Blood Plasma and Erythrocytes and the Neurodevelopment Quotient

	Formula 1	Formula 2	Mother's Milk
DHA in milk (g/100 g fat)	0.3	0	01–0.6
DHA in plasma phospholipids (%)	2.7	0.9	2.8
DHA in erythrocyte phospholipids (%)	4.1	1.8	4.1
Neurodevelopment quotient	105	96	102

Formula 1 = milk enriched in long-chain fatty acids; Formula 2 = standard cow's milk. The ALA content of the 3 milks was equivalent to 0.7 g/100 g fat.

Source: From Agostoni, C., et al. (1994). *J. Am. Coll. Nutr.* 13: 658–664; Agostoni, C., et al. (1997). *Arch. Dis. Child.* 76: 421–424.

lactation have improved mental performance measured at 4 years old (Helland et al., 2003). Other authors (Birch et al., 2002) suggest that babies should be given supplemented formula for at least 6 weeks to ensure maturation of the cerebral cortex and optimal visual performance in later years. A quantitative analysis of the influence of the prenatal intake of omega-3 fatty acids on cognitive development estimated that increasing maternal DHA intake by 100 mg/day increased child's IQ by 0.13 points (Cohen et al., 2005).

These studies used multiple tests, and it is quite possible that only some of them are influenced by dietary omega-3 fatty acids, as has been shown in animal studies (Frances et al., 1996b). They do demonstrate the advantage of breast-feeding over feeding formula containing DHA or ARA.

Several studies have shown the importance of breast-feeding and some have justified it by the amount of polyunsaturated fatty acids, particularly omega-3 fatty acids, in mother's milk. It improves the neurological development of newborns, especially if continued for over 6 weeks, as evaluated by motor activity (Bouwstra et al., 2003), and this effect can be measured in infants up to 1 year old. Some believe that this is due to the presence of omega-3 fatty acids, especially DHA (Agostoni et al., 2001). Others have found that babies thus fed have an IQ 8.3 points higher at 18 months (Lucas et al., 1992). One study using the Bailey test showed that breast-feeding had a positive effect, measured at 1 year old, on baby boys, but not on girls (Paine et al., 1999). The effect on neurological development is measurable in children up to the age of 7 or 8 (Horwood et al., 2001) or even 9 years (Lanting et al., 1994). But others have found that the effect cannot be measured at 13 months or 5 years (Angelsen et al., 2001). These widely differing results may be due to the different tests used.

In fact, small differences in brain concentrations of DHA, such as might occur between infants fed unsupplemented or supplemented formulas, may result in small, subtle effects that are currently difficult to detect, but could be significant. This is sustained by animal studies linking a decrease in the brain concentration of DHA to altered performance in cognitive and behavioral tests. Thus, the published literature shows that long-chain polyunsaturated fatty acids are important for the growth and development (cognitive) of human infants (Fleith and Clandinin, 2005).

III. ADULT HUMANS, EPIDEMIOLOGICAL AND EXPERIMENTAL EVIDENCE

A. Mental Health, Cognition, and Mood

It has been reported that omega-3 fatty acids improve cognitive development and learning under certain conditions. Dietary omega-3 fatty acids may improve intentional and physiological functions

involving complex cortical processing. Studies on the relationship between cognition alterations and mood and fatty acids (particularly omega-3 fatty acid) are scarce, but there has been a lot of speculation. These fatty acids are essential for normal general health and well-being, mainly because of their biochemical properties. An Australian study concluded that regular normal meals (including omega-3 fatty acid), including a good breakfast, improved mood and cognitive performance (Lombard, 2000). A New Zealand study found that the personal perception of good mental and physical health varied with the consumption of fish, thus of omega-3 fatty acids, so that they were considered to stabilize mood (Silvers and Scott, 2002). However, an English study found that the consumption of fish did not improve the mood of people who were not depressed (Ness et al., 2003). The food consumption and nutrient intake of subjects with depressed mood was studied, using anxiety and insomnia as indices of compromised mental well-being. Those subjects reporting anxiety or depressed mood had higher intakes of omega-3 fatty acids and omega-6 fatty acids (Hakkarainen et al., 2004b). It is thus too early to say that omega-3 fatty acids modulate cognitive processes by altering mood.

B. Stress

As fish oil is known to influence behavior cognition, at least indirectly, its influence on aggressive behavior was assessed. Anxiety is an incapacitating syndrome. Subjects were given capsules of vegetable oils to determine the optimum dietary ratio between omega-3 and omega-6 fatty acids needed to counteract stress. The ratio was found to be 4 for LA and ALA. This protected against changes in the hippocampus in response to excess cortisol and corticosteroids, avoiding impaired learning (Yehuda et al., 2000). Aggression was found to vary inversely with the consumption of fish; daily doses of 1.5–1.8 g DHA (found in fish oil) helped to reduce stress and decreased the aggressive tendencies of young adults, perhaps by modulating stress. Fish oils reduced the aggressiveness of subjects aged 50–60 years, when given for 2 months, by 30%, but doses of 150 mg per day were insufficient (Hamazaki et al., 2002). An adequate DHA intake could prevent aggression from increasing at times of mental stress; this may indicate how fish oils prevent diseases such as coronary heart disease.

If increasing the intake of omega-3 fatty acids is favorable, what happens if the intake of linoleic (omega-6) acid is reduced? There is evidence for a correlation between the consumption of seed oils, the major dietary source of LA, and a greater incidence of homicide in western countries with similar socioeconomic and background seafood intakes. Thus, a diet poor in LA may prevent behavioral disorders that are now the concern of correctional institutions, social services, and mental health providers (Hibbeln et al., 2004).

C. Hyperactive and Dyslexic Children, Dyslexia in Adult

The cluster of age-inappropriate behavioral abnormalities including inattention, impulsiveness, and hyperactivity are the hyperkinetic disorders. Fatty acids seem to be important for attention deficit associated with hyperactivity (Richardson and Puri, 2000). A pilot study carried out on 50 children showed that supplementing their diet with essential fatty acids (daily doses of 480 mg DHA, 80 mg EPA, 40 mg ARA, 96 mg γ-linolenic acid, and 24 mg tocopherol acetate) improved their symptoms (Stevens et al., 2003). However, another study showed that dietary supplements improved the blood parameters of hyperactive children suffering from attention deficit, but not their clinical symptoms (Voigt et al., 2001). Polyunsaturated fatty acids, including omega-3 fatty acid, could be implicated not only in disorders of cerebral development, attention deficit, hyperactivity, but also in dyslexia and even in autism (Richardson and Ross, 2000). The severity of dyslexic signs was found to vary with the lack of polyunsaturated fatty acids in boys, but not in girls (Richardson et al., 2000). Dyslexia in adults is accompanied by indications of a lack of polyunsaturated fatty acids (Taylor et al., 2000).

D. Depression

The major mood disorders can be roughly divided into unipolar major depression and bipolar disorder. Bipolar disorder, also known as manic-depressive illness, causes shifts in a person's mood and ability to function. It is a complex and chronic condition associated with considerable morbidity and mortality, including a high suicide rate. The increased prevalence of depression over the past half century seems to parallel fundamental changes in dietary habits, with a reduced intake of foods containing omega-3 fatty acids (Colin et al., 2003). The frequency of depression in British Columbia increased as the traditional dietary habit of fish eating was lost, and then fell as these dietary elements were reintroduced (Bates, 1988). There is a relationship between the drop in omega-3 fatty acid consumption (in fish) and the risk of depression, particularly as the incidence of the disorder varies from 1 to 50 per 1000 population, depending on the country, in parallel with fish consumption (Hibbeln and Salem, 1995; Tanskanen et al., 2001). This was not confirmed by another study (Hakkarainen et al., 2004b). Yet another study showed that, in Crete, there is an inverse relationship between the DHA concentration in adipose tissue and the risk of depression (Hibbeln, 2002). An overview of 41 published studies covering 23 countries shows that a fish-poor diet leads to a low-DHA concentration in mothers' milk (which is undesirable for newborns) and an increased risk that the mothers will suffer from postnatal depression (Hibbeln, 2002). However, there was no such relationship between dietary EPA and ARA.

There is still, however, little hard evidence showing a relationship between changes in dietary fatty acids and the risk of depression with aging, although plasma composition of fatty acids and depression are associated during aging (Tiemeier et al., 2003). Regular fish consumers in a community of elderly French people aged 65 years and above, had fewer depressive symptoms and scored higher on the Mini Mental Status Examination. The regular fish consumers (at least weekly) were better educated (OR 1.19–1.65) and had better incomes (1.37–1.89) than the controls (Barberger-Gateau et al., 2005).

Two clinical studies have used doses of 2 g per day of EPA ethyl ester to successfully treat cases of depression that responded only partially to classical psychiatric treatment (Peet and Horrobin, 2002; Puri et al., 2000). One patient was given EPA together with conventional treatment; this not only improved the clinical signs (suicidal tendencies, social phobias), but also resulted in morphological changes (reduced volume of the lateral ventricles) (Puri et al., 2001). DHA is also a successful treatment for minor depression (Mischoulon and Fava, 2000). A patient suffering from postnatal depression was successfully treated with omega-3 fatty acids (Chiu et al., 2003), while another study using fish oil (2.69 g per day, EPA/DHA = 1.4) starting at the 34–36 week of pregnancy and continuing until 12 weeks after birth obtained negative results (Marengell et al., 2004). Yet another study found that a dietary supplement of 200 mg DHA per day for 4 months after childbirth prevented the drop in plasma DHA but did not alter the patients' self-evaluated state of depression (Lombard, 2000). This could be because they measured mood changes rather than real depression and/or because the doses of DHA were too low. Treatment with 9.6 g omega-3 fatty acid per day for 8 weeks gave positive results with cases of major depression (Su et al., 2003), but DHA alone (2 g per day for 6 weeks) did not seem to be effective (Marangel et al., 2003). In fact, EPA seems to increase the action of antidepressant drugs (Murck et al., 2004).

A study of bipolar disorder patients (manic depressives) in 14 countries showed a correlation between the prevalence of the disorder and low fish consumption, with the threshold of vulnerability being 65 g per day (Noaghiul and Hibbeln, 2003); treatment with omega-3 fatty acids could be useful under certain specific conditions (Stoll et al., 1999).

Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes, thus dietary, genetic and hormonal factors may all play a role in both depression and coronary heart disease (Frasure-Smith et al., 2004).

Depressive symptoms are among the most prevalent psychiatric symptoms and are strongly associated with the development of depressive disorders; individuals with depressive symptoms are

at four times greater risk of developing major depression within 1 year. Depression is a major source of functional disability. This has been consistently demonstrated in epidemiological, primary care, and out-patient studies, at least in Western Europe and North America.

E. Drug Addiction

Drug addiction alters cognition. Omega-3 fatty acids, and perhaps omega-6 fatty acids, may also be involved in drug addiction. Ex-cocaine addicts more rapidly return to addiction if they lack polyunsaturated fatty acids (Buydens-Branchey et al., 2003a,b). It is possible that a diet-related change in the membranes of certain cerebral neurons makes some people more unstable, so that they may be more predisposed to drug addiction in response to other impulses. Whatever the cause, drug addicts often have very poor diets, but how this can aggravate their condition, interfere with curing their addiction or make re-addiction more likely remains a major question. Animal experiments have shown that a lack of ALA alters the response to morphine (Frances et al., 1996a).

F. Autism

Autism remains one of the very few conditions classified as a syndrome, defined only in terms of observable symptoms. This is largely due to the lack of accepted biochemical diagnostic markers. Sufferers from autism often have a range of physiological problems, and alterations in their fatty acid metabolism, particularly those related to eicosanoid production. An autistic boy aged 11 years was successfully treated with fish oil (540 mg EPA per day) for 4 weeks (Johnson and Hollander, 2003). The plasma phospholipids of autistic children contain 23% less DHA than do those of normal children, while the total omega-3 fatty acid concentration is 20% lower and their omega-6 fatty acids are unchanged (Vancassel et al., 2001). Another study found that the phospholipids in erythrocytes were 70% below normal (Bell et al., 2000).

G. Dementias

Alzheimer's disease is a neurological disorder that is characterized by progressive memory loss, intellectual decline, and eventually global cognitive impairment. Major symptoms include short-term and long-term memory loss, impaired speech and language, visual-spatial changes, impaired abstract reasoning, and poor judgment. Several epidemiological studies have shown that omega-3 fatty acids play a role in the prevention of dementia, as expanding knowledge in the area of lipid biochemistry suggests that Alzheimer's disease is associated with brain lipid defects. For example, the Rotterdam study found that the risk of dementia with vascular features is positively correlated with the consumption of saturated fatty acids, but negatively correlated with the consumption of omega-3 fatty acid-rich fish (Kalminjn et al., 1997a); these results are discussed in (Engelhart et al., 2002). A diet rich in unsaturated fatty acids and unhydrogenated fat was found to protect against Alzheimer's disease, in contrast to a diet rich in saturated fatty acids and *trans* fatty acids (Morris et al., 2003b). Some epidemiological studies have shown that the consumption of finfish also seems to protect against dementia, including Alzheimer's disease. In France, the consumption of meat, because of its saturated fatty acid content, is only poorly correlated with an increased risk of dementia, while the consumption of fish has a protective effect. Those subjects who ate fish at least once a week were 34% less likely to develop any form of dementia, and 31% less likely to suffer from Alzheimer's disease. The effect was still present when socioeconomic factors were taken into account, as these factors are linked to both the reduced risk of Alzheimer's disease and the fish consumption (Barberger-Gateau et al., 2002). A study carried out in the United States found that Alzheimer's disease was 60% less common in people that consumed about 60 mg DHA per day (at least one fish meal a week) than in people that ate very little fish (Morris et al., 2003a). The overall findings in Japan are the same (Otsuka et al., 2002).

A low plasma concentration of omega-3 fatty acids (including DHA) is an indication of risk of cognitive deficiencies and other types of dementia, besides Alzheimer's disease (Conquer et al., 2000).

Cardiovascular risk often clusters into a metabolic syndrome that may increase the risk of dementia. Epidemiological studies have implicated cerebrovascular disease and its antecedent as risk factors for Alzheimer's disease. Vascular dementias and Alzheimer's disease have nutritional factors in common: an excess of omega-6 fatty acids and a lack of omega-3 fatty acids; this leads to changes in the microvasculature, chronic inflammation, platelet aggregation, and endothelial dysfunction (Otsuka et al., 2002). This provides at least a partial explanation of why the cognitive disorders in very elderly people are positively correlated with the consumption of LA, and negatively correlated with the consumption of fish (Engelhart et al., 2002). The cardiovascular risk increases the risk of dementia, particularly vascular dementia (Kalmijn et al., 2000). Inflammatory processes may well be implicated in all these disorders (Simopoulos, 2002). It also seems clear that insufficient fish consumption is a significant risk factor, there has not yet been any published report on the use of omega-3 fatty acids in the preventive treatment of dementia of any type. One-fourth mixture of ALA and LA, given as a capsule, was shown to improve the quality of life for those suffering from Alzheimer's disease, as measured by tests of spatial orientation, cooperation, mood, appetite, short-term and long-term memory, sleep, and hallucinations (Yehuda et al., 1996). There have been no studies to date on the implication of omega-3 fatty acids in cognitive alterations due to alcoholism and in alcoholic dementia, although studies on experimental animals have shown that dietary ALA modulates the effects of alcohol on such things as the nerve terminals (Zerouga et al., 1991).

H. Schizophrenia

Antipsychotic drugs are the most effective treatment of schizophrenia, particularly the most disturbing symptoms such as agitation and psychosis. Treatment with new antipsychotics seems to have improved the negative symptoms and cognitive deficits that are considered difficult to treat. However, their side effects significantly reduce the patient's quality of life, so that it is important to develop adjunctive treatment strategies. Adjuncts of essential fatty acids, and their metabolites, such as eicosanoids, have been considered to improve the outcome of this disease. For instance, schizophrenics whose diet includes plenty of fish have less severe clinical signs (Peet et al., 1996).

Patients who ate 10 g of fish per day for 6 weeks seemed to have improved symptoms (Laugharne et al., 1996), as did patients given a combination of 120 mg EPA, 150 mg DHA, 500 mg vitamin C, and 400 IU vitamin E twice a day for 4 months, but the improvement was relatively modest (Arvindakshan et al., 2003). A single patient given EPA alone showed improved symptoms; the turnover of brain phospholipids (as measured by ^{31}P NMR) returned to normal and brain atrophy had receded after treatment for 6 months (Puri et al., 2000). This clearly needs to be confirmed. Some patients who had been treated and stabilized were then given EPA for 3 months (Peet et al., 2001). EPA has also been used to supplement a 6-month course of antipsychotic drugs, but it left residual symptoms (Emsley et al., 2002). One author reported that treatment with 3 g EPA per day produced no results (Fenton et al., 2001), perhaps because the dose was too low or because the patients were nonresponders (Horrobin, 2003). There have been several proposals as to how omega-3 fatty acids are involved in schizophrenia (Peet, 2003), including the modulation of neurotransmission, particularly dopaminergic transmission, but there is as yet no hard evidence (Peet et al., 2001).

Fatty acids differentially affect serotonin receptors and the binding of its transporters in the rat brain; this may be important for understanding neuropsychiatric diseases such as schizophrenia, where there seems to be an association between altered fatty acids levels and the serotoninergic system (Du Bois et al., 2006).

The erythrocyte membranes of schizophrenic patients contain subnormal concentrations of DHA and EPA, and a link has been found between the change in fatty acid profile and the severity of the clinical disease (Assies et al., 2001). Another study did not find these results (Hibbeln et al., 2003). However, there could well be subgroups of patients for whom the omega-3 fatty acids are

particularly important, which would explain why clinicians find divergent results in which the DHA content is either elevated, depressed, or normal, depending on the publication. In fact, there is a relationship between membrane phospholipids composition, alterations in neurotransmitter systems and schizophrenia (Du Bois et al., 2005), and hence cognitive alterations.

I. Cognition and Aging

Dietary factors might modify the cognitive decline that results from aging. Several studies have shown that cognitive deterioration in the elderly is associated with deficiencies of macronutrients and micronutrients. Fatty acids are the prime candidates, as they are quantitatively and qualitatively extremely important in the brain. The biochemical and physiological roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during aging have been reviewed (Bourre, 2004b). The changes that occur with advancing age are complex, in both animals and humans. Omega-3 fatty acids may be involved either directly or indirectly, depending on the part of the body, the structure, cells, and organelles, or lipids concerned. A recent French study showed that age-related cognitive deficit is linked to a reduction in the omega-3/omega-6 fatty acid ratio in erythrocytes (Heude et al., 2003); an excess of nutritional LA was also coupled to a decline in cognitive performance, while the reverse is true for fish oils (Kalmijn et al., 1997a,b). Studies on an aging population in southern Italy found that an elevated unsaturated fatty acid intake (monosaturated, unsaturated, and polyunsaturated), high intakes of antioxidant compounds, and a diet very low in saturated fatty acids could act synergistically to improve cognitive performance (Solfrizzi et al., 2005). Fish consumption may be associated with a slower cognitive decline with age: the rate was 10% slower among persons who consumed one fish meal per week, and 13% slower in persons who consumed two or more fish meals per week (Morris et al., 2005). Phosphotidyl-choline improves the memory, learning, concentration, vocabulary recall, and mood of elderly people suffering from cognitive loss. Phosphatidyl-choline, together with vitamin B_{12}, improves learning in aging mice (Hung et al., 2001). There appears to be no question but that an adequate intake of omega-3 fatty acids could ensure the turnover of membranes, so helping to protect against brain aging. However, a dietary supplement of high concentrations of omega-3 fatty acids produces behavioral changes that vary with the age of the individual, improving learning in young animals, but reducing learning and motor activity in older ones (Carrie et al., 2000). This should be borne in mind when considering dietary supplements.

There are well-established benefits of omega-3 fatty acids (from fish or fish oils) for vascular health, and theses advantages may explain better cognitive performances in those with high omega-3 fatty acids concentrations in their erythrocytes. (The evidence obtained from self-reported consumption of fish or fish-oil supplements is generally a less informative indicator than are measurements of erythrocyte omega-3 fatty acid contents.) Some vascular risk factors and cardiovascular diseases have been linked to cognitive decline (and dementia). Hence, fatty acid intake, mainly omega-3 fatty acids, might affect the development of cognitive impairment by influencing the development of thrombosis and atherosclerosis. Cerebrovascular disease is associated with a cognitive decline and progression of dementia, and this association could be weakened by a high intake of omega-3 fatty acids (from finfish or fish oils) to slow or prevent the development of age-related cerebrovascular disease. The vascular risk factors that predict stroke are associated with a wide range of cognitive alterations that occur around the age of 60 years; moreover, a lower childhood IQ is associated with an increased incidence of cardiovascular diseases.

These observations could be expanded to other stages of life. For instance, the consumption of oily fish and marine omega-3 fatty acids is associated with a reduced risk of impaired cognitive function, while a diet rich in cholesterol and saturated fat has been linked to an increased risk in a middle-aged population (Kalmijn et al., 2004). The spatial memory of rats fed sub-normal amounts of omega-3 fatty acids in the Morris maze is compromised. These animals seem to have fewer endothelial mitochondria, and a lower ratio of micro-vessels to degenerative pericytes (de Wilde et al., 2002).

Aging is associated with an increased production of free radicals and a decrease in antioxidant defences, both of which lead to increased oxidative stress that is implicated in altered cognition and the development of dementia. On the contrary, a diet rich in antioxidants such as vitamins E, A, and C, β-carotenoids, and flavonoids could protect against such changes. But only α-tocopherol is effective, at least in animals (Clement et al., 1995). It has been speculated that the loss of DHA in Alzheimer's disease may reflect its propensity for free radical-mediated lipid peroxidation. Thus, either a decreased intake or increased oxidative stress (or both) could contribute to brain DHA depletion and hence to altered cognitive function.

Food supplements (and erythrocyte content) are associated with better cognitive aging. If associations with omega-3 fatty acid are causal, optimizing the omega-3 and omega-6 fatty acid intakes could help preserve cognitive function in old age (Whalley et al., 2004). Greater fish oil intake is associated with better cognitive function in late adulthood. Supplementation is associated with improved attention and physiological functions, particularly those functions involving complex cortical processing, even in healthy subjects (Fontani et al., 2005).

IV. DISCUSSION

Omega-3 fatty acids have two broad actions, one long-term and the other short-term. Their long-term actions are on membrane composition and function. This is supported by studies on brain development and probably the role of dietary omega-3 fatty acids in the prevention of dementia, including Alzheimer's disease. Their short-term actions could involve phospholipid metabolism, and hence the modulation of signal transduction. The evidence for this includes the effect of EPA on depression, schizophrenia, and autism. But the two types of action can occur simultaneously, as in inflammation during Alzheimer's disease. It is too soon to state that omega-3 fatty acids prevent depression by treating inflammation, even though there are good indications that inflammation occurs during depression. Although there is clear evidence that omega-3 fatty acids do prevent and reduce these symptoms, to some extent, these relationships are not necessarily causal. Epidemiological studies showing that dietary fish, or fish oil capsules alleviate some psychiatric diseases, have flaws inherent in this type of epidemiological survey (e.g., association does not imply causation).

The timetable of cerebral development means that any short-term perturbation can result in long-term changes in the biochemistry, physiology, and function of the brain, and the possibilities of recovery are relatively restricted, because of the genetically programmed brain development, although a dietary lack of omega-3 fatty acids results in their replacement by other fatty acids. Dietary omega-3 fatty acids contribute to the construction and maintenance of the brain (Bourre, 2004a,b,c).

Dietary omega-3 fatty acids, especially DHA, have probably had a major influence of the evolution of the human brain. It is found in a few special vegetables, oily fish, all seafood (fish and shellfish), and certain eggs (Broadhurst et al., 2002). The lack of dietary ALA can readily be overcome by using rapeseed oil (canola oil) and walnut oil ("noix de Grenoble" oil) (soybean oil has too much omega-6 fatty acids) and a special variety of eggs (omega-3 eggs, Columbus® or Benefic®)—all other eggs, including organic ones, are unsuitable. Walnuts, mixtures of oils containing at least 50% rapeseed oil, or 10% linseed oil, and other plant-based foods rich in ALA are also useful (Bourre, 2005b). Thus, it is not difficult to find food rich in omega-3 fatty acids (Bourre, 2003, 2005b).

An adequate intake of the precursor ALA enables the body to regulate interconversion, as dietary DHA results in a regulatory systems being short-circuited, requiring precise definitions of nutritional requirements for all physiological and pathological situations, which is not presently possible. Dietary intake of DHA varies enormously and depends on where people live. Children are at particular risk of such deficiencies. Thus, those with low-dietary intakes need to be identified and counseled. Wild oily fish are generally very rich in DHA and EPA, as are farmed fish, provided they are fed correctly. The human nutritional value of meat and eggs, in terms of lipid (amounts of

omega-3 fatty acids), may vary considerably depending on the fats fed to the animals/hens (Bourre, 2003, 2005b).

Although a good dietary intake of omega-3 fatty acids is fundamental, it is not enough. The presence of omega-6 fatty acids must also be taken into account, to ensure that the omega-6/omega-3 ratio is not above 5. And this ratio is far too high in the diet of most people in the Western world. It should only be reduced by increasing the intake of omega-3 fatty acids, and most certainly not by decreasing the intake of omega-6 fatty acids, as our intake of omega-6 fatty acids is only slightly above the recommended value.

V. SUMMARY AND CONCLUSIONS

The effects of fatty acids, essentially omega-3 fatty acids, on cognition and behavior have been examined mainly during perinatal brain development and, to a lesser extent, during aging and in terms of some psychiatric diseases. Fatty acids control the structure and function of biological membranes, including membranes in the nervous system. The high omega-3 polyunsaturated fatty acid content of the brain clearly indicates that these lipids are involved in brain biochemistry, physiology, and functioning, and thus in cognitive performances during development and in some cognitive changes due to neuropsychiatric diseases and aging. Animal studies have provided convincing and consistent evidence linking a decrease in brain concentrations of DHA to altered performances in cognitive and behavioral tests. Some studies on perinatal cerebral development and omega-3 fatty acids have focused on ALA, while others have examined long-chain derivatives such as DHA and EPA. Other studies have examined the influence of ALA plus DHA, sometimes with the omega-6 fatty acid, ARA.

Studies on ALA first showed the effect of a dietary component on the structure and function of the brain. These include (1) cultures of dissociated brain cells, analyses of the fatty acids and lipids in whole organ, regions, cell types in the brain, and classes of phospholipids; (2) physicochemical studies on brain membrane fluidity, and biochemical and enzymological studies on enzymes such as ATPase; (3) physiological studies on dopaminergic, serotoninergic, and cholinergic neurotransmission; (4) toxicology of heavy metals and trans fatty acids; (5) studies on vision, hearing, and taste; and (6) electrophysiological studies (ERG, EEG) and cognitive and behavioral studies, memory, and habituation being specifically affected. For instance, dietary omega-3 deficiency influences specific neurotransmitters systems, particularly the dopamine systems of the frontal cortex, which is related to cognition.

The accumulation of considerable conclusive experimental evidence led to the inclusion of ALA in baby formulas. This decision was due to many studies on newborns, including those on cognition. The nature of the polyunsaturated fatty acids (particularly the omega-3 fatty acids) in baby formulas for both full-term and premature infants influences the infant's visual, neurological, cerebral, intellectual capacities and cognition. Infants fed vegetable oil-based formulas (poor in ALA) may have poorer visual functions, lower cognitive scores, and acquiring learning tasks are slower in comparison with those breast fed or those fed formulas supplemented with DHA. Despite a lack of exhaustive experiments, DHA and EPA were also added to baby formula, which may have a limited or even negative effect because of competition with omega-6 fatty acids. A combination of DHA, EPA, and ARA was later shown to improve membrane composition and cognition.

Dietary omega-3 fatty acids may also help prevent psychiatric disorders such as depression and dementia, particularly Alzheimer's disease. They probably directly influence major depression, bipolar disorder (manic-depressive illness), and schizophrenia. Omega-3 fatty acids may be important in diseases such as dyslexia and autism. Dietary omega-3 fatty acids deficiency can alter membrane turnover, and thus accelerate cerebral aging and cognitive decline. The lack of omega-3 fatty acids in today's occidental diet suggests that consumer must be persuaded to select foods that are rich in omega-3 fatty acids, such as rapeseed and walnut oils and oily fish.

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40 Fatty Acids and Aging

José L. Quiles and M. Carmen Ramírez-Tortosa

CONTENTS

I. CONCEPT AND PRINCIPLES OF AGING

Aging is defined as the progressive loss of function accompanied by decreased fertility and increased mortality with the advance of age (Kirkwood and Austad, 2000). In addition, it is said to be a common phenomenon to all multicelled organisms and has been described as an endogenous and a progressive decline in the effectiveness of physiological processes after the reproductive phase of life (Camougrand and Rigoulet, 2001; Barja, 2002; Sohal et al., 2002). The aforementioned decline has been attributed either to a genetic program present in all the individuals of a species or else to the stochastic accumulation of errors in the somatic cells, which would give rise to the progressive loss of cell functions (Camougrand and Rigoulet, 2001). The study of aging confronts, among others issues, the problem of separating the process itself from diseases related to aging.

The determinants of longevity have captured the attention of researchers of a great variety of disciplines, including sociology, biology, psychology, and medicine (De Benedictis et al., 2001). Many studies made on the human species have concluded that humans have steadily increased their average life expectancy, owing to improved living conditions and advances in medicine, particularly the control of infectious diseases.

As a living being ages, the probability of death augments, reaching a certain characteristic age for each species at which all the individuals have died. In the case of the human, this maximum lifespan has been established at 110–120 years. The life expectancy requires a multifactorial treatment, given that it is affected by genetic as well as environmental factors, in addition to the stochastic component resulting from the interaction between individual changes for survival and unpredictable events that occur over the course of a lifetime (De Benedictis et al., 2001). At present, there are many

countries in which only a few individuals are capable of reaching this maximum lifespan, owing to infectious disease or inadequate nutrition. In industrialized countries the problem is different. Since 1840, the life expectancy has grown at a rate of approximately 3 months per year, and at the moment there are no signs that this tendency is going to change. The good news is that health has improved; however, the cost of health has also grown, and previously unknown diseases have appeared in the past 100 years, such as Alzheimer's, age-associated macular degeneration (currently the main cause of blindness), diverse cardiovascular diseases, cancers, and so forth. Therefore, it can be said that the average lifespan has lengthened in developed countries with respect to undeveloped ones, although the maximum duration of lifespan appears to be the same (Halliwell and Gutteridge, 1999; Partridge and Gems, 2002).

In relation to mean and maximum lifespan, Sanz et al. (2006) suggests that the endogenous character of aging is useful to distinguish between maximum and mean lifespan, two concepts too frequently mixed. In that sense, the maximum lifespan is determined mainly by genes, meanwhile mean lifespan is governed primarily by the environment and to a lesser extent by the genotype.

Until not long ago, scientists asked: if aging is harmful for individuals, why does it occur to everyone? The answer could be that aging is the collateral effect of something else. That is, the genes that delay aging could do so by repressing the cause that generates damage associated with aging. A source of this type of damage appears to be reproduction. Fertility is frequently reduced both evolutionary when aging decreases as well as by the presence of punctual mutations that extend the lifespan. Food appears to be another source of damage, since many of the genes that are involved in the reduction of aging also participate in the response to the change in nutrient levels. Furthermore, it is known that the reduction in the ingestion of food diminishes aging in a variety of organisms from yeasts to mammals (Masoro, 2000; Partridge and Gems, 2002).

II. SOME AGING THEORIES

A large number of theories have been proposed in order to explain how and why we age, although many lack the scientific bases to be seriously considered (Medvedev, 1990). Overall, any theory must explain, according to Sanz et al. (2006) some characteristics of aging: it is progressive, endogenous, irreversible, and deleterious for the individual. Other authors also stress that any theory should be able to fulfill the following conditions (Sohal et al., 2002):

- It should be able to explain how an organism loses the capacity of maintaining its homeostasis in the latter part of its life.
- It should clarify the bases for the wide variations in the duration of life of cohorts, genetic stocks, and species.
- It should be able to identify the factor or factors responsible for extending the lifespan by simple mutations or through experimental regimes such as caloric restriction in rodents and environmental temperature changes in poikilotherms.
- It should be able to demonstrate that the degree of aging can be manipulated by variations in the factors suspected of causing senescence.

The existing theories on aging can be grouped into two large blocks: stochastic theories and genetic-evolutionary theories. In general, it can be said that there is no single, unified theory that by itself can explain the phenomenon of aging in all its dimensions. This is because the mechanisms of aging can vary considerably in different organisms, tissues, and cells.

A. Stochastic Theories

Stochastic theories propose that aging is caused by random damage in different biological molecules. The damage accumulates little by little with age until reaching a level where the damage triggers the physiological decline known as aging.

Pearl (1928) proposed the *rate-of-living theory*, based on the observation that species with high metabolic rates had shorter lives, stating that life expectancy is inversely proportional to the metabolic rate of the species. Although initially the link between metabolism and longevity was unknown, Harman (1956, 2006) presented the biochemical explanation for this interrelationship, putting forward the *theory of free radicals*, this becoming one of the most widely accepted theories on aging. This theory suggests that normal aging is the result of the random damage to tissues by free radicals. With time, Harman began to focus his theory toward the mitochondrion as the primary source of the production of free radicals and the target of damage that these elements cause. Later, Miquel et al. (1980) proposed the *mitochondrial theory* (progressive damage to mitochondrial DNA (mtDNA) by reactive oxygen species, ROS). Given that at present many ROS are not known to be free radicals, the main theory today concerns oxidative stress (Yu and Yang, 1996).

There is another group of stochastic theories that bear similarities to the theory of oxidative stress and that in a certain way could be explained by it (Halliwell and Gutteridge, 1999). This group includes the *crossing-over theory* (the random crossing-over of proteins and DNA alter cell functioning); the *theory of the catastrophic error* (accumulation of random damage in protein synthesis), the *theory of glycosylation* (the formation of glycosylate proteins give rise to a serious disruption of cell functions); the *theory of longevity determinants* (aging is caused by the products of metabolism and the degree of aging is determined by the capacity to protect oneself against such products); the *membrane hypothesis* (damage to the cell membrane diminishes the capacity to eliminate waste products, a decreased protein synthesis, and a loss of water from the cytoplasm, thereby diminishing enzyme activity); and *entropy theory* (mechanisms such as the restriction of caloric intake, reducing the degree of entropy production, releasing energy more slowly, and delaying molecular deterioration).

B. Evolutive and Genetic Theories

This group of theories considers the aging process to be part of a phenomenon of continuous development and maturation, genetically controlled and programmed. Although these concepts are very attractive, such strict control with respect to developmental phenomena proves contradictory with regard to such a diverse degree of expression in aging effects.

According to the *immunological theory* (Walford, 1969), aging is brought about by the decline in the capacity of the immune system to produce antibodies. That is, as the immune response diminishes, the capacity of the system to discriminate between its constituents and foreign elements also declines, with an increase in autoimmune reactions. This theory has the drawback that it is applicable to the immune system and does not rule out the possibility that these changes might be secondary to other earlier ones, such as hormonal types.

The *neuroendocrine theory* (Medvedev, 1990; Weinert and Timiras, 2003) is based on the fact that no part of the body can act in isolation from the nervous and endocrine systems and, therefore, if one of these is disturbed, the other systems are affected in some way. However, as with the immunological theory, this theory lacks universality, as not all living beings have a neuroendocrine system but nevertheless undergo aging.

The *genetic-evolutionary theory* (Finch and Ruvkun, 2001) proposes that aging is the continuation of the developmental and differentiation process, unraveling a sequence of events encoded in the genome. This theory postulates that aging would be the late consequence of the expression of genes selected by evolution for increasing reproductive success. The power that favors the selection of beneficial genes is manifested more in the young, as these are the ones in charge of reproduction and genetic transmission, so that what occurs to these genes at later ages is not taken into account. Thus, a gene that favors reproduction but is harmful in the long term is not selected for elimination.

Within this group of theories, the *hypothesis of disposable soma* was proposed by Kirwood (2000). According to this theory, the utilization of energy over a lifetime should be used preferentially for reproduction, at the expense of repair mechanisms, which lose capacity soon after surpassing the reproductive age. This theory suggests that among the main candidates determining the life

expectancy of a species from the genetic standpoint are genes that regulate the repair and maintenance of somatic cells. The study of gene expression in aging rodents reveals the presence of genes that alter their expression with age, or their expression is altered with the intervention of the type of caloric restriction that affects the aging rate. It is not surprising that most of these genes are involved in the pathways of cell response to oxidative damage. According to the hypothesis of disposable soma, natural selection favors those genes that act in the early stages of life, thereby permitting reproduction of the species, against those genes that are in charge of preserving nongerminal cells or available soma. Therefore, the somatic lines (as opposed to the germinal cells) of all animals that decline and degenerate with age, provoking the phenotypic changes that we recognize as aging.

Some scientists believe that the keys to aging should be sought in the process of cell division, an idea that has led to the *telomeres theory*. According to this hypothesis, formulated by Olovnikov (1973), the shortening of the telomeres in each of the cycles of cell division is the factor responsible for the limitation in the proliferation of the cell cultures, the so-called Hayflick limit (1998).

III. THE ROLE OF MITOCHONDRIA IN AGING

Progressive loss of mitochondrial functionality is one of the common events associated with aging. For this reason, these organelles are considered the biological clock of aging (Salvioli et al., 2001). In fact, mitochondria have been proposed as the link between the age-dependent accumulation of oxidative damage produced by ROS and the physiological alterations associated with aging (Van Remmen and Richardson, 2001). In this sense, several experimental studies suggest that mitochondria is one of the main targets of the aging process. According to Salvioli et al. (2001) this evidence includes (1) accumulation of deletions and certain mutations of the mtDNA in addition to the decreased mtDNA copy number in some tissues; (2) age-dependent decline in the activity of some enzymes in the mitochondrial electron-transport chain (mtETC); (3) greater production of free radicals, presumably as a consequence of the above-described alterations; and (4) changes in the morphology of mitochondria and collapse of the mitochondrial membrane potential (ψ_{mt}).

Variations in the functionality of mitochondria may determine adequate or inadequate aging, according to studies of specific population groups around the world. For example, an inherited variation in an mtDNA germ line (halogroup J) has been associated with a more adequate aging process and to an extended lifespan in the Italian population (De Benedictis et al., 2000). On other hand, in Japan, three mutations associated with an mtDNA germ line have been found with a high frequency in centenarians in this part of the world (Tanaka et al., 1998).

Another compelling question is the role of mitochondria as a key element in cell-signal transduction. Thus, mitochondria may also be considered an element of control for the nuclear gene expression. In this sense, a number of adaptation or regulation proteins have been found at the mitochondrial level or are translocated to the mitochondria to cope with this role, as with Nur77/TR3, p53, PKCδ, JNK/SAPK, some caspases and several members of the bcl2 family, such as Bid, Bax, or Bim (Finkel and Holbrook, 2000).

Regarding mitochondria in the aging process, the control of apoptosis is vital. This control is frequently lost in aged cells, which in addition are more prone to suffer from oxidative stress. ROS decrease ψ_{mt}, allowing the opening of the transition pore and the subsequent escape to the outside of calcium and other substrates. This sequence of reactions leads to apoptosis in lymphocytes as well as liver and the brain cells of aged mice (Watson et al., 2000).

IV. MITOCHONDRIA AS THE CELLULAR SOURCE OF REACTIVE OXYGEN SPECIES

In recent years, it has been ascertained that although ROS are produced through a large number of pathways of the aerobic metabolism, the main source of these species is the mitochondria (Lenaz, 1998; Halliwell and Gutteridge, 1999; Cadenas and Davies, 2000; Sastre et al., 2000; Salvioli et al., 2001;

Van Remmen and Richardson, 2001). The inner mitochondrial membrane is markedly different from the rest of biological membranes since its protein content goes beyond 80% (most of biological membranes do not exceed of 50%) (Quiles, 1995). Because of its importance and significance in the context of oxidative stress, the protein complexes within the mtETC deserve to be mentioned. In aerobic organisms, the mtETC produces the energy needed for life support. Basically, food is oxidized through the loss of electrons that are accepted by such electronic carriers as nicotinamide adenine dinucleotide (NAD^+) and flavins (flavin mononucleotide, FMN, and flavin adenine dinucleotide, FAD). The reduced nicotinamide adenine dinucleotide (NADH) and the reduced flavins ($FMNH_2$ and $FADH_2$) are oxidized again by oxygen, producing great amounts of ATP. Oxidation occurs with small jumps in which energy is gradually released (Lenaz, 1998). The mtETC is composed mainly of five lipoprotein complexes (Quiles, 1995; Lenaz, 1998; Cadenas and Davies, 2000): (1) Complex I, NADH dehydrogenase complex; (2) Complex II, succinate dehydrogenase; (3) Complex III, bc_1 complex; (4) Complex IV, cytochrome *c* oxidase (CCO); and (5) Complex V, ATPase. The mtETC fraction that metabolizes oxygen is Complex IV. This enzyme uses four molecules of reduced cytochrome *c* to remove one electron from each one and to pass them to an oxygen molecule. This tetraelectronic reduction of oxygen is not tenable in a single step but rather must be done electron by electron. Because of this gradual reduction, the protein complex must be sure that partially oxidized oxygen, highly toxic, will not leak into the medium before being transformed into water.

Superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are, respectively, the product of the monovalent and bivalent reduction of oxygen. Both species are usually produced during aerobic metabolism, mainly at the mitochondrial level (Cadenas and Davies, 2000). It has been estimated that a 1%–5% of the oxygen consumed by the mitochondria is not fully reduced to water. In turn, this small oxygen percentage is transformed to $O_2^{\bullet -}$, which spontaneously or as the result of the action of superoxide dismutase enzymes is transformed to H_2O_2. Although CCO is the enzyme involved in the oxygen reduction, it hardly generates free radicals. In contrast, the two main sites of free-radical production at the mtETC are complex I and complex II (Cadenas et al., 1977; Ksenzenko et al., 1983; Shimomura et al., 1985; Cross and Jones, 1991). What happens is that during the pass from one complex to the other, some electrons escape and directly join the surrounding oxygen, resulting in the generation of $O_2^{\bullet -}$. Moreover, at the external mitochondrial membrane there is an additional source of ROS. This source comes from the deamination process of biogenic amines by monoamine oxidases, which through a bi-electronic reduction produce H_2O_2 from O_2 (Hauptmann et al., 1996). The physiological level of ROS production at the mtETC depends on the metabolic state of mitochondria. Thus, the state of mitochondrial rest (state 4), characterized by a low respiration level and no ADP availability, is associated with a high rate of ROS production, probably as a consequence of the high degree of reduction of the chain components. The active mitochondrial state (state 3), characterized by high oxygen expenditure and elevated ADP availability, shows relatively low-ROS production. In the state of anoxia (state 5), distinguished by a limitation in the oxygen delivery and absence of respiration, no ROS production is observed (Cadenas and Davies, 2000). From the standpoint of aging, there seems to be a consensus on the major importance of complex I as the main site for ROS production (Sanz et al., 2006).

Biological membranes are overall very sensitive to oxidative stress because of the presence of a double carbon–carbon bond in the lipid tails of its phospholipids (PL) (Montine et al., 2002). Oxidative damage to membrane lipids may be directly generated through initiation by ROS as hydroxyl radicals or the superoxide anion, or indirectly by some products of the same lipid peroxidation such as some highly reactive aldehydes that maximize the phenomenon (Esterbauer et al., 1991). Irrespective of the way, oxidative damage of membrane lipids leads to its alteration and to changes in membrane fluidity, and as a consequence of all these changes to alterations in membrane function (Halliwell and Gutteridge, 1999). Moreover, there is a particular mitochondrial lipid called cardiolipin. This is a highly unsaturated lipid and consequently highly prone to oxidation. Cardiolipin oxidation is extremely important to mitochondria since it is involved in the function of mtETC proteins such as CCO or the adenine nucleotide transporter (ANT) (Paradies et al., 1998).

Since lipids and proteins are physically very close, oxidative damage to mitochondrial proteins, as result of direct oxidative stress or as a consequence of lipid peroxidation, may lead to cross-linking, degradation, and loss of function of such proteins. Several membrane proteins, such as ATPase, ANT, CCO, and so on, are easily inactivated by oxidative stress. Moreover, protein oxidation leads to the opening of the permeability transition pore, a key step in the process of apoptosis. In summary, mtETC protein alteration has as a direct consequence, the loss of mitochondrial functionality and indirectly a rise in the ROS production (Lippe et al., 1991; Forsmark-Andree et al., 1997).

Mitochondria have their own genome, which differs in structure and organization from the nuclear genome. It is composed of a variable number of copies of identical circular double-strand DNA (up to ten copies). It is located in the mitochondrial matrix, near specific areas of the inner mitochondrial membrane (i.e., close to the main source of ROS). It has a small size (16.5 kb) and codifies for 13 mitochondrial proteins: 7 subunits from complex I, 1 protein from complex III, 3 proteins from complex IV, 2 from complex V (ATPase), 22 trRNA and 2 rRNA (Lenaz, 1998; Cadenas and Davies, 2000; Van Remmen and Richardson, 2001). As opposed to nuclear DNA, mtDNA is not protected by histones and it has been traditionally considered of a high susceptibility to be oxidatively attacked (Richter et al., 1988). For a long time, it was considered that mitochondria did not have a system to repair damaged DNA. Recently, the existence of such a system was reported, although it is not yet well known (Bohr and Anson, 1999).

Many reports suggest that oxidative damage to mtDNA is more important from the perspective of aging than that involving lipids or proteins. This fact is due to the ability of mtDNA to be spread because of the division capacity of mitochondria and cells, which allows amplification of the physiological consequences of the damage inflicted. Furthermore, oxidative damage to mtDNA might be even more important than the damage to the nuclear DNA as the entire mitochondrial genome codifies for genes that are truly expressed, while the nuclear genome contains a huge amount of nontranscribed sequences (Van Remmen and Richardson, 2001). Oxidative stress may affect mtDNA in several ways, among which the most typical are the oxidative alteration to bases, the rise in the number of deletions and the occurrence of certain mutations. At the moment, the most popular approach to studying oxidative alterations to bases is through the analysis of 8-hydroxy-2-deoxyguanosine by HPLC attached to electrochemical detection. Using this procedure, several labs have reported higher levels of this biomarker at the mitochondrial level with respect to the values found in the nucleus during aging (Chung et al., 1992; Agarwal and Sohal, 1994). Concerning DNA deletions, rise has been reported in the frequency of these events with aging among a wide variety of postmitotic tissues from several species, including humans, monkeys, rodents, and nematodes (Yoneda et al., 1995). Moreover, the rise in the percentage of deletions has been directly correlated with oxidative damage. Among the most studied deletions, one has been called the "common deletion" because of its frequency, which increases twofold to threefold with aging in some tissues such as the brain (Cortopassi et al., 1992). Nonetheless, since the percentage at which the level of deletions increases does not exceed 2%–3%, the physiological significance that this phenomenon could have for aging is speculative (Van Remmen and Richardson, 2001). Mutations of mtDNA are the basis for a high number of human pathologies. This has opened a new field in mitochondrial research. This type of DNA is transmitted maternally and, moreover, there are many copies of the molecule in single cell (polyplasmy) so that there is the chance that a mutation undergoes different degrees of heteroplasmy. All these questions imply that a lesion is manifested only when around 80% of all the mtDNA in the cell become mutated (Lenaz, 1998; Michikawa et al., 1999).

V. MEMBRANE UNSATURATION AND LONGEVITY

One of the most important findings concerning fatty acids and aging comes from the collaborative work between Dr. Pamplona, at Lleida, and Dr. Barja, at Madrid, both in Spain. These scientists revealed over the past decade something that appears to be crucial to understand—that is, to explain and to update the mitochondrial free radical theory of aging. Briefly, it has been proven from

comparative studies that long-lived mammals and birds have low rates of mitochondrial ROS production and low levels of oxidative damage in their mtDNA (Pamplona et al., 2002). Now it is also possible to affirm that long-lived mammals and birds possess a low degree of unsaturation in their cell membranes (Sanz et al., 2006). In other words, animals with higher lifespan have membranes containing fatty acids less susceptible to lipid peroxidation. This leads to lower levels of DNA damage from lipid peroxidation and of lipoxidation-derived protein modification in such species (Pamplona et al., 1998, 1999, 2000a,b, 2002, 2004; Herrero et al., 2001; Sanz et al., 2006).

As stated above, oxidative damage to membrane lipid is extremely important in aging. Polyunsaturated fatty acids (PUFA) from membrane PLs are highly sensitive to oxidation. Thus, in this sense, membranes very rich in PUFA should be more prone to oxidation than those having a lower level of highly unsaturated fatty acids (UFA). A study on the fatty acid profile of liver mitochondrial membranes from eight animal species ranging in maximum lifespan from 3.5 to 46 years revealed that the degree of fatty acid unsaturation is inversely correlated with maximum lifespan (Pamplona et al., 1998). Authors stressed that this was not due to a low content of UFA in long-lived mammals, but mainly to redistribution between components of the polyunsaturated n-3 fatty acid series. What happened was that docosahexaenoic acid (22:6n-3) was predominant in short-lived animals, meanwhile in long-lived species the dominant n-3 fatty acid was linolenic acid (18:3n-3). This difference may explain why fatty acids from long-lived animals had the lowest peroxidizability index (the total number of double bonds present in UFA in the membrane). Something similar has also been found by these authors in the fatty acid profile of heart mitochondria in eight different species (mouse, rat, guinea pig, rabbit, sheep, pig, cow, and horse), in which the highly unsaturated 22:6n-3 predominated in the short-lived species meanwhile 18:2n-6 was the dominant in long-lived ones (Pamplona et al., 1999). These fatty acid redistributions among species may be attributable to differences in the elongase and desaturase systems, mainly at the levels of the biosynthetic pathways of 20:4n-6 (arachidonic acid) and 22:6n-3 from their dietary precursors, 18:2n-6 (linoleic acid) and 18:3n-3. As is well known, a fine control in the elongation and desaturation pathways is crucial for maintaining the membrane function through proper fluidity, signaling, and other means (Sanz et al., 2006).

What is the physiological meaning of the lower degree of unsaturation in long-lived animals? Couture and Hulbert (1995) have stated that the low double bond index (DBI) found in large body size mammals is related to the need to decrease their metabolic rates. This is assumed to be because a low DBI leads to a lower permeability to ions, ion pumping being one of the main determinants of metabolic rate. Body size has also been negatively correlated with permeability to Na^+ and K^+ in liver hepatocytes (Porter and Brand, 1995) and to H^+ in the inner mitochondrial membranes (Porter and Brand, 1993). However, although this explanation could be plausible for mammals of different sizes, Pamplona et al. (2002) state that it cannot explain the low DBI of birds since they have a similar or even higher metabolic rate than that of mammals of similar size. These authors hypothesize that instead a relation exists between DBI and metabolic rate, a true correlation between DBI, lipid peroxidation, and lipoxidative damage.

Differences in peroxidizability between species should lead to differences in oxidation at the level of lipids, DNA, or protein. In fact, this has been confirmed. For example, studies have been made of the fatty acid differences between a short-lived species such as the rat (maximum lifespan potential (MLSP) = 4 years) vs. a long-lived species such as the pigeon (MLSP = 35 years) in relation to lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria (Pamplona et al., 1999). Results were as expected, in the sense that the lower double bond content present in different lipids of the pigeon (with substitution of the highly unsaturated 22:6n-3 or 20:4n-6 for less unsaturated 18:1n-9 or 18:2n-6) were associated with a lower concentration of lipid peroxidation products *in vivo*, and a lower level of malondialdehyde–lysine protein adducts in heart mitochondria of pigeons than in rats. Following these results, authors suggested that the constitutive lower level of unsaturation in long-lived animals helps to protect their tissues and mitochondria against lipid peroxidation and oxidative protein modification and can be a factor contributing to their slow rate

of aging. Comparable results have been also described for liver mitochondria of mouse, rat, guinea pig, dog, pig, cow, and horse (maximum lifespan potential ranging from 3.5 to 46 years) in relation to lipid peroxidation, as well as the presence and steady-state levels of malondialdehyde–lysine and N^{ε}–carboxymethyllysine adducts.

In summary, the above mentioned studies provide a correlation between the maximum longevity of animals and the degree of unsaturation of membrane fatty acids. That correlation joins the previously stated one between the rate of mitochondrial oxygen radical generation and the maximum longevity of animals. In long-lived homeothermic vertebrates, both free-radical production and the membrane fatty acid unsaturation are lower, offering an explanation for some of the main causes of the low aging rate peculiar to these animals. No studies have been carried out on these aspects in relation to dietary fat and, as it will be stated below, this is another notable aspect of fatty acids and aging.

VI. DIETARY FAT, MITOCHONDRIAL OXIDATIVE STRESS, AND AGING

The previous section discusses the relationship between the membrane fatty acid profile and longevity, in particular between the DBI and MLSP in different homeothermic species. Authors of these studies argue that diet has a moderate effect, if any, in establishing a particular fatty acid profile in a given biological membrane leading to the final MLSP. However, although the low impact of dietary fat on issues related to interspecies longevity, a different viewpoint may be taken in relation to human nutrition and health-related issues concerning dietary fat intake. In this sense, the importance of a particular dietary fat profile is well known in relation to avoiding cardiovascular disease, certain types of cancer, and alterations at the immunological level (Quiles et al., 2006b). In addition, in terms of aging, the dietary fatty acid profile has been demonstrated to be important to the mitochondrial oxidative stress associated with the aging process (Quiles et al., 2002b, 2004b, 2006a; Ochoa et al., 2003).

Nutrition has been related to aging for some time, a link that identified mainly at the level of caloric restriction and supplementation with antioxidants. The role of caloric restriction, that is, a limitation in food intake, was first described in 1935 by McCay et al. (1989). Since then, caloric restriction has been demonstrated to enhance mean lifespan in a wide range of species, and decreasing the development of age-related disease in rodents (Finkel and Holbrook, 2000; Masoro, 2000). These effects are suggested to be mediated by a reduction in the level of oxidative stress. This is supported by the fact that calorically restricted mice undergo less oxidative stress than their counterparts fed ad libitum (Finkel and Holbrook, 2000; Masoro, 2000). In addition, caloric restriction prevents many of the changes found at the level of gene expression during aging (e.g., increase in the expression of heat-shock proteins). Caloric restriction might be a powerful therapeutic tool to fight against aging since, a priori, it shows effectiveness against oxidative stress and aging (Roth et al., 1999). Nevertheless, the possible use of caloric restriction as an antiaging therapy in humans would involve practical and ethical difficulties that make it almost impossible to consider feasible or desirable (Finkel and Holbrook, 2000).

As described in previous sections, oxidative stress plays a very important role in the global process of aging. Thus, nutritional supplementation with molecules or substances endowed with antioxidant capacity would be useful as a possible antiaging therapy. Miquel and Economos (1979) performed some of the first studies in this field. These authors studied the capacity of thiazolidine carboxylate to enhance vitality and mean lifespan in mice. Later, Furukawa et al. (1987) showed the role of glutathione in the protection against the decline in immune function associated with aging. Many other antioxidants (including vitamin E, vitamin C, coenzyme Q, herbal extracts rich in polyphenols and flavonoids, and others) have been tested in relation to aging, showing more or less positive results (Halliwell and Gutteridge, 1999; Huertas et al., 1999; Watson et al., 2000). Although results found with these antioxidants have been successful in relation to the attenuation

of the age-related oxidative stress, they have had low or no success in extending lifespan. The reasons are not clear, but perhaps require a deeper understanding of the pharmacological properties of the molecules studied, particularly in relation to absorption, tissue distribution, metabolism, and dosage. Furthermore, it should be remembered that ROS play a role in cell signaling, and therefore the antioxidant dosage must be carefully adjusted in order to avoid changes in the redox state that could alter the cell function. These problems are being addressed through the use of a new generation of synthetic antioxidant substances, mimetic of the superoxide dismutase and catalase enzymes. These substances are being assayed with some success, for example, in relation to the extension of longevity in mice and *Caenorhabditis elegans* (Melov et al., 1998; Rong et al., 1999).

For the past 15 years, our group has been investigating changes in dietary fat with regard to oxidative stress. Dietary fat type influences several biochemical parameters at the mitochondrial membrane level (Mataix et al., 1998; Quiles et al., 1999a). The importance of fatty acids resides in the fact that the mitochondrial membrane (as with other biological membranes) adapts its lipid composition to some extent in response to dietary fat (Huertas et al., 1991a; Quiles et al., 1999c; Ochoa-Herrera et al., 2001). Thus, humans and animals fed on olive-oil-based diets have membranes richer in oleic acid than those fed on sunflower-oil-based diets, in which case the membranes are richer in linoleic acid. In addition, adaptations of the electron-transport system in response to dietary fat type have been widely reported (Huertas et al., 1991b; Quiles et al., 2001; Battino et al., 2002a). Moreover, oxidative stress is related to biological membrane composition. In that sense, a polyunsaturated fat source (e.g., sunflower oil) will make membranes more prone to oxidation than will a saturated (e.g., animal fat) or a monounsaturated (e.g., olive oil) source. This has been widely demonstrated under a wide range of physiological and pathological situations using both animal models and humans (Quiles et al., 1999b, 2002b; Ramírez-Tortosa et al., 1999; Battino et al., 2002b; Ochoa et al., 2002).

According to the above mentioned premises, the dietary-fat type affects mitochondrial structure and function as well as susceptibility to oxidative stress. In this way, if we build "customized" biological membranes according to a particular dietary fat type, it would be possible to alter in a positive way the manner in which different organs undergo the aging process. This working hypothesis represents a new approach to the study of aging from a nutritional perspective and it could have far-reaching implications for the study of the aging phenomenon.

We have conducted studies comparing virgin olive oil and sunflower oil as the single life long dietary fat source in rats (Table 40.1). All of the studies started at weaning and ended at 24 months of age of the animals, with samples being collected at different age periods (from 6 to 24 months). The results may be grouped into those related to general aspects of oxidative stress status in blood

TABLE 40.1
Fatty Acid Composition of Edible Oils Used in Most of Our Experiments

Fatty Acid Composition	Virgin Olive Oil (g/100 g)	Sunflower Oil (g/100 g)
C16:0	8.9	12.6
C16:1n-7	1.1	0.2
C18:0	1.9	1.9
C18:1n-9	78.7	24.1
C18:2n-6	8.4	60.1
C18:3n-3	0.9	1
Total saturated	10.9	14.6
Total unsaturated	89.1	85.4
Total monounsaturated	79.8	24.3
Total polyunsaturated	9.3	61.1

(Quiles et al., 2004b), those concerning the effects on liver mtDNA deletions and ultrastructural abnormalities (Quiles et al., 2006a) and those concerning differences between mitotic and nonmitotic tissues in relation to different aspects of mitochondrial function (Quiles et al., 2002b; Ochoa et al., 2003).

A. Studies on Fatty Acids, Oxidative Stress, and Aging in Blood

Concerning studies on blood and plasma aspects of oxidative stress during aging (Quiles et al., 2004b), the first question to answer is whether the dietary manipulation determines the plasma fatty acid profile. We followed the quantitative determination (mg/dL) of fatty acid profile in plasma-cholesterol esters (CE), triglycerides (TG), and PL. Although some studies suggest that fatty acids become more saturated with aging, this being associated with a decrease in the degree of polyunsaturation (Ulmann et al., 1991; Imre et al., 2000). Most authors (e.g., Engler et al., 1998) reported lower levels of saturated fatty acids (SFA) with aging as well as increased levels of selected PUFA. In the present study, concerning total SFA, animals fed only on olive oil led to cholesterol esters, TG, and PL more saturated with age. No changes in SFA were found with age in animals fed on sunflower oil. These results paralleled net increases in all classes of PUFA for cholesterol esters, TG, and PL for both dietary groups with aging (except for TG in the olive-oil group). These results suggest that net changes in fatty acids during aging depend to some extent on the specific dietary fat, although the general feature of increased polyunsaturation is maintained.

Consequences of the intake of PUFAn-6 or monounsaturated fatty acids (MUFA) on health are well established, apart from those related to the effects on blood lipids. Among these, consequences are those related to oxidative stress. In this sense, it has been reported that plasma and subcellular-membrane lipid profiles of animals fed on diets with PUFAn-6 fatty acids are associated with higher levels of oxidative stress at different levels than when animals are fed on virgin olive oil. Examples of such a situation include the stress produced by xenobiotics such as doxorubicin (Huertas et al., 1991a), the performance of physical exercise (Mataix et al., 1998) or the intake of thermally oxidized fats (Battino et al., 2002b; Quiles et al., 2002a). Even under the situation of aging, it has been demonstrated that lipid peroxidation is lower in animals fed on olive oil (Ochoa et al., 2003). Moreover, in an attempt to test whether antioxidant supplementation of PUFA-rich diets would help preserve the beneficial aspects of PUFAn-6 in health, avoiding their higher susceptibility to oxidation and other deleterious aspects during aging, rats were fed lifelong on a PUFAn-6 plus coenzyme Q_{10} diet. This treatment resulted in lower levels of DNA oxidative damage and to an improvement in the mean and maximal lifespan of animals compared with rats fed on a PUFAn-6-alone diet (Quiles et al., 2004a).

Opposing results concerning free radical damage and aging have been reported in recent years (Halliwell and Gutteridge, 1999). However, overall, it appears that free radical damage (and particularly the levels of DNA damage) increased during aging (Halliwell and Gutteridge, 1999; Chevanne et al., 2003). In our study, when studying DNA damage in peripheral blood lymphocytes (in terms of DNA double strand breaks), we found the lowest levels in young animals fed on virgin olive oil, which were approximately 50% lower than the damage found in the sunflower-oil group. Aging raised the levels of DNA oxidative damage in both dietary fat groups, with the highest values found in the sunflower-oil group. This finding agrees with the above mentioned assumption of higher DNA damage with aging, and is additionally consistent with the free radical theory of aging of Harman (2003).

B. Studies on Mitochondrial DNA Deletions, Oxidative Stress, and Ultrastructural Alterations in Liver

The liver is the central metabolic organ of the body; therefore, dietary changes can have a major impact on aging liver and on general health (Anantharaju et al., 2002). Moreover, the liver is critical in the

protection from oxidative damage and plays a major role in the breakdown of potentially harmful lipophilic toxins (Thomas et al., 2002). Although the aging liver appears to preserve its function relatively well (Anantharaju et al., 2002), several changes have been associated with this organ during the process of aging. We therefore investigated possible effects on the frequency of liver mtDNA deletions, oxidative stress, and mitochondrial abnormalities in liver mitochondria during aging by following the previously described model of feeding rats lifelong with two different dietary-fat sources (virgin olive or sunflower oils).

The study of the fatty acid profile of mitochondrial PL revealed the following. In relation to SFA, no differences were found for this fraction between both dietary treatments. A net increase was found in the amount of these types of fatty acids, individually as well as for total fatty acids, with aging (except for C24:0 in olive-oil group). MUFA were higher in animals fed on olive oil both at 6 and at 24 months. Aging increased these fatty acids in the virgin-olive-oil group. In animals fed sunflower oil, only the total amount of MUFA were affected by aging, showing a net increase. PUFAn-6 were higher in animals fed on sunflower oil for both time periods. Aging led to a net increase in all studied PUFAn-6 in both dietary groups. For PUFAn-3, virgin-olive-oil groups had a higher content in these fatty acids, both individually and as a total. Aging increased PUFAn-3 in both dietary groups. As a consequence of results on n6 PUFA and PUFAn-3, the total PUFA index showed no differences between dietary groups, but aging resulted in an enhancement. Something similar was found for the total fatty acid content. Finally, concerning the ratio between PUFAn-3 and PUFAn-6, animals fed on sunflower oil led to an index sevenfold higher than animals fed on virgin olive oil; aging did not alter this index. In summary, the different lipid profile of the diets were properly reflected in liver mitochondrial PL of young and old animals, suggesting, as for plasma, a proper adaptation of the rats to dietary fats.

There is substantial evidence from human and animal studies linking mtDNA deletions and aging. Deletion frequency is affected by age, tissue of origin, species, and the presence of some age-related diseases (such as Alzheimer's) and these aspects also vary widely depending on the laboratory (Kang et al., 1998). We have found increased frequency of the so-called common deletion at the mtDNA levels in aged animals (Quiles et al., 2006a), with those fed on sunflower oil being twice as frequent as those fed on virgin olive oil. This finding demonstrates that the age-related rise in mtDNA deletions can be modulated by the dietary-fat profile. A similar effect was previously demonstrated for caloric restriction (Kang et al., 1998). In addition, mtDNA deletions corresponded to increased levels of oxidative stress with aging in both dietary groups, although this increase was greater in animals fed on sunflower oil. We investigated whether changes in the frequency of mtDNA deletions and oxidative stress status could affect mitochondrial ultrastructure under our experimental conditions. We found that sunflower oil led to deterioration in mitochondrial structure, as suggested by the lower percentage of cristae per μm of mitochondrial contour found in old animals fed on sunflower oil compared to the young animals fed on the same oil (Quiles et al., 2006a). In addition, animals fed on virgin olive oil had a higher number of mitochondrial cristae in both age periods. Mitochondrial circularity (the increase of which represents control loss) was higher in old animals fed on sunflower oil compared to those fed on virgin olive oil (Quiles et al., 2006a,b). These results demonstrate that the age-related increase in liver mtDNA-deletion frequency is differentially modulated by the intake of different dietary fats, with virgin olive oil leading to a lower frequency of deletions than the n-6 polyunsaturated sunflower oil. On other hand, mtDNA-deletion frequency could be correlated with mitochondrial oxidative-stress status and ultrastructural alterations.

C. Differences between Mitotic and Nonmitotic Tissues

The third group of studies concerns differences between mitotic (liver) and nonmitotic (skeletal and cardiac muscles) tissues in relation to aging from the standpoint of mitochondrial oxidative stress and function. Regarding the fatty acid profiles, we found (Ochoa et al., 2003) that the proportion

of SFA in liver did not change with age in either group, although there was a higher proportion of these fatty acids in the sunflower group at 12, 18, and 24 months. Only dietary fat affected liver SFA. In heart, the sunflower group increased SFA with age. Differences between diets were found at 12 and 18 months, with animals fed on sunflower oil showing the highest levels. In skeletal muscle, differences between groups were detected only at 6 months, with the olive group registering the highest SFA proportion. The effect on age differed in the rats fed virgin olive oil having lower SFA levels at 12, 18, and 24 months. Age also affected skeletal muscle SFA. Animals fed on olive oil had higher MUFA levels in all tissues, except for 6 months in skeletal muscle. Aging affected the proportion of MUFA differentially, depending on the tissue. In liver, age did not affect olive group, while in the sunflower group the rats 18 and 24 months old showed significantly lower values than in rats 12 months old. In heart, MUFA decreased in both dietary groups at 18 and 24 months with respect to 6 months. In skeletal muscle, age led to increased MUFA in both groups. The lipid source significantly affected the three tissues studied, while age was significant only for heart and muscle. The proportion of PUFA, for all the tissues studied, proved higher in the sunflower group (except for heart at 18 months). No age effect was detected in liver. In heart, the olive group increased in PUFA at 18 and 24 months with respect to 6 and 12 months. The sunflower group registered a significant decrease at 12 months with respect to 6 and 24 months. In skeletal muscle, both groups showed a similar response, with the highest value at 18 months.

Oxidative stress in these animals was studied through the analysis of mitochondrial hydroperoxides (Ochoa et al., 2003). In liver, differences between diets were found only at 12 months, with the sunflower group reaching the highest levels. Both groups showed a significant decrease at 24 months compared with 6 and 18 months. Nonmitotic tissues (i.e., heart and skeletal muscle) increased hydroperoxide levels with age. Animals fed on sunflower oil reached higher values for all time periods except for 6 months. A two-way ANOVA revealed an effect of age in the three tissues studied. The lipid source had a direct effect only on heart and muscle hydroperoxides.

Mitochondrial function in these animals was investigated through the study of some elements of the mtETC (Quiles et al., 2002b), namely, cytochrome b, coenzyme Q (CoQ), and the amount and activity of the complex IV (the CCO system). In liver, CoQ levels for animals fed olive oil compared with sunflower oil were higher for 6 months of age and lower for 24 months. Age affected only the sunflower-oil group, with an increase in animals of 24 months of age. In heart, for both age periods, olive oil led to lower levels of CoQ than sunflower oil. A time-course increase was found for olive oil fed animals. In skeletal muscle, the olive-oil group registered lower levels of CoQ than did the sunflower-oil group at both ages. In terms of age, this molecule increased at 24 months for both dietary groups. Concerning cytochrome b in liver, the olive-oil group showed higher values for 6 months and lower levels for 24 months, compared with the sunflower-oil group. Age affected only the sunflower-oil group, with a sharp increase at 24 months. For heart, no differences were found between dietary treatments for 6 months of age, and higher levels were found in sunflower oil at 24 months. For skeletal muscle, both groups were similar at 6 months and the olive-oil group differed from the sunflower group at 24 months (with lower values). Aging boosted cytochrome b values in both groups at 24 months. Regarding the cytochrome a + a3 concentration for liver, dietary treatment had an effect only at 24 months, with higher levels found in rats fed sunflower oil. Age affected only the sunflower-oil group, with higher values at 24 months. In heart, no differences between diets were found for both age periods. Age affected both dietary groups, which decreased cytochrome a + a3 levels at 24 months. In skeletal muscle, no differences were found between dietary treatments. The age effect was reflected as higher values at 24 months for both groups. In terms of CCO activity in liver, differences were found between the diets at 24 months (higher activity for sunflower oil). No age effect was found for olive-oil-fed animals, but CCO activity increased at 24 months in sunflower-oil group. In heart, no differences between diets were found. Aging led to a decrease in both groups. For skeletal muscle, diet had an effect only at 6 months, with higher activity for the sunflower-oil group. Aging led to a reduction in CCO activity in the sunflower-oil group. Complex IV turnover for liver registered no differences with regard to diet or age. In heart,

a higher turnover was found at both age periods for sunflower-oil rats. Aging led to a sharp decrease in turnover for both dietary groups. In skeletal muscle, diet resulted in differences only at 24 months, with higher levels for the sunflower-oil group. Age decreased the turnover in both dietary groups, showing lower values for 24 months.

Aging, considered as an endogenous and progressive phenomenon (Barja, 2002), leads throughout the lifespan to different disturbances in mitochondria and their components, such as mtDNA (Sohal and Dubey, 1994; Lee et al., 1997; Michikawa et al., 1999). These disturbances (which have a high oxidative component) have a negative impact on mitochondrial structure and function. Depending on the capacity of the affected tissue to repair the damage or to replace the altered cell, tissue function is affected to a greater or lesser extent (Quiles et al., 2002b). In this way, tissues with the ability to regenerate their cells, such as liver, appear to be able to buffer the damage, at least in part, as suggested by the lack of alterations of mitochondrial function in terms of CCO activity (Quiles et al., 2002b). However, a loss in function is found in postmitotic tissues such as skeletal muscle, heart, or brain. These tissues lack the opportunity to replace damaged cells and are likely to have a less effective repair system (differences between liver and heart regarding repair mechanisms for mtDNA damage have previously been reported [Souza-Pinto et al., 1999]). This loss in function is reflected in the substantial decrease in CCO activity, which leads to the uncoupling of the mtETC, with the further bioenergetic inefficacy and increased ROS production (Quiles et al., 2002b; Ochoa et al., 2003). Mitochondria from postmitotic tissues buffer the unfavorable situation by increasing in some elements of the mtETC, such as cytochrome b or PUFA. The increase in polyunsaturation could enhance membrane fluidity and CCO activity by the presence of a more polyunsaturated cardiolipin, as has been suggested previously (Huertas et al., 1991b; Quiles et al., 2001). However, both actions raise ROS production. The role of dietary fatty acids in this mechanism could reside in the building of an environment more or less prone to the generation and propagation of ROS, especially when as the result of events such as aging, failures in the mtETC start to appear. Moreover, dietary fat could modulate the phenomenon through variations in the antioxidant system and overall upregulate or attenuate the process. Thus, as postmitotic tissues are the most affected by aging, the influence of diet should be particularly important in these tissues.

Summarizing the results from our group concerning dietary fat, mitochondrial oxidative stress and aging, one of the most important findings is that it is possible to modulate mitochondrial membranes through dietary fatty acid changes and that these changes are maintained lifelong if the diet is not changed. As a consequence of such manipulation, changes in mitochondrial oxidative stress, mtDNA deletions, mitochondrial ultrastructural alterations, and mitochondrial electron-transport function take place. In this sense, dietary fats rich in MUFA are more positive than fats rich in PUFA, in agreement with findings related to membrane unsaturation and evolution. Thus, from an evolutionary perspective or from a nutritional perspective, the way to extend the human lifespan is the same: to ameliorate reactive-oxygen production by reducing the unsaturation of the mitochondrial membranes. However, at least in the short term, we cannot manipulate evolution, but we can modulate our diet and this is a very attractive opportunity to improve our health.

VII. DIETARY FATTY ACIDS AND COGNITIVE FUNCTION IN AGING

Cognitive impairment is a major component of dementing syndromes in humans and influences the individual's ability to function independently (Solfrizzi et al., 2006). Cognitive decline in old age is typically attributed to a certain amount of brain atrophy, but there is growing evidence that the process is influenced by a complex interplay of health-related biological and nonbiological factors (Winocur and Greenwood, 2005). Previous work has linked cognitive decline to cardiovascular disease and generalized atherosclerosis (Breteler et al., 1994), diabetes mellitus (Naor et al., 1997), hypertension, and high and low blood pressure (Launer et al., 1995; Guo et al., 1997). Dietary effect on cognitive decline has been only partially studied and among dietary components, fat has focused

the attention of scientists (Winocur and Greenwood, 2005; Solfrizzi et al., 2006). In this sense, the effects of high-fat diets on cognitive function in rats have been studied mainly by the group of Dr. Winocur, while Dr. Solfrizzi and his colleagues have focused on the role of UFA on age-related cognitive decline (ARCD) in the Italian population.

In a series of studies, rats, administered diets high in saturated or polyunsaturated fat, were impaired on various tests of learning and memory (Greenwood and Winocur, 1990; Winocur and Greenwood, 1993; Gold, 1995). Tasks found to be sensitive to the negative effects of high-fat diets included Olton's radial arm maze, a nonspatial test of conditional associative learning, the Hebb–Williams complex maze series, and a variable-interval, delayed alternation (VIDA) test that highlighted deficits in rule-learning and specific memory function. The range of cognitive processes sampled in these diverse tests suggests that the dietary effects on brain function were not confined to specific brain regions and could be best described as nonspecific in nature. The impairments observed in rats fed high-fat diets were linked, to some extent, to the type of dietary fat. Thus, while impaired performance was associated generally with a high level of fat intake (20% by weight in high-fat diets compared to 5% in standard laboratory chow), rats fed dietary fats high in SFA, such as beef tallow or lard, typically were more impaired than rats fed dietary fats high in PUFA, such as soybean oil. Indeed, when fats and oils were blended in order to distinguish between the roles of SFA, PUFA, and MUFA, the impairment on the VIDA task was positively associated with the dietary level of SFA, and independent of the PUFA or MUFA levels (Greenwood and Winocur, 1990). The mechanism whereby SFA impairs cognitive performance has not been identified, but data suggest that broad changes to bulk-membrane fatty acid composition are probably not a primary factor (Greenwood and Winocur, 1990).

In the identification of the pathways that mediate the fat-induced effects on cognitive function, one possibility relates to the well-established fact that high-fat diets contribute to increased insulin resistance and decreased glucose uptake in the brain (Winocur and Greenwood, 2005). Impaired glucose regulation is an important factor in the cognitive decline associated with type-2 diabetes and normal aging (Gold, 1995). Since glucose treatment can temporarily improve memory function in aged rats and humans (Messier et al., 1999), presumably by facilitating glucose metabolism in specific brain regions (e.g., hippocampus), Greenwood and Winocur set out to determine whether effects would be similar in rats with cognitive impairment resulting from high-fat ingestion (Greenwood and Winocur, 2001). Once again, groups of rats fed diets high in saturated fat, unsaturated fat, or lab chow for 3 months were administered the VIDA test and, once again, the high-fat groups were impaired on all aspects of the task. Following baseline testing, rats were maintained on their respective diets and, for the following days, alternately received intraperitoneal injections of glucose (100 mg/kg) or an equal volume of saline. Glucose treatment significantly improved performance in both high-fat groups (but not in the chow group), with the effect being most pronounced at the longer inter-trial intervals where performance was sensitive to hippocampal impairment. The finding that glucose significantly improved memory function under hippocampal control was instructive and suggested that the memory component of dietary fat-related cognitive impairment is linked to insulin resistance and/or glucose intolerance. With this in mind, the authors administered the VIDA test to genetically obese Zucker fa/fa rats that present insulin resistance, along with a range of endocrinological characteristics that appear to model type-2 diabetes (Gold, 1995). The obese Zucker rats did not differ from their lean counterparts in terms of learning the basic alternation rule or in performing at short inter-trial intervals. However, the obese rats were significantly and selectively impaired at the long intervals where hippocampus-controlled memory function was reduced. These results, together with those of the previous studies from this group, provide convergent evidence that the hippocampus is particularly sensitive to the effects of insulin resistance, and that impaired glucose regulation, resulting in reduced glucose uptake in the hippocampus may account for the long-term memory loss in rats fed high-fat diets. If confirmed by subsequent research, this would constitute a promising beginning in describing the neurobiological mechanisms underlying the full range of cognitive impairment that can result from chronic high-fat ingestion.

The role of the diet in cognitive decline has not been extensively investigated in humans, with few data available on the role of macronutrient intake in the pathogenesis of dementia and ARCD (Solfrizzi et al., 2003). Since several dietary factors affect the risk of cardiovascular disease, and cardiovascular disease has been linked to cognitive decline (Breteler et al., 1994), it can be assumed that they also influence the risk of dementia. Some recent studies have suggested that dietary fatty acids may play a role in cognitive decline associated with aging or dementia. Only a few epidemiological and clinical studies have addressed the link between the intake of UFA and cognitive function, most being cross-sectional. In a recent cross-sectional French study, the association among macronutrient intake, functional variables, and cognitive functions was studied in 441 free-living elderly subjects aged 65 or over. A positive relationship was found in women between lipid intake and the Mini Mental State Examination (MMSE) score, which evaluates overall cognitive functions. A positive relationship was also found between PUFA intake and mobility in men, and between functional variables and alcohol intake in the whole sample.

The cross-sectional association between dietary macronutrients and cognitive impairment was also examined in 278 nondemented elderly subjects aged 65–84 years from the Italian Longitudinal Study on Aging (ILSA) (Solfrizzi et al., 1999). Dietary intake was assessed with a 77-item food-frequency questionnaire. Authors included MUFA, PUFA, and SFA in the analysis and found a positive Spearman correlation coefficient between MUFA intake and the MMSE (0.12; $p < .05$) and the Digit Cancellation Test (DCT) (0.16; $p < .01$), assessing visual selective attention. Also, the correlation coefficient between PUFA intake and the DCT was positive (0.12; $p < .05$). After adjustment for educational level, the odds ratios (ORs) of cognitive decline (MMSE score <24) decreased exponentially with the increase in MUFA energy intakes. Despite the lower education (%3 years), MUFA energy intake over 2400 kJ/day was associated with a reduction in OR of cognitive impairment (OR 0.6, 95% confidence interval [CI] 0.1–4.5). Age as a confounder of the interaction term "education by MUFA" was associated with a further increase in OR of cognitive impairment (OR 0.7, 95% CI 0.1–4.5). Furthermore, selective attention performances evaluated by DCT were independently associated with MUFA intake (OR 0.99, 95% CI 0.98–0.99) (Solfrizzi et al., 1999).

Conquer et al. (2000) measured the plasma fatty acid composition of various PL in blood samples from 84 subjects with different degrees of cognitive impairment, including Alzheimer's disease and other types of dementia. Without considering confounding factors, this study showed a statistically significant lower level of nKs3 PUFA in the plasma of subjects with cognitive impairment. Finally, in the cohort of the Etude du Viellissement Arteriel (EVA) Study, moderate cognitive decline (a O2-point of MMSE decrease) and erythrocyte membrane fatty acid composition were evaluated in 264 elderly subjects aged 63–74 years, during a 4-year follow-up. In this study, a lower content of PUFAn-3 (OR 0.59, 95% CI 0.38–0.93) was significantly associated with a higher risk of cognitive decline. In this study, after adjusting for age, gender, educational level and initial MMSE score, stearic acid [OR 1.91, 95% CI 1.16–3.15 for a 1 standard deviation (SD) difference] and total n-6 PUFA (OR 1.59, 95% CI 1.04–2.44 for a 1 SD difference) were consistently associated with an increased risk of cognitive decline. Moreover, a lower content of PUFAn-3 (OR 0.59, 95% CI 0.38–0.93 for a 1 SD difference) was significantly associated with cognitive decline, but after adjustment this association remained significant only for docosahexaenoic acid, and not for eicosapentaenoic acid (Hende et al., 2003).

Only a few epidemiological studies are available on the association between fatty acids and cognitive functioning (Kalmijn et al., 1997; Capurso et al., 2003; Morris et al., 2004), indicating a crucial need for prospective studies that could confirm initial observations. On the basis of the previous significant suggestions, Solfrizzi et al. (1999) tested the hypothesis that high-MUFA and PUFA intakes may protect against the development of cognitive impairment over time in a median follow-up of 8.5 years of the ILSA. The major finding of this study was that the MMSE performance over the course of an 8.5 years follow-up due to high PUFA ($R \geq 220$ kJ/day) and MUFA (>2000 kJ/day) intakes were significantly better than those due to low PUFA (<220 kJ/day) and MUFA (≤2000 kJ/day) intake, in all subjects and completers (Capurso et al., 2003).

Findings from the Chicago Health and Aging Project (CHAP) showed that in a large population-based sample of 2560 persons, aged 65 years and older, a high intake of saturated and *trans*-unsaturated fat was associated with a greater cognitive decline over a 6-year follow-up. Intake of MUFA was inversely associated with cognitive change among persons with good cognitive function at baseline and among those with stable long-term consumption of margarine, a major food source. Slower decline in cognitive function was associated with higher intake of PUFA, but the association appeared to be due largely to its high content of vitamin E, which shares vegetable oil as a primary food source and that is inversely related to cognitive decline. Finally, cognitive change was not associated with intakes of total fat, animal fat, vegetable fat, or cholesterol (Morris et al., 2004).

Therefore, several studies have suggested that increased SFA could negatively affect cognitive functions. Furthermore, a clear reduction of risk of cognitive impairment in cross-sectional studies and a cognitive decline in longitudinal studies have been found in population-based samples with high intakes of PUFA and MUFA. The mechanisms by which high-UFA intake could be protective against cognitive decline and dementia in healthy older people are, at present, unknown. However, Several hypotheses could explain the association between fatty acids and cognitive functioning, including mechanisms through the copresence of antioxidant compounds in food groups rich in fatty acids, via atherosclerosis and thrombosis, inflammation, accumulation of b-amyloid, or via an effect in maintaining the structural integrity of neuronal membranes, determining the fluidity of synaptosomal membranes that thereby regulate neuronal transmission.

VIII. SUMMARY AND CONCLUSION

Aging represents a great concern in developed countries because of the high number of people included in this group and because of the number of pathologies related with this phenomenon. According to the free-radical theory of aging and its further mitochondrial extension, aging is the result of the oxidative insult to the organism throughout life. Some of the damages are not entirely repaired and are accumulated, leading to organism malfunction. Such oxidative-stress-related events are particularly important in the mitochondria and especially at the mtDNA level. Oxidative stress is related to the fatty acid composition of membranes and a correlation has been established between the maximum longevity of animals and the degree of unsaturation of membrane fatty acids. This correlation joins to the previously established one between the rate of mitochondrial oxygen-radical generation and the maximum longevity of animals. In long-lived homeothermic vertebrates, both free-radical production and the membrane fatty acid unsaturation were lower, offering an explanation for some of the main causes of the low aging rate peculiar to these animals. In relation to results found on dietary fat, mitochondrial oxidative stress and aging, one of the most important finding is that it is possible to modulate mitochondrial membranes through dietary fatty acid changes and that these changes are maintained lifelong. These manipulations trigger changes in mitochondrial oxidative, dietary fat rich in MUFA being more positive from that viewpoint than those rich in PUFA, which agrees with events related to membrane unsaturation and evolution. From an evolutionary perspective or from a nutritional perspective, the way to extend human life is the same: by ameliorating reactive-oxygen production through reducing the unsaturation of the mitochondrial membranes. Finally, several studies have suggested that an increase in SFA could have negative effects on cognitive functions. Furthermore, a clear reduction of risk of cognitive impairment in cross-sectional studies and cognitive decline in longitudinal studies has been found in population-based samples with high intakes of PUFA and MUFA. The mechanisms by which high intake of UFA could protect against cognitive decline and dementia in healthy older people are, at present, unknown. In conclusion, we cannot, at least in the short term, manipulate evolution, but we can modulate our diet and this is a very attractive opportunity to improve our health.

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41 Dietary Fat, Immunity, and Inflammatory Disease

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CONTENTS

I. INTRODUCTION

Although the effects of dietary deficiency on risk of infection have long been known and studied, only in the relatively recent past the influences of dietary fat have been systematically investigated. Thus, the first review of the literature surfaced as recently as 1978 (Meade and Mertin, 1978) and others quickly followed (Vitale and Broitman, 1981; Beisel, 1982; Gurr, 1983; Johnston, 1985, 1988; Erickson, 1986; Perez and Alexander, 1988; Hwang, 1989; Kinsella et al., 1990). The field expanded to include examinations of the immune-modulating effects of specific fatty acids, fat level, cholesterol, plasma lipoproteins, and oxidized fats. Moreover, the role of fat and fat metabolism in the genesis, regulation, and management of various disease states is receiving increased notice. This chapter will review the literature addressing this rapidly expanding field of research.

The immune system imparts protection against threatening agents or situations, both internal and external to the organism. These threats include invading microbes such as bacteria, fungi, viruses, rickettsia, and mycoplasma; abnormalities of host tissue such as cancer; and tissue trauma. Under certain conditions, the immune system can itself cause disease by unwittingly responding against host tissues as in cases of autoimmunity or contribute to the etiology of other diseases, such as atherosclerosis and psoriasis. For in-depth coverage of immunity, autoimmunity, and host response I refer the reader to one of the many fine immunology textbooks available today.

II. DIETARY FAT AND IMMUNITY

A. Dietary Fat Level and Degree of Saturation

Numerous studies have demonstrated that diets high in fat suppress immune function. Diets rich in polyunsaturated fatty acids (PUFA), particularly of the n-6 family, have proven to be especially suppressive, at least in experimental animal studies. Dietary fats were shown to influence the fatty acid composition of membrane phospholipids in numerous studies, principally by increasing the amounts of the provided fatty acids (Marshall and Johnston, 1983; Prescott et al., 1985; Tiwari et al., 1986, 1987; Terano et al., 1987; Bankey et al., 1989; Chandrasekar et al., 1996). In addition, however, consumption of certain fatty acids affects not only their membrane concentrations directly but may also have significant effects on other, physiologically relevant fatty acids. For example, Calder (1998a,b) described how increasing amounts of the n-3 fatty acids in immune cell membrane phospholipids via dietary availability reduced the proportion of arachidonic acid (an n-6 fatty acid) while simultaneously increasing the proportion of n-3 fatty acids. These direct and indirect alterations in membrane phospholipids composition are believed to be in large part responsible for changes in immune function, either through influences on membrane-bound enzyme activity, availability of fatty acid precursors of immune-modulating eicosanoids (Stulnig, 2003; Fritsche, 2006), or other influences on intracellular signaling such as phosphatidylinositol pathways (Prosko and McColl, 2005), membrane lipid raft composition (Laethem and Leo, 2002), and nuclear receptor activation (Nencioni et al., 2003).

The literature generally shows that diets containing high levels of PUFA reduce responsiveness of most aspects of immunity. T cell function in many of its facets is diminished. For example, consumption of high-PUFA diets reduced the response of mouse lymphocytes to T cell mitogens relative to low fat control diets (Erickson et al., 1980, 1983; Morrow et al., 1985; Kraus et al., 1987) to a greater extent than high levels of dietary saturated fatty acids (SFA) (Erickson et al., 1980; Olson et al., 1987). Other investigators were, however, unable to demonstrate a depression in T cell mitogenesis by high dietary fat levels in rats (Alexander and Smythe, 1988; De Deckere et al., 1988) or by high PUFA vs. high SFA in rabbits (De Deckere et al., 1988), mice, or rats (Alexander and Smythe, 1988). The proliferative response of spleen T cells to cell surface antigens of allogeneic lymphocytes (mixed lymphocyte response [MLR]) was depressed as the dietary corn oil level increased from 0.5% to 8% and 20%, with the observed reduction being statistically significant only at 20% dietary fat (Erickson et al., 1983). In this study, MLR was affected by dietary fat level but not by saturation.

Kelley et al. (1992) reported that lowering the total dietary fat content from 41.1% to 31.1% or 26.1% resulted in an increase in several indices of immunity such as T cell mitogen response and serum complement components. They did not see an effect of changing total PUFA content from 3.2% to 9.1% of calories within the noted ranges of total dietary fat. In a more recent report, Santos et al. (2003) reported that ten hyperlipidemic human volunteers were fed three 6-week diet plans containing 35%, 26%, and 15% fat. Following completion of these three maintenance diets all subjects were subjected for 12 weeks to the 15% fat diet at calorie levels to induce weight loss. At the end of each diet phase the following parameters were measured: delayed-type hypersensitivity (DTH); T lymphocyte proliferation to phytohemagglutinin (PHA); and interleukin-1 (IL-1), interleukin-2 (IL-2), and prostaglandin E2 production. DTH was significantly increased following the weight loss phase compared to the 35% maintenance diet, however, other parameters were not influenced by any of the dietary manipulations.

Bedoui et al. (2005) reported that tumor necrosis factor-α (TNF-α) production by blood monocytes and alveolar macrophages, as well as circulating TNF-α levels, was not different in rats made obese by up to 10 weeks of high-fat feeding. They went on to suggest that while obesity caused by severely elevated or reduced leptin levels present in leptin-deficient or leptin-receptor-deficient animals was related to altered immune function, leptin level variation in diet-induced obesity was unlikely to significantly alter immune function. Other studies have shown that leptin binding to

its receptors on T and B lymphocytes enhances signal transducer and activator of transcription-3 (STAT3) activation and suppresses Fas-mediated apoptosis (Papathanassoglou et al., 2006). Thus, within the range of fat recommended and typically consumed in the United States it is unlikely that dietary fat intake significantly affects T cell-mediated immune competence, although some studies suggest the possibility that significant reductions in total fat intake may be beneficial, presumably through influences independent of circulating leptin levels.

Other T cell functions that are influenced by dietary fat saturation include DTH (Thomas and Erickson, 1985; Kelley et al., 1997), cytotoxicity (Erickson, 1984), and generation of graft-vs.-host disease (Thomas and Erickson, 1985). However, there remains some inconsistency in the literature regarding the effects of high-fat and high-PUFA diets on these immune functions. For example, DeWille et al. (1981) reported no difference in the DTH response in mice fed 13% of energy (en%) corn oil vs. 50 en% corn oil.

B cell responses to mitogens or antigens appear to be influenced to a lesser extent by dietary fat level or degree of saturation. For example, varying the level of dietary corn oil from 13 en% to 70 en% did not influence the primary or secondary plaque forming cell (PFC) response to sheep red blood cells (sRBC) in mice after 35 or 70 days feeding (DeWille et al., 1979). The later report of Crevel et al. (1992) supports this finding. In contrast, Friedman and Sklan (1995) reported that antibody production developed more rapidly, reached a higher level, and was more persistent in chicks fed lower levels of linoleic acid.

Feeding mice 20% fat diets, compared to controls fed 5% fat, resulted in decreased lymphocyte binding to high endothelial venules in Peyer's patches and lymph nodes (Twisk et al., 1992). This effect was of the same magnitude with either sunflower oil (high PUFA) or lard (lower PUFA). The authors suggest that dietary fatty acid level and composition may affect expression of adhesion molecules responsible for lymphocyte homing.

Feeding rats a high-PUFA diet resulted in enrichment of neutrophil membranes with linoleic acid (the major PUFA in the oil source), whereas the levels of other elongation and desaturation products were not altered (Ringertz et al., 1987). The activity of these neutrophils was altered as evidenced by an increase in chemiluminescence to *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and leukotriene (LT) B4, and depressed aggregation (to LTB4 and calcium ionophore A23187) and chemotaxis to LTB4.

Tappia and Grimble (1996) reported that macrophages isolated from rats fed diets containing butter, coconut oil, corn oil, fish oil, or olive oil as fat source (also containing 10% of the total fat as corn oil to prevent essential fatty acid [EFA] deficiency) for 8 weeks resulted in differential enrichment of membrane phosphatidylethanolamine fatty acids with linoleic acid. Both linoleic acid intake and content of membrane phosphatidylethanolamine fatty acids were correlated with TNF-stimulated production of IL-1. IL-6 production showed a similar though less significant trend. These relationships were only in part explained by eicosanoid effects.

Gallai et al. (1995) reported that supplementing multiple sclerosis patients with 6 g of fish oil per day for 6 months reduced endotoxin-stimulated production of the cytokines IL-1 and TNF, and the eicosanoids PGE2 and LTB4 by blood mononuclear cells. They also reported reduction in PHA-induced production of IL-2 and γ-interferon, and the levels of serum soluble IL-2 receptors. These authors suggest that the reduced production of these proinflammatory cytokines may be beneficial in individuals with multiple sclerosis.

The consumption of diets rich in fat, particularly n-6 PUFA, increases many aspects of autoimmunity in mouse models (Fernandes, 1994). Examples of these effects include higher antidouble stranded DNA (Lin et al., 1996) and anticardiolipin (Lin et al., 1997) antibody levels, reduced lifespan, and worsened proteinuria in high-fat fed NZB/W F1 mice. High-fat fed mice also exhibited a higher percentage of cyclodextrin-5+ (CD5+) B cells (and greater mean fluorescent intensity of major histocompatibility antigens on the their surfaces), greater helper T cell (type 2) function, and elevated production of IL-6, TNF, and PGE2 from endotoxin-stimulated peritoneal macrophages (Lin et al., 1996). In contrast, diets rich in n-3 fatty acids appear to offer protection

(Simopoulos, 1991; Venkatraman et al., 1994; Chandrasekar et al., 1996); however, Tateno and Kobayashi (1994) did report fish oil supplementation to increase bovine serum albumin induced immune complex nephritis in NZB/W F1 mice. There are numerous mechanisms whereby n-3 fatty acids may reduce expression of autoimmune syndromes, detailed in a following section of this chapter.

B. Essential Fatty Acid Deficiency

Deficiency of dietary EFAs, that is, linoleic acid and its longer and/or more unsaturated derivatives, is known to alter immune function. Huang (1989) reviewed this field. As stressed by Huang, one of the major effects of EFA deficiency is to modulate the production of immunomodulatory eicosanoids including prostaglandins (PG), thromboxanes (TX), LT, hydroxy-fatty acids (HETE and HEPE), hydroperoxy-fatty acids, and lipoxins. It is also probable that alterations in membrane phospholipid fatty acid composition influence various membrane functions such as receptor recognition of ligands and subsequent signal transduction across the membrane.

The T cell response to mitogens was reported to be stimulated by consumption of EFA-deficient hydrogenated coconut oil as the sole fat source (Erickson et al., 1980, 1983; Marshall and Johnston, 1985; Kelley et al., 1988). However, the proliferative response of T cells to B16 melanoma cells was depressed in mice fed a fat-free EFA-deficient diet (Erickson et al., 1983). Several investigators reported that EFA deficiency reduced the DTH response (DeWille et al., 1981; Thomas and Erickson, 1985; Piegari et al., 2001) and DeWille et al. (1981) showed that refeeding EFA-deficient mice an EFA-adequate diet resulted in complete recovery of the DTH response within 7 days. Similar to the DTH response, EFA deficiency depressed Tc-mediated cytotoxicity against P51 melanoma target cells (Erickson, 1984). EFA deficiency was reported to have no effect on the graft-vs.-host response (Thomas and Erickson, 1985), although EFA deficiency was shown to prevent rejection of renal allografts in another study (Schreiner et al., 1988). Feeding of fat-free diets to mice resulted in depressed generation of concanavalin A (con A)-induced Ts and Keyhole Limpet hemocyanin-induced Th activity (Wee et al., 1988).

EFA deficiency has been reported to enhance (Boissonneault and Johnston, 1983; Boissonneault and Johnston, 1984) or depress (DeWille et al., 1979; Erickson et al., 1986) generation of antibody producing PFC, apparently dependent on whether the deficiency was induced using an EFA-free hydrogenated fat or a fat-free diet, respectively. Boissonneault and Johnston (1983, 1984) reported a direct correlation between inhibition of *in vivo* PG synthesis and elevation of the PFC response in Lewis rats, thereby linking alteration of eicosanoid production with changes in B cell function.

Macrophage killing of P51 melanoma cells was depressed by feeding a fat-free diet (Erickson et al., 1983; Erickson, 1984). Receptor-mediated pinocytosis was reported to be depressed in macrophages from EFA-deficient mice whereas phagocytosis was unaffected (Lefkowith et al., 1987). EFA deficiency was shown to reduce macrophage PGE2 (Adams et al., 1997) and nitric oxide (Boutard et al., 1994) formation. Neutrophils isolated from EFA-deficient rats exhibited decreased adherence to plastic in response to the calcium ionophore A23187 but not to LTB4 or fMLP (Lindstrom and Palmblad, 1988). Likewise, chemotaxis to fMLP was depressed whereas chemotaxis to LTB4 was unchanged.

Erickson and Schumacher (1989) reported that EFA deficiency was without effect on the activity or number of NK cells in mice fed fat-free diets.

C. Specific Fatty Acids

The roles of the eicosanoids in immune regulation have been widely studied and new eicosanoids, as well as new roles for these eicosanoids, continue to be discovered. Of dietary fatty acids, those of the omega 3 and omega 6 (or n-3 and n-6) families are most capable of influencing eicosanoid production. n-6 Fatty acids are precursors to the 1 and 2 series PG and the 3 and 4 series LT. n-3 Fatty acids are precursors to the 3 series PG and the 5 series LT. In addition, n-3 fatty acids inhibit

desaturation of linoleic acid (18:2n6) to its longer, more unsaturated derivatives, and also compete for transformation to eicosanoids. Hence, the ratio of dietary n-3 and n-6 fatty acids, as well as their absolute amounts, can have profound effects on immune function. Besides the prostaglandins and LTs, other eicosanoids have profound effects on immune modulation, including the hydroxyl- and hydroperoxy-fatty acids, lipoxins, and endocannabonoids. For background on the roles of the eicosanoids on immune function we refer the reader to several reviews (Peplow, 1996; Peters-Golden et al., 2004; Cabral, 2005; Parkinson, 2006).

Virtually all the studies of high dietary PUFA levels have been conducted using linoleic acid as the major PUFA. Generally, however, n-6 fatty acids were shown to depress T cell function while having little effect on B cell and NK cell function. Neutrophil responses to various stimuli were reported to change.

The effects of dietary n-3 fatty acids on immune function are currently under great scrutiny and have been reviewed elsewhere (Calder, 2003; Wu, 2004; Arterburn et al., 2006; Fritsche, 2006). Therapy with n-3 fatty acids may prove useful in the modulation of a number of disease states including heart disease and cancer, and in inflammatory diseases such as arthritis and psoriasis (Fernandes et al., 1998; Ergas et al., 2002; Harbige, 2003). As mentioned earlier, one of the primary mechanisms of action of n-3 fatty acids appears to be as a modulator of n-6 fatty acid-derived eicosanoid production and as precursor to other eicosanoid series.

Consumption of n-3 fatty acids, either as α-linolenic acid (18:3n3—primarily found in certain plant oils such as linseed oil) or eicosapentaenoic (EPA), docosapentaenoic (DPA), and docosa hexaenoic (DHA) acids (20:5n3, 22:5n3, and 22:6n3, respectively—primarily found in marine animal oils), resulted in incorporation of these fatty acids into membrane lipids of all tissues studied, including cells of the immune system (Marshall and Johnston, 1983; Lee et al., 1985a; Conroy et al., 1986; Chapkin et al., 1988; Bankey et al., 1989; Chandrasekar et al., 1996). These changes in membrane composition depressed production of PGE2 (Marshall and Johnston, 1985; Fritsche et al., 1993), 5-HETE (Lee et al., 1985a), and LTB4 (Lee et al., 1985a; Payan et al., 1986; Fritsche et al., 1993) by peripheral blood mononuclear cells whereas the production of the n-3 derived LTB5 was increased (Payan et al., 1986). Similar alterations of eicosanoid production were reported in spleen leukocytes (Lokesh et al., 1986a; Meydani et al., 1988; Chang et al., 1989a), peritoneal macrophages (Magrum and Johnston, 1985; Lokesh et al., 1986b, 1988, 1990; Lokesh and Kinsella, 1987; Cleland et al., 1990), Kupffer cells (Bankey et al., 1989), and neutrophils (Prescott et al., 1985; Lee et al., 1985a; Payan et al., 1986; Terano et al., 1987).

Marshall and Johnston (1985) reported that stimulation of T cells by the mitogens con A and PHA was unchanged in linseed oil-fed rats. Payan et al. (1986) reported that a dietary supplement of EPA to human volunteers increased the PHA response of T cells without modifying the numbers of Th or Ts. Similarly, Endres et al. (1989) reported that T cell proliferation in response to PHA was increased in n-3 fatty acid-supplemented humans. Supplementing human diets with arachidonic acid (n-6) did not influence the mitogen response to PHA, con A, or pokeweed mitogen (Kelley et al., 1997). Meydani et al. (1985) reported that spleen leukocyte mitogenesis was elevated in mice fed n-3 fatty acids. Likewise, the con A response of spleen leukocytes from 9-month-old, but not 5-month-old, autoimmunity-prone NZB/NZW mice was elevated. The response was, however, depressed in splenocytes from 4-month-old BALB/c mice (Alexander and Smythe, 1988). Meydani et al. (1991) reported that women supplemented with 2.4 g of n-3 rich oil for 3 months experienced a reduction in stimulated production of IL-1, TNF, IL-6, and IL-2, with older women experiencing a more dramatic reduction than younger women. Older women also have lower mitogenic responses to PHA than younger women prior to n-3 oil supplementation, and it was further reduced following n-3 supplementation. The authors questioned the desirability of further depressing immune activity in older women by regular use of n-3 fat supplements.

Other aspects of T cell function have been noted to be altered in animals fed diets enriched in n-3 fatty acids. Yoshino and Ellis (1987) reported that the DTH response to intradermal bovine serum albumin was reduced in n-3 fed rats. Although T cell subsets were not altered in two studies

of humans supplemented with n-3 fatty acids (Payan et al., 1986), Taylor et al. (1988) reported a transient reduction in the number of circulating Ts in n-3 fed rats. Wee et al. (1988) reported that supplementing EFA-deficient mice with n-3 fatty acids for 20 days did not alter con A-induced Ts. However, Th activity induced by Keyhole Limpet hemocyanin was enhanced. PGE1, PGE2, and LTB4 are known to depress T cell function (Rola-Pleszczynski et al., 1982; Payan and Goetzl, 1983; Payan et al., 1984; Linn et al., 1989) whereas LTB5 is less effective (Goetzl et al., 1986). Thus, alterations in T cell function may be a direct effect of modulation of eicosanoid synthesis.

The B cell proliferative response to the mitogen endotoxin was not altered in mice by dietary n-3 fatty acids (Alexander and Smythe, 1988; Wee et al., 1988). However, *in vitro* culture of B cells in the presence of EPA markedly depressed IgG/IgM production in response to PWM (Virella et al., 1989). Capping of B cell surface-bound immunoglobulin, a step preliminary to B cell response to antigen, was not changed (Alexander and Smythe, 1988). In their studies of n-3 supplemented EFA-deficient mice, Wee et al. (1988) reported restoration of antibody production against sRBC. The *in vitro* anti-sRBC response was also improved by n-3 supplementation; however, no change was noted in n-3 supplemented B cell antibody production in response to bacterial endotoxin. Prickett et al. (1982) likewise reported an increase in serum IgE and IgG levels in rats fed n-3 rich diets. In contrast, Virella et al. (1989) reported a time-dependent reduction in total serum IgG and IgM in humans fed supplemental n-3 fatty acids whereas IgA levels were not affected. Likewise, Watanabe et al. (1994) reported that linolenic acid suppressed antigen-induced IgE production and subsequent anaphylactic shock in mice. Finally, Huang et al. (1992) reported that n-3 feeding significantly reduced Ia antigen expression on peritoneal macrophages and splenic B cells. Others also report fish oil supplementation to reduce Ia antigen expression on peritoneal macrophages of mice and rats (Mosquera et al., 1990).

Graft rejection was reported to be suppressed by feeding n-3 rich lipids (Grimm et al., 1996). IL-6 production by blood mononuclear cells was also depressed by this treatment. A study investigating the effect of supplementing human renal transplant patients with 8 g of n-3 rich fat per day over 1 year showed a significant improvement in glomerular function (Maachi et al., 1995). Thus, n-3 fatty acid supplements have been proposed as nontoxic immunomodulatory agents for autoimmune, transplant, and other patients (Donadio, 1991; Calder, 1996).

As the prime producers of immune regulatory eicosanoids, the effect of n-3 fatty acids on monocytes/macrophages is of relevance to our discussion. Indeed, n-3 fatty acid supplementation influenced macrophage eicosanoid production, as previously mentioned (Magrum and Johnston, 1985; Lokesh et al., 1986b, 1988, 1990; Lokesh and Kinsella, 1987; Bankey et al., 1989; Cleland et al., 1990). Perez et al. (1989) reported that provision of rats with an n-3 oil supplement significantly reduced the T cell-dependent one-way MLR response. However, addition of control macrophages to the MLR cultures resulted in a 20-fold increase in the MLR response of spleen lymphocytes from n-3 supplemented rats whereas the MLR response from control rats was unchanged. In this experiment, oleic acid (18:1n9—a fatty acid of the n-9 family found in high concentrations in olive oil) had a similar effect to n-3 fatty acids, that is, it depressed the MLR, and the MLR response was elevated fivefold when cultures were enriched with control macrophages.

Phagocytosis and killing of ingested cells is a central function of monocyte/macrophages, and these combined functions may be altered by dietary n-3 fatty acids. Chemotaxis of human blood monocytes to fMLP or to C5a was not altered (Payan et al., 1986; Schmidt and Dyerberg, 1989) or was decreased (Schmidt and Dyerberg, 1989) by supplementation of human volunteers with EPA. Chemiluminescence in response to phagocytic signals, an index of oxygen radical production, was decreased (Magrum and Johnston, 1985) or not altered (Schmidt and Dyerberg, 1989) in monocytes or macrophages isolated from rats or humans, respectively. Magrum and Johnston (1986) reported that phagocytosis of yeast or carbon particles was not altered in peritoneal macrophages from n-3 supplemented rats, however, arginase activity was depressed. More recently, Boutard et al. (1994) reported a slight depression of arginase activity of macrophages from rats fed EFA-deficient or n-3 supplemented diets. Fish oil supplementation was also shown to reduce nitric oxide synthesis by

rat macrophages (Boutard et al., 1994). In contrast, Chapkin et al. (1992) reported that peritoneal macrophages from fish oil-supplemented mice phagocytosed zymosan at a greater rate than cells from n-6 fatty acid-supplemented animals. Macrophage killing in n-3 supplemented animals may be depressed, as this treatment resulted in depressed killing of *Mycobacterium tuberculosis* in guinea pigs (Mayatepek et al., 1994).

Macrophages obtained from n-3 containing fat-fed rats significantly increased clotting time of blood (van Dam-Mieras et al., 1986); this macrophage procoagulant activity may require macrophage plasma membrane involvement. Macrophage production of platelet-activating factor (PAF) was reported to be unchanged (Payan et al., 1986) or decreased (Sperling et al., 1987a,b) by supplementation of humans with n-3 fatty acids, whereas changes in intracellular calcium and inositol phosphate concentrations characteristic of the signal transduction response to PAF exposure was reduced (Bankey et al., 1989).

Lokesh et al. (1990) reported that IL-1 and TNF production by peritoneal macrophages was enhanced by feeding dietary n-3 fatty acids. Supporting this finding was the report of Hardardóttir et al. (1992a,b), who reported increased TNF production in resident, but not elicited, peritoneal macrophages from n-3 supplemented mice. Likewise, Ertel et al. (1993) reported that n-3 fatty acid supplementation increased IL-1 production by mouse peritoneal macrophages and suppressed PGE2-mediated immunosuppression following hemorrhagic shock. In contrast, Endres et al. (1989) reported that supplementation of human volunteers with large amounts of n-3 fatty acids diminished levels of IL-1 and TNF produced by peripheral blood monocytes. Guinea pigs fed an n-3 rich diet for 6 weeks exhibited a blunted fever response to injected recombinant IL-1 (Pomposelli et al., 1989).

Unlike the case with monocytes, neutrophil chemotaxis to fMLP (Payan et al., 1986; Terano et al., 1987; Ferrante et al., 1994), C5a (Payan et al., 1986), and LTB4 (Lee et al., 1985a; Payan et al., 1986; Terano et al., 1987; Sperling et al., 1993) was depressed by n-3 fatty acid supplementation. Culture of human neutrophils with EPA resulted in depressed release of lysozyme and elastase from cytosolic granules in response to immune complexes (Virella et al., 1989). Moreover, 6 weeks of n-3 fatty acid supplementation to a human volunteer markedly reduced neutrophil ingestion of latex beads and antibody-coated sRBC and decreased lysozyme release in response to fMLP and antibody-coated sRBC. In a similar vein, chemiluminescence and superoxide production was depressed in phagocytosing neutrophils isolated from human volunteers consuming n-3 fatty acid supplements (Fisher et al., 1986).

Prescott et al. (1985) reported that human neutrophil aggregation or adherence to nylon wool fibers was not affected by dietary treatment with n-3 fats, although Lee et al. (1985a) found that neutrophil adherence to bovine endothelial cell monolayers pretreated with LTB4 was inhibited by a similar dietary treatment. Thus, characteristics of the substrate may be important in determining the effects of dietary lipids on neutrophil adherence.

LTB4 is known to augment NK cell activity while LTB5 is less potent (Chang et al., 1989a). PGE2, on the other hand, is inhibitory to NK activity (Brunda et al., 1980; Goto et al., 1983). Thus, it is difficult to predict the effect of n-3 fatty acids on NK cells. In fact, several studies have demonstrated that NK cell activity is depressed by supplementation with n-3 fatty acids. NK activity was depressed in splenocytes from rats supplemented with EPA for 4 weeks (Chang et al., 1989a,b). Likewise, consumption of diets containing n-3 containing PUFA for 6 weeks depressed NK activity of 3-month-old mice but not of 24-month-old mice (Meydani et al., 1988). A single intraperitoneal injection of EPA (Yamashita et al., 1988) or *in vitro* incubation of splenocytes with EPA (Yamashita et al., 1986) resulted in an inhibition of NK cell activity.

Although the majority of research on specific fatty acids as influences of immune responsiveness have focused on the n-6 and n-3 fatty acid families, a recent paper by Stentz and Kitabchi (2006) reported that human T lymphocytes responded to the SFA palmitic acid differentially than other PUFA. In particular, human T cells responded to palmatic acid, but not to oleic, linoleic, linolenic, or arachidonic acids, demonstrated enhanced time and concentration-dependent expression of CD69, insulin receptors, insulin-like growth factor 1 receptors, GLUT 1 and 4 receptors, insulin receptor

substrate 1, generation of reactive oxygen species and the lipid peroxidation product MDA, and cytokine secretion (IL-1, IL-2, IL-6, IL-8, IL-10, and TNF-α). At this time these data have not been confirmed by other laboratories.

D. Cholesterol and Plasma Lipoproteins

Cholesterol is an important component of plasma lipoproteins and plays a critical role in cellular membrane function. A large number of studies have examined the immune-modulating effects of dietary cholesterol, oxidized cholesterol derivatives, *in vivo* exposure to cholesterol or membrane cholesterol depletion, and normal and pathologic lipoproteins. These studies were previously reviewed (Boissonneault, 2000). This section will address two current areas of study that illustrate cholesterol's contributions to regulating immune function: acute phase regulation of cholesterol availability to immune cells and membrane lipid rafts.

The acute phase response (APR) is a concerted response of the body to acute and chronic inflammatory stimuli that result in profound changes in bone marrow production of leukocytes, regulation of specific protein production by liver, macrophages and other immune cells, cytokine production, changes in tissue metabolism, and other changes. The specific proteins whose levels increase or decrease are known collectively as positive or negative acute phase proteins (APP), respectively. The primary positive APP in humans are C-reactive protein (CRP) and serum amyloid-A (SAA) (Gabay and Kushner, 1999; Pepys and Hirschfield, 2003; Manley et al., 2006). These and other APP enhance immune reactivity, divert metabolic substrates for support of essential bodily functions, including support of immune activity, and reduce availability of substrate from invading microorganisms.

The "APR" is a term used to describe the plethora of changes resulting from trauma or other events leading to a systemic inflammatory response. One "acute phase protein" produced in this response is SAA, a liver apoproprotein that displaces apolipoprotein A1 from the surface of HDL (Husebekk et al., 1987). Kisilevsky and Subrahmanyan (1992) reported that SAA binding to HDL reduced HDL's binding affinity for normal hepatocytes by twofold. It also increased binding of SAA-HDL to macrophages by threefold to fourfold. Thus, this change in binding of HDL was suggested to result in a shift of HDL to the role of reverse cholesterol transport from inflammatory sites. In a later report, Kisilevsky and coworkers reported that SAA also changed cholesterol metabolism in macrophages such as cholesteryl ester hydrolase activity (Lindhorst et al., 1997). The role of acute phase SAA and reverse cholesterol transport during the APR has been reviewed (Kisilevsky et al., 1996).

The major lipoprotein associated with HDL, apolipoprotein A1, is a negative APP (Burger and Dayer, 2002). The positive APP SAA circulates in the blood bound to high-density lipoprotein (HDL), forming what is known as "acute phase HDL" or SAA-HDL. This form of HDL binds with very high affinity to macrophages, displacing apolipoprotein A1 from the surface of HDL (Husebekk et al., 1987). Kisilevsky and Subrahmanyan (1992) reported that SAA binding to HDL reduced HDL's binding affinity for normal hepatocytes by twofold. It also increased binding of SAA-HDL to macrophages by threefold to fourfold (Husebekk et al., 1987), and consequently enhanced phagocytosis of these modified HDL particles (Kisilevsky and Subrahmanyan, 1992; Banka et al., 1995; Artl et al., 2000).

Thus, whereas noninflammatory HDL participates principally in the process known as "reverse cholesterol transport" (Lewis, 2006), SAA-HDL alters macrophage pathways of cholesterol metabolism. SAA was shown to inhibit macrophage acyl-CoA cholesterol acyl transferase (ACAT) and enhance the activity of neutral cholesterol ester hydrolase (nCEH) (Tam et al., 2002, 2005; Kisilevsky and Tam, 2003), making cholesterol available for export via the ABCA1 transporter system. In addition to phospholipids delivered to macrophages via SAA-HDL, additional phospholipids are actively transported to macrophages via CRP during the APR (Manley et al., 2006). Thus, SAA-HDL may contribute to redistribution of cholesterol and other lipids to support the markedly augmented requirement for these lipids to support cell proliferation during the ongoing immune

response, rather than storing the ingested cholesterol and other lipids in inert cytosolic lipid droplets. The roles of SAA-HDL in the APR, however, remain only partially understood. A recent editorial elegantly discusses some of the remaining questions and controversies in this rapidly developing field of research (Van Lenten et al., 2006).

The concept of discreet membrane lipid domains termed "lipid rafts" was first described by Simons and Ikonen in 1997. This field has expanded rapidly over the ensuing years, with over 777 related published articles indexed as of late 2006 (www.pubmed.gov, accessed September 17, 2006). Lipid rafts are densely packed lipid domains enriched with cholesterol, sphingomyelin, and glycosphingolipids (Simons and Ehehalt, 2002), which play an integral role to transmembrane signaling by virtue of their ability to restrict the movement of transmembrane signaling proteins and enhance the rate of association between complimentary proteins of signaling cascades. The cholesterol concentration influences the fluidity and liquid crystal structure of lipid rafts, potentially affecting their function (Ikonen and Parton, 2000). In order for cells of the immune system to respond to stimuli they, similar to most other cells, must transmit external signals through their lipid membranes, and lipid rafts are now known to be vitally important to this function.

A number of recent review articles describe the current state of knowledge surrounding lipid rafts in the regulation of lymphocyte activation (Pizzo and Viola, 2003, 2004; Becher and McIlhinney, 2005; Meiri, 2005). Rather than reiterate what is described in these and other excellent reviews, I will here present a description of how membrane cholesterol enrichment and depletion affect lipid raft function.

Becher et al. (2001) reported that saponin depletion of cholesterol from lipid rafts isolated from rat cerebellar membranes resulted in the disassociation of the membrane proteins Thy-1 and the GABAB receptor, as well as ganglioside GM1 into the supernatant fluid. Treatment of cerebellar membrane fragments with the cholesterol solubilizer methyl-β-CD likewise resulted in dissociation of Thy-1 and GABAB receptor into the supernatant fluid, confirming their first finding.

Methyl-β-CD depletion of cholesterol from lipid rafts isolated from 3T3-F442A cells reduced growth hormone (GH) receptor activation of extracellular signal-regulated kinase (ERK) by 45% ; ERK activation upon GH receptor stimulation could be reversed in these preparations by re-enrichment with cholesterol (Yang et al., 2004). In this study, GH receptor stimulation of signal transducer and activator of transcription (STAT5) tyrosine phosphorylation was unaffected by methyl-β-CD depletion of lipid raft cholesterol. The authors interpreted these divergent results as suggesting that these two signaling pathways may reside in different subregions of the plasma membrane, which are therefore differentially affected by cholesterol content.

Takebayashi et al. (2004) reported that methyl-β-CD treatment of wild-type PC12 cells reduced epidermal growth factor (EGF) receptor concentration associated with lipid rafts. In addition, methyl-β-CD depletion of cell cholesterol prior to EGF exposure resulted in an enhancement of EGF-induced phosphorylation of the EGF receptor, and functionally this translated as an increased rate of neurite sprouting. The authors reported that the increase in methyl-β-CD-induced PC12 neurite spouting was attenuated by simultaneous exposure to cholesterol. These data suggest that while some signaling proteins are activated by translocating into cholesterol-enriched lipid raft domains, the activity of others, such as the EGF receptor, may be suppressed in such an environment.

Nguyen et al. (2004) enriched Jurcat T cells with cholesterol to about four times normal cholesterol concentrations. This treatment was shown not to influence transferrin uptake, and it also did not interfere with or significantly alter the clustering of ganglioside GM1 on CEM-R5 cells. Antibody-mediated cross-linking of CD3 on the surface of cholesterol-enriched Jurkat T cells was likewise not altered compared to un-enriched cells, and it only weakly inhibited the ability to phosphorylate proteins upon αCD3 mAb stimulation. On the other hand, cholesterol enrichment significantly reduced intracellular calcium accumulation in response to αCD3 mAb stimulation. Similarly, cholesterol loading almost completely inhibited SDF-1α-induced intracellular calcium mobilization in Molt-4 T cells and Sup-T1 cells. Finally, preloading naive human T cells with cholesterol inhibited cell enlargement in preparation for cell division as well as IL-2 production when the T cells

were stimulated by cross-linking of surface CD3 and CD28 molecules with monoclonal antibodies. These results support the vital role that membrane lipid raft cholesterol concentration plays in supporting appropriate T lymphocyte function.

This brief review of selected reports illustrates the potentially far-reaching impacts of cholesterol enrichment or depletion from membrane domains such as lipid rafts on immune cell function. Immune modulation by statin drugs may also be mediated in part via modulation of lipid raft composition.

E. Parenteral Lipids

Parenteral use of fats in the United States began in 1977 with the FDA approval of Intralipid®, a linoleic acid-rich lipid emulsion made with soybean oil, phospholipids, and glycerol (Wretlind, 1981). Other lipids such as fish oil emulsions (Mascioli et al., 1988), MCT (Shils, 1988), and structured lipids (Babayan, 1987) have also been used for nutritional therapy of parenterally fed patients.

Providing lipids via total parenteral nutrition (TPN) poses the greatest potential for influencing the immune system because the route of their administration bypasses normal physiological mechanisms for lipid processing, for example, digestion and circulation in the form of lipoproteins made in the intestinal mucosa (chylomicrons) and liver (very-low-density lipoprotein [VLDL]). Similar to other intravenously injected lipid emulsions, there is the potential for stimulating massive uptake of the lipid particles by macrophages and other components of the reticuloendothelial cell system (Friedman et al., 1978; Lanser and Saba, 1981; Fraser et al., 1984), effectively diminishing the phagocytic and bactericidal capacity of macrophages and neutrophils (Di Luzio and Wooles, 1964; Berken and Benacerraf, 1968). This could profoundly increase risk for developing infection (Fischer et al., 1980; Fraser et al., 1984; Hamawy et al., 1985).

Early studies, mostly conducted *in vitro*, suggested that parenteral fat emulsions could result in impairment of critical phagocyte functions such as neutrophil and monocyte chemotaxis (Nordenström et al., 1979; Fraser et al., 1984), neutrophil cytotoxicity toward virus-infected cells (Loo et al., 1982), and neutrophil bactericidal activity (Fischer et al., 1980; Lanser and Saba, 1981). Later reports of work conducted *in vivo*, however, generally failed to support these findings. For example, there are now numerous reports that neutrophil chemotaxis (Palmblad et al., 1982; Ota et al., 1985; Escudier et al., 1986; Usmani et al., 1986), bactericidal activity (Palmblad et al., 1982; Ota et al., 1985), and chemiluminescence (Palmblad et al., 1982; Usmani et al., 1986) were not influenced in patients infused with traditional long-chain fatty acid-containing triglyceride (LCT), linoleic acid-rich parenteral fat emulsions such as Intralipid®. Likewise, no negative alteration of phagocytic activity of rat Kupffer cells (Romano Carratelli et al., 1986) or human peripheral blood leukocytes (PBL) (Szeinberg et al., 1986; Padeh et al., 1987), PBL bactericidal activity (Padeh et al., 1987), PBL generation and/or secretion of H_2O_2 (Padeh et al., 1987), superoxide (Strunk et al., 1985; Szeinberg et al., 1986), lysozyme, or PGE2 (Szeinberg et al., 1986) was reported to result from *in vivo* or *in vitro* exposure to parenteral lipids. Allen and Murray (1986) reported that long-term administration of Intralipid® to mice did not result in reticuloendothelial blockade, unlike phosphatidylcholine/cholesterol liposomes.

There have been reports of lung granulomas forming in pigs (Aksnes et al., 1994) and rats (Nordstrand et al., 1987) maintained on long-term soybean-based TPN lipids. This finding might be related to the activation of macrophages reported to occur in human (Gogos et al., 1992) and rat (Dahl et al., 1992) macrophages following administration of TPN lipids. Aksnes et al. (1995) reported that maintaining pigs on TPN with soybean-based lipids for 7 weeks resulted in a spontaneous twofold increase in nitroblue tetrazolium reduction in PBL and a 1.6-fold increase of this function in alveolar macrophages. Nitroblue tetrazolium reduction is indicative of phagocyte activation with production of oxygen radicals. In addition, the spontaneous rate of lymphocyte proliferation was increased threefold in TPN animals. Membranes from PBL, isolated from TPN animals, contain higher levels of linoleic acid and somewhat lower levels of arachidonic acid.

The effect of LCT fat emulsions on synthesis of complement components C2 and C4 by macrophages have been studied. Guinea pig peritoneal macrophages and human PBL incubated with Intralipid® produced less C2 and C4 than control cells (Strunk et al., 1978, 1983). On the other hand, Strunk et al. (1985) reported normal serum levels of C2 and C4 in infants receiving Intralipid® infusions, and Ota et al. (1985) found no change in serum C3 and C4 levels after Intralipid® administration. Etzioni et al. (1987) reported that generation of the anaphylatoxin C5a was depressed in serum from preterm infants infused with Intralipid®, although there was a large amount of variation in the results. No significant alterations in the opsonizing capacity of the sera were noted, suggesting that Intralipid® administration had no effect on complement activation.

Similar to the data just reviewed, most studies of nonphagocyte-mediated immunity indicate no influence or augmentation by parenteral LCT emulsions. Several laboratories have reported an elevation or no change in the number of blood T cells (Helms et al., 1983; Ota et al., 1985; Monson et al., 1986), Th, Ts (Ota et al., 1985; Monson et al., 1986), B cells, NK cells, and monocytes (Ota et al., 1985). Similarly, mitogenesis to PHA (Helms et al., 1983; Fraser et al., 1984; Ota et al., 1985) or PWM (Helms et al., 1983), and basal and stimulated IL-2 production (Monson et al., 1986) were reported to be unchanged or enhanced by parenteral lipids, although others reported that antigen-driven lymphoproliferation was inhibited by *in vitro* culture with Intralipid® or with hypertriglyceridemic plasma from humans or monkeys infused with Intralipid® (Ladisch et al., 1982). De Chalain et al. (1994) reported a greater mitogenic response to con A from rats, previously treated with surgery and hemorrhagic shock, provided intravenous Intralipid®, compared with control and enteral Intralipid® groups. In contrast, in this study bacterial translocation to mesenteric lymph nodes was greatest in rats provided intravenous Intralipid®.

Antibody-dependent cell-mediated cytotoxicity (Monson et al., 1986) and NK cytotoxicity (Almond et al., 1985; Kurzer et al., 1990) were also unaffected or augmented with host treatment with parenteral lipids. Thus, it is unlikely that phagocyte function is markedly affected by administration of parenteral LCT emulsions, particularly when fat provides less than 50% of non-nitrogen calories, especially during acute illness (Dahlstrom et al., 1988).

In 1999, Furukawa and coworkers reported on the effects of fat free, Intralipid®-containing TPN (20% of calories), or Intralipid®-containing and EPA-containing TPN (1.8 g/day) on immune and stress responses of humans undergoing esophagectomy with thoracotomy. Patients provided with Intralipid®-containing TPN following surgery had significantly elevated serum IL-6 compared with the fat-free TPN group. Provision of the EPA supplement to subjects provided with Intralipid® resulted in a reduction of IL-6 to levels at or below fat-free TPN subjects. Proliferative responses of subject T lymphocytes to mitogens con A and PHA were similar between fat-free and Intralipid® groups; however, provision of the EPA supplement to Intralipid®-fed subjects resulted in significantly elevated proliferation on postoperative day (POD) 21. In a second study, Furukawa et al. (2002) examined the effects of fat-free or Intralipid®-containing (20% of calories) TPN on immune response of humans undergoing moderate (gastric or colorectal surgery) or severe (esophagectomy) surgical stress. Although immune response was not affected by TPN energy source in subjects undergoing moderate surgical stress, parenteral lipid-fed subjects undergoing severe surgical stress showed similar results to their 1999 study, including elevated IL-6 levels that persisted through POD 10. Likewise, C-reactive protein levels were elevated at POD day 3 and 10, and serum glucagon was elevated at POD 1, indicative of a more robust APR. Whereas the proliferative response of T lymphocytes to con A and PHA were not different in severely stressed subjects before surgery, these responses were significantly depressed in subjects provided parenteral lipids on POD 7. Thus in these studies, provision of soybean-based TPN lipids resulted in augmentation of the stress response and diminished immunity, whereas supplementation with EPA rectified these effects.

Other parenterally administered lipids were studied regarding their impact on immune competence. Alexander et al. (1986) reported that administration of n-3 fatty acid-containing fish oil to burned Guinea pigs resulted in less weight and skeletal muscle mass loss, and lower resting metabolic rate than did other n-6 fatty acid-enriched parenteral lipids. Moreover, the DTH response and

opsonic index were both elevated, along with spleen weight, with the fish oil supplement. Mascioli et al. (1988) reported improved survival to an intraperitoneal injection of endotoxin in Guinea pigs provided with a fish oil emulsion vs. a safflower oil emulsion. As stated above, parenterally administered EPA eliminated the immune suppression related to parenteral Intralipid® administration in humans (Furukawa et al., 1999, 2002).

Similar to n-3 fatty acid-containing emulsions, those containing MCT have been investigated. Inclusion of MCT into parenteral fat emulsions was reported to have no effect on neutrophil function or morphology (Monico et al., 1988). On the other hand, Garnacho-Montero et al. (2002) described a study in which rats were assigned to one of five dietary treatments (chow, starvation, long-chain triglyceride TPN, long- and medium-chain triglyceride TPN, and oleic acid-rich olive oil TPN), followed 2 days later by intravenous inoculation with *E. coli*. Two days following infection, rats were killed and the extent of infection of liver and lungs was determined. Bacterial growth in the long- and the long-/medium-chain triglyceride TPN groups was significantly greater than in the chow-fed group, and the olive oil and starvation groups were intermediate. Other studies of the effects of parenteral MCT and structured lipids (Babayan, 1987) are warranted, given the paucity of data available concerning their influence on immune function.

Olive oil emulsions have now entered the clinical arena. A study by Moussa et al. (2000) investigated the effects of providing rats with an oleic acid-rich olive oil-based TPN lipid emulsion (ClinOleic®) or a linoleic acid-rich TPN lipid emulsion (Ivelip®) for 6 days, after which they measured expression level of CD25, the IL-2 receptor α-chain. Lymphocytes from rats provided with the oleic acid-enriched emulsion demonstrated greater CD25 expression compared with those provided with the linoleic acid-rich emulsion, and expression was correlated with oleic acid content of lymphocyte membrane phospholipids.

Granato et al. (2000) studied the effects of the same oleic acid-rich TPN emulsion, ClinOleic®, compared with linoleic acid-rich TMP emulsions Ivelip® or Intralipid®, on *in vitro* PHA-induced human lymphocyte activation (measured by thymidine incorporation, expression of CD25, and IL-2 release) and LPS-induced monocyte/macrophage activation (measured as HLA-DR expression and TNF-α release). Compared with control cells, PHA stimulation of lymphocytes incubated with Ivelip® or Intralipid® resulted in depressed thymidine incorporation compared with control or ClinOleic® incubated cells. Similarly, CD25 expression was nonsignificantly depressed and IL-2 release significantly reduced in Intralipid®-incubated but not ClinOleic®-incubated lymphocytes, as compared with control. TNF-α and IL-1β release were similarly reduced by both lipid emulsions.

Reimund et al. (2004) studied the effect of three lipid emulsions on human peripheral blood mononuclear cell TNF-α and IL-1β release in response to PHA and LPS stimulation. They used Intralipid® (long-chain triglycerides), Medialipid® (50:50 long- and medium-chain triglycerides), and ClinOleic® (olive oil based). Compared to control, each of the lipid emulsions reduced basal TNF-α and IL-1β production, although the effects of ClinOleic® were lesser in magnitude. Unlike the results reported by Granato et al. (2000), Reimund et al. (2004) found no impact of lipid emulsion incubation on PHA- or LPS-induced *in vitro* cytokine production.

In a more recent study by Buenestado et al. (2006), the effects of Intralipid® and ClinOleic® on human neutrophil function were evaluated. Neutrophils were incubated *in vitro* with various concentrations of lipid emulsions and stimulated with *N*-formyl–Met–Leu–Phe (FMLP). ClinOleic® had no effect on FMLP-induced rise in intracellular calcium, oxidative burst, chemotaxis, or elastase release, whereas Intralipid® incubation decreased intracellular calcium and chemotaxis while increasing oxidative burst compared with control. LTB4 production was augmented by both lipid emulsions whereas basal and FMLP-induced CD11b expression and serum-opsonized zymosan-induced phagocytosis were unchanged. LPS-induced TNF-α, IL-1β, and IL-8 mRNA and protein expression were unaffected by ClinOleic® whereas incubation with Intralipid® and a 50:50 long- and medium-chain triglyceride emulsion, Lipofundin®, decreased IL-1β mRNA and protein. Overall, the authors concluded that ClinOleic® had a reduced impact on neutrophil function compared with Intralipid® or Lipofundin®.

III. DIETARY LIPIDS AND INFLAMMATORY DISEASE

A. Psoriasis

Psoriasis is one of the most common skin ailments, affecting approximately 2% of the population (Champion, 1986; Baadsgaard et al., 1990). Grossly it is characterized by the eruption of raised, discrete, and confluent papules or a reddish color, and covered with flaking scales of shed skin. Rapid proliferation of keratinocytes results in the thickened, scaly plaques. The formation of macropapules is the result of fibroblast activation, vascular expansion, and infiltration of the area with neutrophils, macrophages, and T cells (Baadsgaard et al., 1990). The immune response responsible for the symptoms of this disease is similar to the DTH response; however, the stimulus for the response is currently unknown. It may be related to microbial antigens or to the anti-self-response of autoreactive T cell clones (Baadsgaard et al., 1990). Cytokines produced by T cells are known to be important effectors of disease symptoms (Quesada and Gutterman, 1986; Ristow, 1987; Baadsgaard et al., 1990; Gottlieb, 1990), including interferon, IL-1, and IL-6. The immunopathology of psoriasis has been reviewed (Christophers, 1996; Wolters, 2005).

The LTs are also known to be intimately involved in the disease process. The epidermis and scale chamber fluid of psoriatic lesions contain several lipoxygenase products including LTB4, LTC4, LTD4, 12-HETE, and 15-HETE. Epidermal and endothelial cells, keratinocytes, and macrophages, among other cells, are capable of synthesizing these eicosanoids. These eicosanoids are chemotactic, cause leukocyte aggregation and degranulation, and promote leukocyte adhesion to the endothelium; they also enhance vascular permeability, cause epidermal hyperproliferation, and vasodilation. Thus, modulation of eicosanoid production, and especially production of lipoxygenase products, may be of therapeutic importance.

Ziboh et al. (1986) reported that an 8-week treatment of psoriatic patients with fish oil (11–14 g of EPA/day) resulted in mild to moderate improvement in their psoriatic lesions. Fatty acids from the fish oil (i.e., EPA and DHA) were incorporated into neutrophils and the epidermis, and the improvement in clinical response was found to be related to incorporation of EPA and DHA into the epidermal tissues. Bittiner et al. (1988) also found that consumption of fish oil tablets for 8 weeks (1.8 g of EPA/day) resulted in a reduction of itching, erythema, and scaling, and a trend toward a decrease in the percentage of total surface area involved.

Both reports (Ziboh et al., 1986; Bittiner et al., 1988) suggested that the mode of action of the fish oil treatments was related to its inhibitory effects on the production of arachidonic acid-derived eicosanoids. Terano et al. (1989) conducted a study similar to those of Ziboh et al. (1986) and Bittiner et al. (1988) in order to gain insight into this question. In their study, patients consumed 3.6 g of EPA ethyl-ester/day for 3–6 months. By the third month both scaling and erythema had significantly improved and continued to improve throughout the remainder of the study. Neutrophil production of LTB4 was depressed from 1 month after the start of the study, whereas LTB5 and 5-HETE production increased significantly over the same time period.

In contrast to these reports, several other investigators failed to find significant improvement of psoriasis symptoms with n-3 fatty acid supplementation. Bjorneboe et al. (1988) supplemented patients with psoriasis with 10 g of fish oil daily containing approximately 1.8 g EPA acid. Control subjects received an olive oil placebo. They failed to find significant improvement in clinical symptoms after 8 weeks of treatment, though they reported an expected increase of n-3 fatty acid and decrease of n-6 fatty acid content in serum phospholipids following treatment. Using a similar experimental design, Gupta et al. (1990) supplemented psoriatic patients with fish or olive oil capsules in addition to traditional topical treatment with betamethasone diproprionate for 3 weeks. When the topical betamethasone was discontinued, symptoms worsened and no significant benefit of fish oil was noted. Finally, in a large human study with 145 patients with moderate to severe psoriasis, Soyland et al. (1993) provided 5 g of highly purified EPA/DHA esters or an equivalent mass of corn oil to treatment and control groups, respectively. Similar to the study of Gupta et al. (1990), although there was significant enrichment of plasma phospholipids with n-3 fatty acids in the treatment group

there was no significant clinical improvement associated with n-3 fatty acid supplementation. A recent literature review of the effects of intravenous n-3 fatty acids as a treatment for guttate psoriasis suggested that there was no firm evidence for its effectiveness (Chalmers et al., 2004).

Therefore, at this time it appears that n-3 fatty acid consumption is likely to have minimal if any benefits in ameliorating symptoms of psoriasis.

B. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints, which appears to be autoimmune in nature, with the participation of cell-mediated and humoral arms of the immune system (Fogh, 1990). It is characterized by inflammation of the synovium and infiltration of the joint by neutrophils, macrophages, and T cells. The presence of IgG "rheumatoid factors" that bind to other IgG molecules, forming complement-activating immune complexes in the joint, is also characteristic. Activation of neutrophils and macrophages by T cell-derived cytokines, immune complexes, and activated complement factors ultimately results in increased vascular permeability, damage to the cells and connective tissues of the joint, and proliferation of synovial cells and chondrocytes. The final result of this inflammatory process is erosion of the cartilage and other components of the joint, eventually making movement exceedingly painful and paving the way for fusion of the joint.

As in all inflammatory responses, eicosanoids are an important component of RA (Fogh, 1990), and obstructing the synthesis of these eicosanoids can slow the progression of the disease. Synovial fluid from patients with RA contains LTB4 and 5-HETE (Klickstein et al., 1980; Fogh, 1990), and these lipoxygenase products serve as a potent source of chemotactic factors, as discussed previously. Interestingly, membranes from peritoneal macrophages isolated from rats with adjuvant-induced arthritis had significantly higher levels of linoleic acid (18:2n6) and lower levels of the elongation and desaturation product 22:5n6, whereas levels of arachidonic acid (20:4n6) were not different (Hawkes et al., 1991). Peritoneal macrophage membrane levels of n-3 fatty acids were not consistently affected by adjuvant-induced arthritis. Therefore, as in the case of psoriasis, the clinical course of arthritis may be influenced by dietary treatment with n-3 fatty acids, and indeed, several laboratories have investigated this area.

Prickett et al. (1984) reported that feeding a diet containing fish oil increased the incidence but not the severity of collagen-induced arthritis in rats. Yet, they found that the serum IgG titer against type II collagen as determined by enzymed-linked immunosorbent assay (ELISA) was depressed in the fish oil group whereas the serum hemagglutinating antibody titer and DTH response against type II collagen was not different from the control group. Moreover, synovial explants from the fish oil-fed rats produced significantly less PGE2 than those from control rats, without a change in PGE3 production. The authors suggested that the increase in incidence of arthritis in the fish oil-fed group was related to alterations in tissue fatty acid composition, although the actual mechanism remained unclear.

Contrary to the findings of Prickett et al. (1984), Leslie et al. (1985) found an increase in the time to onset of collagen-induced arthritis in mice, as well as reduced incidence and severity of the disease. Similar to the report of Prickett and coworkers, macrophages from fish oil-supplemented mice produced less PGE2 and PGI2 than macrophages from control mice. The difference between the results reported by Leslie et al. (1985) and Prickett et al. (1984) is unclear; however, all studies conducted with humans have corroborated the results of Leslie and coworkers.

Kremer et al. (1985) reported that RA patients who were supplemented with 1.8 g of EPA/day experienced fewer clinical symptoms after 12 weeks. However, the absolute effectiveness of the treatment was somewhat unclear, and the patients who received EPA supplements declined more rapidly than control patients did after a 1–2-month washout period. The authors concluded that this further deterioration also supported their interpretation that the EPA treatment was beneficial. In later studies (Kremer et al., 1987, 1990), these authors demonstrated significant improvement in mean time to onset of fatigue and number of tender joints in patients consuming fish oil supplements.

Other parameters improved with fish oil supplementation, although not significantly. Sperling et al. (1987b) and Magaro et al. (1988) also reported alleviation of symptoms of RA in patients supplemented with n-3 fatty acids.

In addition to alleviation of arthritis symptoms, neutrophil production of LTB4 was reduced (Kremer et al., 1987, 1990; Sperling et al., 1987b), and LTB5 (Kremer et al., 1987, 1990; Sperling et al., 1987b) and 5-HEPE (Sperling et al., 1987b) were produced only by neutrophils from fish oil-supplemented patients. Production of PAF by macrophages was depressed by fish oil treatment (Sperling et al., 1987a,b). Also, chemiluminescence decreased and chemotaxis to LTB4 increased (Sperling et al., 1987b) in neutrophils from fish oil-supplemented arthritic patients. Lastly, fish oil supplementation reduced macrophage IL-1 production (Kremer et al., 1990). Of interest in this regard, Hom et al. (1988) found that administration of IL-1 accelerated the development of collagen-induced arthritis in mice.

In a more recent study by Remans et al. (2004), 55 RA patients were supplemented with a cocktail of 1.4 g EPA, 0.211 g DHA, and 0.5 g γ-linolenic (n-6) acid plus a wide assortment of vitamins and minerals, or placebo each day for up to 4 months. At both 2 and 4 months in the study, the investigators noted no significant differences between control and experimental treatments, including the measurements of C-reactive protein, sedimentation rate, medication use, or indices of clinical RA status.

Overall, the evidence is modestly supportive for a beneficial role of n-3 fatty acids in RA treatment (Fortin et al., 1995; MacLean et al., 2004). Additional studies may clarify the reason for the variable results noted by investigators.

C. Asthma

Asthma is characterized by recurrent, reversible obstruction of pulmonary airflow resulting from pathologic bronchoconstriction. Approximately 5% of the Western population suffers from this disorder (Fleming and Crombie, 1987), which results from smooth muscle contraction, enhanced intraluminal secretion, and bronchiolar edema (Kaliner et al., 1987). A large number of stimuli for the asthmatic response exist including exercise, stress, and ingestion of certain compounds such as aspirin. The eicosanoids, including the prostaglandins, thromboxanes, hydroxy-fatty acids, and LTs are intimately involved in this multifaceted inflammatory response. Antagonists of the synthesis or actions of certain of these eicosanoids, particularly the LTs, are among the most effective treatment modalities. In contrast, PGE2 and PGI2 have bronchodilatory effects (reviewed in Thien and Walters, 1995).

Lee et al. (1985b) and Picado et al. (1988) suggested that by inhibiting cyclooxygenase activity, n-3 fatty acids might divert arachidonic acid toward synthesis of 5-lipoxygenase products, which augment the smooth muscle contraction and bronchoconstriction of asthma. A study by Broughton et al. (1997) found that somewhat greater than 40% of subjects with asthma responded to n-3 fatty acid supplementation with a reduction in methacholine-induced impairment of respiratory function. In this study, a significant increase in urinary 5-series LTs was found in responsive subjects, particularly at 1:2 ratio of dietary n-3 to n-6 fatty acids. On the other hand, an equally large fraction of subjects had no change in respiratory function as a result of n-3 fatty acid supplementation, and in some cases n-3 supplementation was related to a further loss of function. Interestingly, a smaller effect on increased excretion of urinary 5-series LTs was noted in nonresponders than in responders, particularly at the higher n-3 supplementation dose. Therefore, differences in responsiveness to n-3 fat supplementation or to the underlying pathophysiology of the disease may influence the response a person with asthma may experience to this dietary manipulation.

Emelyanov et al. (2002) provided 46 patients with atopic asthma two capsules of lipid extract from the New Zealand green-lipped mussel (each experimental capsule contained 50 mg n-3 fatty acids and 100 mg olive oil) or with placebo capsules (containing 150 mg olive oil) for a period of 8 weeks. At the end of the experiment, subjects receiving the experimental capsules reported

significant decreases in daytime wheezing and use of β2-agonists. Although measured forced expiratory volume in 1 s (FEV1) and evening peak expiratory flow (PEF) were not significantly altered, the researchers noted significant improvement in morning PEF and exhaled H_2O_2 in expired breath condensate (a marker of airway inflammation). The significance of the treatment effect from 100 mg n-3 fatty acids per day suggests that perhaps other active ingredients are present in New Zealand green-lipped mussel lipid extract in addition to their n-3 content.

Okamoto et al. (2000a,b) supplemented 14 patients with asthma with 10–20 g/day of Perilla seed oil (rich in α-linolenic acid, an n-3 fatty acid) or corn oil for a period of 1 month. The investigators reported that PEF, forced vital capacity (FVC), and FEV1 were significantly improved by the Perilla seed oil treatment compared with the corn oil placebo. In addition, they noted that generation of LTC4 by leukocytes from the treatment group was significantly reduced compared with the placebo group.

Hodge et al. (1998) conducted a study with 39 children, aged 8–12, with asthma. Over an experimental period of 6 months they consumed supplements containing fish oil, canola oil, and margarine (the n-3 fatty acid group, 1.2 g n-3 fatty acids per day) or safflower and sunflower oil (the n-6 group). At the conclusion of this 6-month trial, there was no significant difference between groups in clinical indices of asthma severity or plasma TNF-α concentration.

Broughton et al. (1997) utilized a crossover design (1 month on each treatment) to investigate the benefits of n-3 fatty acid supplementation on reducing methacholine-induced respiratory distress in an adult asthmatic population. For 1 month subjects consumed diets containing n-3 to n-6 fatty acid ratios of 0.1:1 (low n-3), followed by diets containing a 0.5:1 (high n-3) ratio. At the end of the low n-3 period, patients experienced increased methacholine-induced respiratory distress whereas at the end of the high n-3 period more than 40% of patients required an increased methacholine dose in order to respond with bronchospasm. Unexpectedly, some patients responded to the high n-3 period with a further loss of respiratory function. The authors suggested that the noted changes were related to changes in de novo production of 4-series vs. 5-series LTs.

Nagakura et al. (2000) supplemented the diets of 29 children with bronchial asthma with fish oil for 10 months in a randomized controlled experimental design. Fish oil supplement capsules contained 84 mg EPA and 36 mg DHA. Control capsules contained 300 mg olive oil. The daily dosages of EPA and DHA were assigned on a body weight basis (17.0 ± 26.8 and 7.3 ± 11.5 mg/kg body weight). The fish oil treatment group showed significant improvements in asthma symptom scores and reduced bronchoconstriction to acetylcholine from 6 to 10 months of treatment. Control subjects did not experience these benefits.

A recent review of the literature by Wong (2005), as well as the studies reviewed above, suggests that n-3 fatty acid supplementation provides benefits in symptom reduction for patients with asthma. On the other hand, a Cochrane review of the literature published in 2002 did not find sufficient support for the benefits of n-3 intake in children with asthma (Thien et al., 2002). Larger, double-blind, placebo-controlled studies may be necessary to clarify these issues.

D. Inflammatory Bowel Disease

Inflammatory bowel disease is a general term used to describe diseases such as ulcerative colitis and Crohn's disease (Kirschner, 1989; Fretland et al., 1990). Inflammation may be restricted to the mucosa of the colon, as in ulcerative colitis, or may involve the submucosa of any segment of the intestinal tract, as in Crohn's disease. Although the etiology of these diseases is unclear, the result of the loss of the mucosa and submucosa is malabsorption. The immunopathology of chronic inflammatory bowel disease has been described (Braegger, 1994; Nielsen and Rask-Madsen, 1996; MacLean et al., 2005). It is interesting to note that the risk for development of arthritis and other inflammatory diseases is increased in individuals with inflammatory bowel disease.

As in other inflammatory diseases, the eicosanoids have been implicated in the etiology of the disease (Fretland et al., 1990; Wallace, 2001). LTB4 was detected in the colonic mucosa of patients

with inflammatory bowel disease at levels greatly exceeding than those in normal individuals (Sharon and Stenson, 1984; Lobos et al., 1987). Moreover, PAF was found in the mucosa of patients with active ulcerative colitis (Eliakim et al., 1988) whereas it was not found in normal mucosa. Finally, Raab et al. (1995) reported markedly elevated levels of PGE2 in perfusates of affected colon specimens from patients with ulcerative colitis. These levels of PGE2 correlated well with levels of tissue eosinophil cationic protein, myeloperoxidase, and TNF.

Given the role of LTB4 and PAF in stimulating neutrophil chemotaxis into inflammatory sites and the inhibitory effect of n-3 fatty acids on the production of these compounds, it is not unexpected that some benefit might be had with this treatment. Evidence has accumulated for n-3 fatty acids ameliorating symptoms of ulcerative colitis (Lorenz et al., 1989; McCall et al., 1989) but not Crohn's disease (Lorenz et al., 1989). Hawthorne et al. (1992) studied the effects of marine n-3 oil supplementation on the course of ulcerative colitis. This blinded controlled study randomized 87 patients to treatments providing 4.5 g EPA or an equivalent volume of olive oil (placebo control) daily for 1 year. Standard drug therapy was continued throughout the trial. The EPA treatment increased rectal mucosa EPA content and also altered LT synthesis patterns by neutrophils, stimulating LTB5 and depressing LTB4 secretion in response to calcium ionophore. Clinical benefits of n-3 supplementation were limited: a significant reduction in corticosteroid requirement after 1 month and 2 months treatment, and a nonsignificant trend toward early remission. There was no significant difference in the rate of relapse between groups. The authors concluded that EPA supplementation provided modest benefit during active disease but was ineffective in maintenance therapy.

Stenson et al. (1992) studied the effects of dietary n-3 fatty acid supplementation with 18 patients with active ulcerative colitis in a double-blinded cross-over study design. Treatment capsules provided 3.24 g EPA and 2.16 g DHA daily whereas control subjects received vegetable oil containing placebo capsules through each 4-month arm of the study (a 1-month washout period occurred between arms of the study). The authors demonstrated a reduction in the dose of prednisone required for symptom management during the n-3 phase compared with placebo. Similar to Hawthorne et al. (1992), this study demonstrated a reduction in LTB4 concentration in rectal dialysates. Finally, histology index and weight gain were significantly improved on the n-3 treatment vs. control. Thus, this study showed significant though modest improvements associated with n-3 fatty acid administration. A related study by Aslan and Triadafilopoulos (1992) resulted in similar results though an improvement in histology index was not significant.

In 1996, Loeschke et al. carried out a 2-year double-blind, placebo-controlled trial of relapse prevention by 5.1 g n-3 fatty acids daily in 64 patients with ulcerative colitis. At the time of entry the patients were in remission and were not using steroid medications. Relapse-free survival was improved by n-3 fatty acid supplementation during months 2 and 3; however, cumulative relapse rate at 2 years was similar for those taking placebo vs. n-3 treated. These authors reported no consistent difference in clinical, macroscopic, and histologic disease activity between treatment groups. Hence, they concluded that while n-3 fatty acids may retard relapse of ulcerative colitis they are unable to prevent such relapse.

Lorenz-Meyer et al. (1996) published the results of a 1-year trial of n-3 fatty acid supplementation with 204 patients suffering from relapsing Crohn's disease. The study investigated the effects of daily 5 g n-3 oil supplementation or a reduced carbohydrate diet on further relapse. In this study, neither the n-3 treatment nor the modified carbohydrate diet showed significant improvement in relapse rate compared to the placebo group.

Inui et al. (1996) also reported that intravenous administration of an n-3 fatty acid-enriched emulsion nonsignificantly reduced indices of tissue inflammation and LTB4 in an acute rat model of inflammatory bowel disease. It is clear, however, that more research must be conducted in order for the therapeutic potential of n-3 fatty acids in inflammatory bowel diseases to be conclusively evaluated.

Belluzzi (2002) reviewed the literature concerning IBD and n-3 fatty acid supplementation, and concluded that there was an overall benefit for n-3 fatty acids as an adjunct treatment for IBD.

In contrast, a recent systematic review of the literature concerning n-3 fatty acid effects on various clinical scores, rates of remission or relapse, or requirements for drug treatment of Crohn's disease or ulcerative colitis found insufficient evidence to draw conclusions as to its effectiveness (MacLean et al., 2005). Other larger and well-controlled studies are called for to better understand the benefits, if any, to be afforded by n-3 fatty acid supplementation for remission control and symptom treatment in patients with IBD.

IV. CONCLUSIONS

Throughout this review I have attempted to present information that portrays the most current knowledge and understanding of each of the areas covered. It is clear that dietary lipids have the capability to modify immune function. The three major mechanisms include alterations in eicosanoid synthesis, changes in lipoprotein levels, and modifications in membrane composition, which affect transmembrane signal transduction. Much work remains to be conducted, especially with regard to the effects of dietary fats on signal transduction across biological membranes.

The potential for modification of inflammatory disease by dietary lipids, particularly n-3 fatty acids, provides exciting possibilities for diet therapy of these diseases. Caution should be exercised in the use of these fatty acids, however, as we are seldom fully aware of the implications of upsetting the natural balance of regulatory factors and processes. In addition, the ease with which n-3 fatty acids are oxidized raises another possible detrimental effect of these lipids—consumption of immuosuppressive, oxidized lipids.

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42 Fatty Acids and Liver Disease

Amin A. Nanji and Bassam A. Nassar

CONTENTS

I. INTRODUCTION

The purpose of this chapter is to bring together information on the role of fatty acids in liver disease. Much of the information presented has been gathered by analysis of fatty acids in liver, liver microsomes, mitochondria, and hepatocytes. It should not, therefore, be assumed to be universal to other cells in the liver such as Kupffer's cells, endothelial cells, or hepatic stellate cells. Quite apart from the effects of fatty acids on liver disease or the effect of disease on fatty acid composition, the fatty acids are of importance as precursors of the prostaglandins, leukotrienes, and hydroxy fatty acid metabolites. Although a wide variety of liver disorders affects fatty acid metabolism, the majority of studies have been devoted to alcohol-induced liver injury. Thus, the major focus of this chapter is on alcoholic liver disease (ALD), which includes the role of fatty acids in the pathogenesis of liver injury as well as their role in the treatment of this disorder.

II. FATTY ACIDS IN THE PATHOGENESIS OF ALCOHOLIC LIVER DISEASE

Dietary fatty acids and alcohol both play an important role in the pathogenesis of ALD. There is considerable evidence to suggest that the amount of fat is an important determining factor in producing the lesions seen in ALD (French, 1993). Studies in rats fed a lipid diet containing alcohol showed that a steatogenic role for dietary fat and that fatty acids in the diet represented a major source of the lipids in the liver of animals fed ethanol (French, 1993). Studies with respect to the intragastric feeding rat model for ALD confirmed the role for dietary fat in ALD (French et al., 1986). When rats were fed a diet in which fat constituted 5% of the calories, only steatosis and focal necrosis

were induced in the centrilobular region of the liver. When the amount of fat was increased to 25% of total calories, over half of the rats developed centrilobular fibrosis similar to that seen in baboons and humans (French et al., 1986).

The role of dietary fat and fatty acids is further supported by epidemiological correlations that indicate that susceptibility to alcohol is related to different types of dietary fat (Nanji and French, 1986). In the first study, the deviation from expected mortality was correlated with the intake of saturated and unsaturated fatty acids and cholesterol (Nanji and French, 1986). The findings showed that saturated fat intake correlated inversely with deviation from expected mortality from cirrhosis suggesting that saturated fatty acids were protective. In a second study, cirrhosis mortality rates were correlated with pork and beef consumption (Nanji and French, 1985). A significant correlation was seen between pork consumption and cirrhosis mortality among countries with low carrier rates for hepatitis. There was no correlation with beef consumption.

To follow up on these epidemiological correlations, further studies were carried out using the intragastric feeding rat model for ALD. Rats that were fed ethanol and tallow (beef fat) developed none of the pathological features of ALD, rats fed ethanol and lard developed minimal to moderate ALD, and rats fed ethanol and corn oil showed the most severe pathological changes (Nanji et al., 1989). In each of the dietary groups, the percentage of calories derived from fat in the diet was similar (25%). Since the severity of histopathological changes was correlated with the content of linoleic acid (LA) in the diet, it was hypothesized that this component of the diet accounted for the differences in the pathology in the tallow-, lard-, and corn oil-fed groups. To confirm the role of LA in the diet, rats were fed ethanol together with tallow to which LA was added (Nanji and French, 1989). The animals supplemented with LA showed all of the pathological features of ALD, thus confirming the importance of this polyunsaturated fatty acid (PUFA) in the pathogenesis of experimental ALD.

The importance of PUFAs is further demonstrated by experiments in which fish oil was used as the source of fat. Feeding fish oil and ethanol produced more severe necrosis and inflammation than corn oil and ethanol (Morimoto et al., 1994; Nanji et al., 1994a).

Although several mechanisms have emerged to explain the development of alcohol-induced liver injury, which include upregulation of lipid peroxidation, increased levels of endotoxin, and induction of nuclear factor κB and its regulation of cytokines and inflammatory mediators that saturated fatty acids downregulate these events, additional mechanisms have also been proposed recently. Chronic ethanol administration is associated with inhibition of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPAR-α), two signaling molecules regulating pathways of hepatic fatty acid oxidation. Both molecules are major mediators of the metabolic effects of adiponectin. You and Crabb (2004) examined the effect of saturated fatty acids on chronic ethanol mice. They showed that chronic ethanol feeding with increased adiponectin levels led to the activation of signaling pathways controlled through AMPK, PPAR, and PPAR-γ coactivator. These alterations were associated with increased rates of hepatic fatty acid oxidation and prevention of steatosis. Importantly, AMPK is the downstream component of a kinase cascade that acts as a sensor of cellular energy change. ZAMPK is activated by rising AMP levels coupled to falling ATP levels, a feature of alcoholic liver injury.

III. FATTY ACIDS AND NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function tests in the general population. NAFLD is the term used to describe a spectrum of conditions characterized mainly by the histological findings of fat in the liver in individuals who consume little or no alcohol. The major feature of NAFLD, both histologically and metabolically, is the accumulation of triacylglycerol (TAG) in the liver.

The plasma nonesterified fatty acids (NEFA) pool contributes the majority of fatty acids that flow to the liver in the fasted state and provides a large proportion of the fatty acids in the very-low-density lipoprotein (VLDL) particles secreted by the liver.

The plasma NEFA pool accounts for approximately 60% of TAG in NAFLD patients thus reflecting the importance of the NEFA pool in NAFLD. Insulin resistance, an important feature of NAFLD, leads to a failure of insulin to fully suppress the activity of the hormone and the release of fatty acids for the TAG pool in adipose tissue is increased leading to an increase in NEFA pool (Step 1). Using nonreactive isotopes, metabolic pathways that lead to the accumulation of fat within the liver in NAFLD have recently been investigated. Important findings suggest that the rate of de novo lipogenesis (DNL) is elevated in NAFLD patients in the fasted state.

Liver cell apoptosis is a prominent feature of nonalcoholic steatohepatitis (NASH) and correlates with disease severity. Lipid-induced apoptosis is related to the degree of saturation of fatty acids. In a cellular model of steatosis and apoptosis, activation of apoptotic pathways was greater in cells treated with saturated than with monounsaturated fatty acids. Cell death was caspase dependent and associated with mitochondrial membrane depolarization.

It has long been known that alcohol abuse is associated with impaired immune function. Fatty acid ethyl esters (FAEEs) inhibit the production of interleukin 2 and influx of intracellular calcium into mononuclear cells. *In vivo* inhibition of the oxidative metabolism of ethanol using 4-methylpyrazole results in increased circulating concentrations of FAEE.

IV. FATTY ACIDS IN HEPATIC FATTY ACID COMPOSITION AND LIVER DISEASE

The fatty acid composition has been studied in a variety of liver disorders, but the most extensive studies have been carried out in ALD. The most consistent finding with respect to changes in the composition of fatty acids in the liver is a decrease in arachidonic acid (AA) (20:4n-6) (Reitz, 1978). Reduced levels of 20:4n-6 are seen in whole liver homogenates as well as mitochondrial and microsomal fractions (French et al., 1970; Alling et al., 1979; Cunningham et al., 1982; Cairns and Peters, 1983). In some studies, the decrease in 20:4n-6 was accompanied by an increase in LA (18:2n-6), although this is not a consistent finding (Reitz, 1978).

Ethanol-induced alterations in fatty acid composition can occur quite early after ethanol exposure. The total pospholipid fractions from liver mitochondria show increased levels of the monoene fatty acids and a decrease in 20:4n-6 after 2 h of exposure to ethanol vapor (Rouach et al., 1984). The magnitude of these changes were greater in the phosphotidylcholine component than in the phosphatidylethanolamine component. In humans, alcohol ingestion also leads to changes in the fatty acid composition that are similar to those seen in experimental animals. Patients with histological liver injury have higher levels of oleic acid (18:1n-9), total monoenoic fatty acids, and lower levels of total PUFAs (de la Muza et al., 1996). The 18:1/18:0 ratio was higher and 20:4/18:2 ratio lower in patients with liver injury compared to those patients ingesting alcohol but with no pathological liver injury.

The effects of different levels and types of dietary fat intake on the fatty acid composition of the liver in alcohol-fed animals have also been studied.

In one study, the effects of dietary fat and ethanol fatty acid composition were compared at two levels of fat intake (high fat, 34.2%; and low fat, 4.6%) in male and female rats (Thompson and Reitz, 1978). In male rats fed the high-fat diet, 16:0, 18:1n-9, and 18:2 were increased in liver mitochondria, whereas 20:4n-6, 22:4n-6, and 22:6n-3 were decreased. In the low-fat group, only the 18:2n-6 was increased and the 20:4n-6 decreased. In female rats, the level of dietary fat did not alter the ethanol effects; ethanol only affected the two saturated fatty acids (16:0 and 18:0) but not the polyunsaturated.

Varying the source of dietary fat in the diet also alters the microsomal fatty acid composition induced by ethanol, and these changes can also be related to the development of liver injury

(Nanji et al., 1994b). In rats fed medium-chain triglycerides and ethanol (no liver injury), the changes in fatty acids in liver microsomes were different from those seen with corn oil and ethanol (liver injury present). In particular, the concentration of AA (20:4n-6) increased and the level of oleic acid (18:1n-9) was decreased in the medium-chain triglyceride–ethanol group. These changes were opposite to what was seen in the corn oil–ethanol-fed animals. In rats fed fish oil and ethanol, it is noteworthy that higher levels of 22:5n-3 and 22:6n-3 were detected in the livers than in the fish oil diet itself (Nanji et al., 1994b). The most probable explanation for this observation is that the increased synthesis and preferential incorporation of 22:5n-3 and 22:6n-3 into liver phospholipids and triglycerides in rats fed fish oil is a compensatory response to the decrease in n-6 fatty acid synthesis in the fish oil-fed group.

Similar changes in fatty acids are also seen in other forms of liver injury due to alcohol-induced liver disease. In rats with chronic iron overload, a significant decrease in PUFAs and a parallel increase in saturated fatty acids (in mitochondrial and plasma membrane phospholipids) was seen after 9 weeks of iron treatment (Pietrangelo et al., 1990). There was no significant difference in the cholesterol/phospholipid molar ratio or in the lipid/protein ratio. Assessment of various membranes in liver using electron spin resonance spectroscopy did not show any change in the molecular order of the membranes suggesting that modification of the fatty acid profile in cellular membranes in *in vivo* chronic iron toxicity does not bring about modification in other physicochemical parameters relevant to cell membrane integrity. Modifications in fatty acid composition of liver plasma membranes in iron overload are also seen after *in vitro* induction of lipid peroxidation by addition of a ADP–Fe^{3+} complex to liver plasma membrane preparations (Pietrangelo et al., 1990).

Cholestasis also produces significant changes in microsomal fatty acid composition. After 2 days of bile duct ligation in rats, microsomal phospholipid fatty acids that were decreased included LA and AA (Bengochia et al., 1992). Phosphatidylcholine showed an increase in oleic and palmitic acids and a decrease in AAs. All fatty acids studied were decreased in the phosphatidylserine portion and were increased in the phosphatidylinositol fraction. Eight days after cholestasis, a global decrease in all fatty acids except oleic acid was seen in the phosphatidylcholine portion; an increase compared to the 2-day values was seen in all fatty acids in the phosphatidylserine fraction. The exact significance of these changes is unclear, and one hypothesis is that intrahepatic availability of fatty acids is distributed owing to bile flow impairment. Furthermore, steps in the synthesis of phophatidylcholine such as lysophosphatidylcholine acylation could be affected.

V. ROLE OF CYTOCHROME P-4502E1

Cytochrome P-450-dependent microsomal monoxygenase enzymes are involved in the oxidative metabolism of a wide variety of xerobiotics (Guengerich, 1995). One isoform of hepatic cytochrome P-450, cytochrome P-4502E1 (CYP2E1), is ethanol inducible and is a major component of the microsomal ethanol-oxidizing system (Lieber, 1988; Morimoto et al., 1993; Kessova and Cederbaum, 2003). The regulation of CYP2E1 by different types of dietary fatty acids has been demonstrated (Caro and Cederbaum, 2004). Corn oil, in comparison to a fat-free diet, increases the constitutive level of CYP2E1 protein and enzyme activity compared to animals fed lard or olive oil (Yoo et al., 1991). Dietary fatty acids also regulate the induction of CYP2E1 by ethanol. Levels of CYP2E1 are higher in the liver of rats fed ethanol with PUFAs compared to rats fed ethanol with saturated fatty acids (Takahashi et al., 1992; Morimoto et al., 1994; Nanji et al., 1994c). In addition to the results obtained in the *in vivo* studies, findings of *in vitro* studies also support a role for unsaturated fatty acids in ethanol-induced toxicity (Caro and Cederbaum, 2004). For example, addition of the substrate AA to a CYP2E1 expression line leads to increased levels of reactive oxygen species (ROS) and cellular cytotoxicity. The combination of iron and unsaturated fatty acids also decreases cell viability when compared to control CYP2E1-expressing cells. French and coworkers have evaluated the role of ethanol-induced CYP2E1 induction in the pathogenesis of changes in hepatic fatty acid composition induced by ethanol (Morimoto et al., 1995). Using inhibitors of

CYP2E1 (diallylsulfide and phenethyl isothiocyanate) in the intragastric feeding model for ALD, these investigators confirmed the observation that ethanol caused an increase in the 18:1/18:0 ratio and a decrease in the 20:4/18:2 ratio compared to pair-fed controls. The inhibitors of CYP2E1 partially prevented the above changes in hepatic fatty acid composition. These results indicate that induction of CYP2E1 could contribute to the decrease in 20:4n-6 after ethanol administration. Possible mechanisms include metabolism of 20:4 by the cytochrome P-450 system and enhanced lipid peroxidation (see below).

VI. EICOSANOIDS AND CYTOCHROME P-450 METABOLITES

To follow up on observation that AA is decreased in the liver after alcohol administration, experiments were conducted to evaluate whether eicosanoid production could, at least in part, account for this decrease in AA. Liver nonparenchymal cells were isolated from rats fed ethanol and different dietary fats. The findings of these studies showed that increased levels of thromboxane B_2 and leukotriene B_4 and decreased levels of prostaglandin E_2 (PGE_2) were seen in rats fed corn oil and ethanol (Nanji et al., 1993, 1994d). Production of the prostacyclin metabolite 6-ketoprostaglandin $F_{1\alpha}$ was also decreased in rats that develop liver injury (Nanji et al., 1994e). The enzyme responsible for the conversion of AA to prostanoids is cyclooxygenase (COX) or prostaglandin H synthase (DeWitt, 1996). It is now evident that two forms of COX exist in many cells: a constitutive enzyme designated COX1 and an inducible isoform known as COX2 (Otto and Smith, 1995; Pairet and Engelhardt, 1995). The expression of the two isoforms is regulated differently. Under physiological conditions, prostaglandin synthesis depends on the availability of AA and the enzymatic activity of COX1. COX2 is induced by a variety of stimuli that are upregulated in inflammatory liver disease (Hla et al., 1993). These stimuli include interleukin-1, tumor necrosis factor α, lipopolysaccharide, and oxidative stress (Hla et al., 1993; Hempel et al., 1994). Increased expression of COX2 in Kupffer's cells is associated with necroinflammatory changes in experimental ALD (Nanji et al., 1997a). COX2 upregulation is also associated with increased synthesis of vasoactive and proinflammatory eicosanoids such as the thromboxanes. The importance of thromboxanes in alcoholic liver injury is further suggested by studies, which show that necrosis and inflammation are attenuated by treatment with inhibitors of thromboxane synthesis and thromboxane receptors (Nanji et al., 1997b). Another possible explanation for the decrease in 20:4n-6 in microsomes after ethanol administration is the metabolism of 20:4n-6 by P-450 isoforms (Makita et al., 1996). These metabolites include the ω and ω-1 hydroxylation products of AA and the epoxide metabolites [epoxyeicosatrienoic (EET) acid] such as 14,15 EET; 11,12 EET; and 8,9 EET. The formation of such metabolites is increased in experimental ALD (French et al., 1997), and the interest in these metabolites is in their potential biological activities such as regulation of blood flow and enzyme activity such as Na, K ATPase (Capdevila et al., 1992).

VII. ROLE OF LIPID PEROXIDATION IN DECREASING HEPATIC ARACHIDONIC AND IN INDUCING LIVER INJURY

A significant body of evidence supports increased production of lipid peroxides in various forms of liver injury (Cederbaum, 1989; Lieber, 1997). The importance of lipid peroxidation and its relationship to fatty acids was suggested by the discovery of isoprostanes (IPs) as markers of peroxidation (Morrow et al., 1992a). IPs are prostaglandin-like compounds that are produced independent of the COX enzyme by free radical-catalyzed peroxidation of AA (Roberts and Morrow, 1996; Morrow and Roberts, 1997). Abstraction of *bis*-allylic hydrogens of AA by free radicals leads to the formation of arachidonyl radicals; subsequent attack by oxygen, endocyclization, and reduction leads to the formation of PGF_2-like compounds.

Measurements of IPs have provided new evidence for a role of oxidative stress in liver injury. In support of this notion, rats were administered carbon tetrachloride and the time course of the increases in levels of F_2-IPs in the liver and plasma were followed. Increased levels of esterified F_2-IPs increased rapidly, whereas the appearance of free IPs was delayed supporting the notion that IPs are initially formed *in situ* on phospholipids and subsequently released in free form (Morrow et al., 1992b). Increased levels of IPs have been demonstrated in the setting of liver ischemia (Mathews et al., 1994). IP levels also correlate with the severity of liver injury in experimental ALD in rats (Nanji et al., 1994f), and urinary F_2-IP excretion increases in a dose-dependent fashion following alcohol ingestion in humans (Lieber, 1997). Dietary iron overload is also associated with increased levels of F_2-IP esterified to lipids in the livers of rats (Dabbagh et al., 1994). Similarly, rats given halothane show increased levels of F_2-IPs esterified to hepatic lipids (Awad et al., 1996). Biliary measurements of IPs have been proposed as a measure of hepatic lipid peroxidation (Awad and Morrow, 1995).

VIII. FATTY ACID ETHYL ESTERS AND LIVER INJURY

FAEEs are esterification products of fatty acids and ethanol (Laposata et al., 1995). They are generated through the enzymatic action of FAEE synthase and, unlike acetaldehyde, represent a metabolic product of ethanol not generated by the oxidation of ethanol. Approximately 30% of the FAEEs in serum are bound to serum lipoproteins and the remaining 70% are bound to albumin (Bird et al., 1997). The LDLs contain most of the FAEEs among the different lipoproteins, and increasing FAEE concentrations result in increasing FAEE binding to lipoprotein relative to albumin. An autopsy study of individuals who were acutely intoxicated at the time of death showed that FAEEs and FAEE synthase, the enzyme responsible for their synthesis, were found predominantly in organs damaged by ethanol abuse (Laposata and Lange, 1986).

Once inside the cell, the mechanism(s) by which FAEEs or their metabolites, such as free fatty acids, produce their toxic effects is unknown. FAEEs have been shown to induce mitochondrial dysfunction by uncoupling of oxidative phosphorylation (Lange and Sobel, 1983), altering the fluidity of cell membranes (Hamamoto et al., 1990); increasing lysosomal fragility (Haber et al., 1993); and decreasing protein synthesis (Szczepiorkowski et al., 1995). Our preliminary studies show that unsaturated FAEEs are associated with alcohol-induced liver injury, whereas saturated FAEEs are not (Nanji et al., 1996a).

IX. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS, FATTY ACIDS, AND LIVER DISEASE

A mechanism common to many diseases associated with fatty acid overload is impairment of mitochondrial fatty acid oxidation (Fromenty and Pessayre, 1995). The term *fatty acid overload* implies a role for long-chain fatty acids in producing the hepatic sequelae associated with these conditions. PPARs are proposed to function as sensors of fatty acid overload (Lemberger et al., 1996). In the liver, the predominant form of PPAR is PPAR-α. PPARs are members of the nuclear receptor family and long-chain fatty acids are important ligands of PPAR-α. Although a key mechanism to fatty acid overload is an increase in peroxisomal and microsomal oxidation of fatty acids (Kaikaus et al., 1993), the metabolism of fatty acids through these pathways may cause toxicity. Peroxisomal β oxidation generates hydrogen peroxide; although peroxisomes contain catalase, decreased catalase activity in certain disorders such as alcoholism may predispose to liver injury.

Sterol regulatory element-binding proteins (SREBPs) are key regulators of cholesterol and fatty acid synthesis. SREBP-1c and SREBP-2 predominate in the liver. The role of SREBPs is suggested by a number of studies which show that overexpressing SREBP-1a or SREBP-1c leads to the accumulation of increased amounts of cholesterol esters and triglycerides in liver. The role of SREBPs

in alcoholic fatty liver has indicated that in addition to increased activation of SREBP-1 ethanol induces the expression of several lipogenic enzymes (You and Crabb, 2004).

X. FATTY ACIDS IN THE TREATMENT OF ALCOHOLIC LIVER DISEASE

Although saturated fatty acids have been shown to be protective against alcohol-induced liver injury, little attention has been paid to the potential role of dietary fat as a treatment for established ALD. Lipid diets high in protein and calories or parenteral nutrition therapy have been used to treat ALD to reverse the protein–calorie malnutrition that often accompanies it (Nanji and Zakim, 2003). The rat intragastric feeding model allows for the evaluation of dietary fat as treatment in established ALD. Data from experimental studies show that a diet low in PUFAs administered to rats with established liver injury leads to near normalization of pathological changes such as fatty liver, necrosis, inflammation, and fibrosis (Nanji et al., 1995, 1996b). Histological improvement was associated with decreased levels of endotoxin, lipid peroxidation, and CYP2E1 activity, and reduced expression of COX2 and tumor necrosis factor-α (Nanji et al., 1997c). It is difficult to determine the relative contributions of each of these factors in ALD, but the results of the different studies show that dietary fat can be used as a pharmacological tool to downregulate the phenomena related to necrosis, inflammation, and fibrosis.

XI. THE ROLE OF FATTY ACIDS IN HEPATOCELLULAR CARCINOMA AND HEPATOCARCINOGENESIS

The role of PUFAs in the promotion and progression of cancers of various tissues has been the subject of many studies and is the subject of some debate (Diggle, 2002; Larsson et al., 2004). There are a number of studies that have reported the beneficial effects of fish and fish oil in cancer prevention, and it is generally considered that the cancer related effects of fatty acids are dependent on their structure. Fatty acids of the n-6 type, such as LA (C18:2n-6) for instance, have a strong promoting effect in some tumors (Sauer et al., 2000), while in contrast, n-3 PUFAs, such as α-linolenic acid (ALA; C18:3n-3) and its metabolites, eicosapentanoic acid (EPA; C20:5n-3), and docosahexanoic acid (DHA; C22:6n-3), may inhibit tumorogenesis (Reddy et al., 1991). ALA only differs from LA by a single double bond at the n-3 position, but this seemingly small structural difference is felt to lead to a remarkable functional contrasting impact between the two molecules.

A. n-6 Fatty Acids and Hepatocellular Carcinoma

Various mechanisms that promote hepatocarcinogenesis and involve fatty acids have been proposed and investigated. n-6 Fatty acids are seen to play a similar stimulatory role in liver cancer as they do in other malignancies. LA for instance, induces cell growth and DNA synthesis in the HTC-R_3T_3 rat hepatoma cell line, and may exert this effect by binding to a specific cellular protein, the liver fatty acid binding protein (L-FABP), an intracellular fatty acid carrier (Keler et al., 1992). *N*-2-fluorenylacetamide (FAA), which induces hepatocarcinoma formation in rats, interacts specifically with L-FABP, to induce its carcinogenic effect. L-FABP is thought to be involved in the biosynthesis of phospholipids by regulating lysophosphatedic transport to the endoplasmic reticulum to be converted to phosphatedic acid, a mitogen proposed to exert this effect through the *ras* protein. In hepatoma cells transfected with L-FABP and treated with FAA, exposure to LA leads to increased DNA synthesis and cellular growth, an effect not seen when those cells were exposed to other n-6 fatty acids, such as γ-linolenic (GLA), dihomo-γ-linolenic (DGLA), AA, or palmitoleic acids. This effect of LA is linked to its oxygenated metabolites, since indomethacin, a COX inhibitor, and α-tocopherol and nordihydroguaiaretic acid (NDGA), both antioxidant inhibitors of lipoxygenase, significantly

decrease the effect of LA on hepatoma cell growth. In rats transplanted with the hepatoma cell line 7288CTC and fed a diet containing LA, tumor growth is directly dependent on plasma concentrations of LA, and to a lesser extent on AA, where LA is converted by the hepatoma lipoxygenase enzyme into the mitogen 13-hydroxy-9,11-octadecadienoic acid (13-HODE) (Sauer et al., 1997). Sauer et al. (1999) also further show that 13-HODE, resulting from converted dietary LA, leads to increased DNA synthesis during *in vivo* perfusion. Inhibition of lipoxygenase activity by NDGA does not inhibit LA uptake by the tumor, but suppresses 13-HODE formation and subsequently tumor growth. In rats sustained on an LA-deficient diet, there was decreased tumor growth.

The effects of other PUFAs on hepatic tumors may differ from that seen with LA. For example, AA induces apoptosis rather than promoting growth. Dymkowska et al. (2006) reported that AA increase the rate of ROS formation in rat hepatoma AS-30D cells. Cell death is accompanied by the release of cytochrome *c* from the mitochondria, activation of caspase-3, and association with the BAX apoptotic protein. Although oleic acid did not induce ROS, it precipitated apoptosis, albeit to a lesser extent than AA.

Conjugated fatty acids, similar to conjugated LA (CLA), have recently been shown to interfere with LA metabolism and its effect in tumor tissue. CLA in mice causes smaller prostatic tumors than LA and a drastic reduction in lung metastases (Cesano et al., 1998). In mammary tissue, Banni et al. (1999) show that CLA interferes with LA metabolism resulting in decreased LA metabolites, including GLA, DGLA, and AA. The CLA dose–response effect on AA suppression corresponds closely with the dose–response CLA effect on cancer protection in the mammary tissue. This effect on tumor suppression, however, is not consistently seen in liver cancer. Yamasaki et al. (2002) show that in rats transplanted with hepatoma dRLh-84 and fed various concentrations of CLA, tumor growth is enhanced in comparison to non-CLA diet group. CLA decreases PGE_2 synthesis and COX2 levels without affecting AA levels. Sauer et al. (2004), in contrast, report that CLA isomers inhibit hepatoma 7288CTC tumor growth via a G-protein-coupled free fatty acid receptor by reducing intratumor cAMP, blocking LA uptake, and decreasing intratumor 13-HODE formation.

B. n-3 Fatty Acids and Hepatocellular Carcinoma

Data on the effect of n-3 fatty acids in hepatocellular carcinoma are mainly on animal studies and have provided evidence of an inhibitory effect on tumor growth. Calviello et al. (1998) show that Morris hepatocarcinoma 3924A grown in rats receiving EPA and DHA exhibit a 50% reduction in weight and volume of tumor in comparison to tumors in rats receiving oleic acid. Interestingly, DHA produced a greater degree of apoptosis than EPA. Sauer et al. (2000) have shown that ALA, EPA, and DHA inhibit the uptake of LA into hepatoma 7288CTC cells. The n-3 fatty acids also inhibit the uptake of saturated, monounsaturated, and other n-6 fatty acids. In addition, ALA and EPA inhibit the *in vivo* conversion of LA into the mitogen 13-HODE, leading to decreased tumor growth. The decrease in fatty acid uptake by ALA and EPA is thought to be due to their binding of an n-3 fatty acid receptor that inhibits a G_i-protein-coupled signal transduction pathway leading to decreased cAMP and LA uptake and its conversion to 13-HODE.

The enzyme fatty acid synthase (FAS) is overexpressed in tumors, and suppression of its activity reduces de novo fatty acid synthesis and is cytotoxic to tumors. Moon et al. (2002) show that n-3 PUFAs can negatively regulate FAS and its transcription factor, the SREBP-1. In addition, n-3 PUFAs induce necrosis and apoptosis of tumors cells and reduce tumor growth and metastatic spread (Diggle, 2002). Vecchini et al. (2004, 2005) show that ALA suppresses Morris hepatoma 3824A FAS, acetyl-CoA carboxylase mRNA, and SREBP-1, and induces apoptosis. Wu (2006) has recently reviewed the interaction between various fatty acid related genes in liver cancer. These include the role of COX2, PGE_2 and its G-protein-coupled E-prostanoid receptors, and their interaction with the epidermal growth factor (EFG) receptor and cross talk with c-Met oncogene. The interplay of COX2 with other cellular messengers such as inducible nitric oxide synthase and vascular EGF and potent gene transcription factors such as the PPARs x and y, which can also be modulated by fatty acids, remains to be further elucidated.

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43 Essential Fatty Acids and Visual Dysfunction

Algis J. Vingrys and Anne E. Weymouth

CONTENTS

I. INTRODUCTION

The association between low omega-3 polyunsaturated fatty acid (PUFA) intake and visual dysfunction has been well documented (Vingrys et al., 2001; SanGiovanni and Chew, 2005; Singh, 2005). This chapter reviews recent research that provides insights into the mechanisms through which these essential fatty acids can act to modulate neural function. A brief overview of the importance of dietary omega-3 fatty acids in sustaining ocular health precedes a discussion of the functional contribution that omega-3 PUFA have as structural components of membranes. The specialized metabolism of these lipids by the retina as well as its accessibility by way of electroretinography render this tissue a useful model in which to consider the functional consequences of omega-3 deficiency. These functional losses are discussed with respect to the roles that omega-3 polyunsaturates are known to have in modulating membrane biophysical characteristics and membrane-bound protein function. Laboratory trials indicate that omega-3 metabolites should impact on membrane-bound protein activity in a predictable fashion, although *in vivo* animal or human studies fail to consistently demonstrate these effects. This review details possible explanations for the lack of concordance between the *in vivo* and *in vitro* outcomes.

II. OMEGA-3 FATTY ACIDS AND OCULAR HEALTH

Many aspects of ocular physiology and vision are affected by omega-3 lipids. Although much attention is paid to the role of these essential fatty acids in sustaining healthy neural function, there is increasing evidence for their benefits in other ocular conditions. This is not surprising given the myriad effects that omega-3 metabolites have in systemic physiology, as detailed in other chapters of this book.

A. Dry Eye

The transparent anterior surface of the eye, the cornea, is composed of connective tissue. Its structure contributes the curvature that underlies the refractive power of the eye in combination with the tear film which provides the optical surface for refraction. Image quality, therefore, is strongly dependent on the composition of the overlying tear film produced by glands within the conjunctiva and eyelids. Insufficient dietary intake of omega-3 fatty acids has been implicated in dry eye syndromes (Miljanovic et al., 2005). The reason for this association remains elusive although there are several possible explanations.

Increased plasma levels of omega-3 fatty acids may directly impact on the lipid profile of the tear film, thereby improving its stability and function. Alternatively, omega-3 fats may optimize meibomian gland health by modulating its secretions as well as suppressing the production of proinflammatory cytokines. It is also possible that omega-3 intake promotes the activity of transmembrane ionic pumps located in the conjunctival epithelium, improving tear quality or quantity (Shi and Candia, 1995). The relationship between membrane omega-3 PUFA composition and transmembrane protein activity will be discussed later. Finally, a greater omega-3 intake should improve the conjunctival and corneal epithelial surfaces, as it does elsewhere in the skin (Ziboh et al., 2000; Sinclair et al., 2002), making them more able to withstand periods of dryness and promoting comfort in cases of inadequate secretion.

B. Retinal Disease

Retinitis pigmentosa is an inherited degeneration of retinal photoreceptors, which has been associated with decreased plasma levels of omega-3 PUFA (Hoffman and Birch, 1995; Hoffman et al., 2001). Despite this altered plasma profile, there is no known causal link between the lower omega-3 PUFA and the retinal degeneration. Moreover, investigations of the functional benefits that dietary supplementation can have in this disease remain inconclusive (Wheaton et al., 2003; Berson et al., 2004a,b; Hoffman et al., 2004).

Apart from systemic complications, diabetes induces vascular anomalies (Stitt and Curtis, 2005; Kalantzis et al., 2006) and neural dysfunction (Phipps et al., 2004) within the retina, by way of diabetic retinopathy. DHA has been shown to decrease the inflammatory processes in diabetic retinopathy (Chen et al., 2005b) and there is ongoing research into the possibility that omega-3 PUFA may inhibit the retinal neovascularization of diabetes. Although fish oil supplementation has been shown to improve the dislipidemia associated with diabetes (Mori et al., 1990), vascular and neural effects have not been evaluated in clinical trials. Recent research from our laboratory has shown that diabetic animals given fish oil supplementation not only returned normal lipid profiles but also had no other signs of diabetic retinopathy (Yee et al., 2006).

The most common cause of vision loss in the elderly is age-related maculopathy (AMD) (Figure 43.1b). Recent findings into the pathophysiology of AMD have shown that the inflammatory cascade is upregulated in this disease (Johnson et al., 2001; Donoso et al., 2006; Gold et al., 2006). Thickening and lipid deposits within Bruch's membrane, a layer that overlies the choroidal

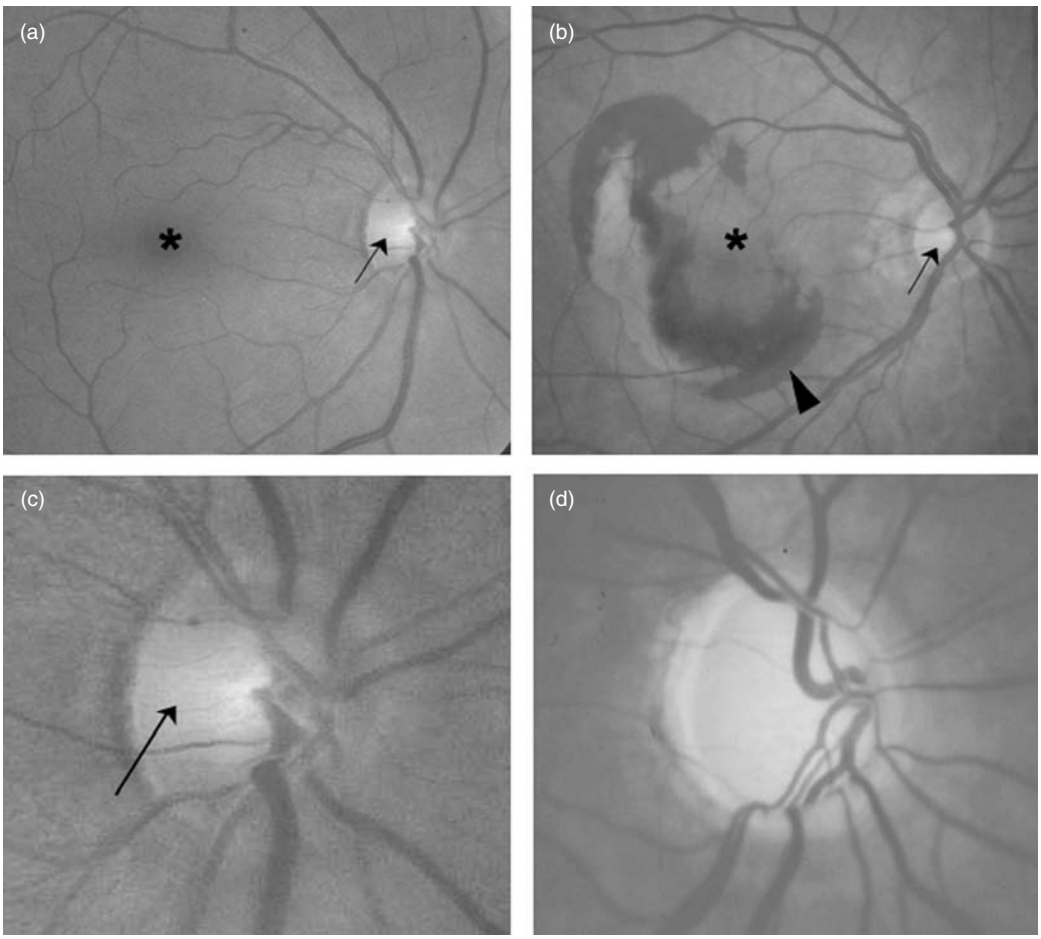

FIGURE 43.1 Retinal fundus images of a normal eye and two age-related diseases related to insufficient dietary omega-3 intake. (a) Healthy fundus showing optic nerve head (arrow) and macula (asterisk). (b) Age-related macular degeneration is evidenced by the macular scar (white area around asterisk) and subretinal hemorrhage (blood surrounding scar as indicated by the arrow head). (c) Enlargement of healthy optic nerve head shown in (a). Arrow indicates healthy neural tissue comprising the neuroretinal rim surrounding a central excavation or "cup." (d) Glaucomatous optic nerve head showing marked cup enlargement, pallor and absence of neural tissue within the optic nerve head.

blood supply to the retinal pigmented epithelium (RPE) (Figure 43.3), compromises photoreceptor integrity causing receptor atrophy and ultimately leads to subretinal neovascularization (Curcio et al., 1996; Mullins et al., 2000; Anderson et al., 2002; Rudolf et al., 2005; Sivaprasad et al., 2005; Tian et al., 2005). Other work indicates that anomalous cholesterol trafficking is associated with the onset or progression of AMD (Malek et al., 2005; Baird et al., 2006). Dietary intake of omega-3 fatty acids reduces the risk of macular degeneration (Smith et al., 2000; Seddon et al., 2001; Elner, 2002) and supplementation has been shown to ameliorate the clinical fundus signs (Feher et al., 2005) although the mechanisms underlying these benefits are unknown.

C. Glaucoma

Glaucoma refers to the syndrome of optic nerve loss with associated visual field deficits (Figure 43.1d). A major risk factor for this disease is elevated intraocular pressure (IOP) (Gordon et al., 2002). Intraocular pressure has been shown to be related to the level of dietary intake of omega-3 PUFA with pressure reduction arising from enhanced aqueous outflow (Nguyen et al., 2005). This improved outflow facility may arise from elevated omega-3 eicosanoid levels within the aqueous humour (Kulkarni and Srinivasan, 1989; Rosch et al., 2005). However, neuroprotective effects of omega-3 metabolites (Miyauchi et al., 2001; Bazan, 2005, 2006) may also impact on the glaucomatous neuropathy. Not surprisingly, the risk of development or progression of glaucoma has been shown to be inversely related to plasma levels of omega-3 fatty acids (Cellini et al., 1998; Ren et al., 2006). The role that omega-3 fatty acid supplements have in IOP modulation and glaucoma modification are not well defined and are in need of well designed clinical trials.

The relationship between the timing and quantity of dietary omega-3 PUFA intake and ocular disease prophylaxis remains to be investigated. As discussed later, there is specific and efficient accretion of omega-3 polyunsaturates, particularly docosahexaenoic acid (DHA) during fetal growth, and adequate levels are required to ensure normal development of the visual system. Less studied is the requisite for ongoing intake of omega-3 PUFA throughout life and the specific effects that insufficient intake can have on ocular aging and pathology.

There is a wealth of literature describing the importance of omega-3 fatty acids in optimizing visual development and ocular health. However, the optimal dietary intake and the mechanisms through which these lipids act are complex and many are yet to be determined. The remainder of this chapter will focus on the structural role of the long-chain omega-3 PUFA, DHA, within the nervous system. As discussed below, the visual pathway provides an accessible route for investigating the means through which omega-3 PUFA can optimize neural function. The fact that omega-3 fatty acids can modulate intraocular fluid flow and neural function bears witness to the complex physiological processes within the eye that are affected by lipid intake. Furthermore, the specialized metabolism of these lipids within the retina creates a unique context for understanding how omega-3 fatty acids maintain healthy neural function. This knowledge can then be projected to the central nervous system and other organs. By relating test-tube results to practical outcomes, the following sections consider the means through which membrane lipid composition can modify retinal function.

III. OMEGA-3 FATTY ACIDS IN THE NERVOUS SYSTEM

The nervous system accumulates and tenaciously retains omega-3 fatty acids more than any other tissue (O'Brien and Sampson, 1965; Sinclair, 1975; Bourre et al., 1992; Makrides et al., 1994; Abedin et al., 1999; Moriguchi et al., 2001; Haag, 2003). Neural retention of PUFA (Bazan et al., 1994) is exemplified by the time course required to create a change in membrane profiles following dietary manipulation (Galli et al., 1971; Neuringer et al., 1986; Watanabe et al., 1987; Connor and Neuringer, 1988; Bourre et al., 1989; Lin et al., 1991; Connor et al., 1992; Weisinger et al., 1995, 1996a, 1998; Moriguchi et al., 2001). Moreover, whereas hepatic fatty acid profiles fluctuate rapidly

subject to dietary intake, the relative omega-3 and omega-6 content of neural membranes remains constant over a wide range of dietary intakes (Abedin et al., 1999). Why the body should go to such lengths for fatty acid profile preservation is not clear given that the sole difference between the two long-chain metabolites, DHA, and its omega-6 counterpart, docosapentaenoic acid (DPA), is the absence of a double bond at carbon 19 in the omega-6 species (Salem et al., 2001). As discussed later, this apparently trivial structural difference can lead to substantial functional alteration.

Omega-3 PUFA in neural membranes comprises little eicosapentaenoic acid (EPA) as this substrate is rapidly recruited to eicosanoid synthesis or elongated into DHA (Hagve et al., 1988). DHA may be obtained directly from the diet or synthesized from one of its omega-3 precursors (Sprecher et al., 1995). Pawlosky et al. (1996) assert that, in addition to dietary sources and placental transfer, there must be local biosynthesis of DHA within nervous tissue for the production of such high local concentrations of DHA. There is increasing evidence of this occurring within the retina (Alvarez et al., 1994; Chen et al., 1999) and brain (Delton-Vandenbroucke et al., 1997; Moore, 2001; Williard et al., 2001a,b).

A. Preferential Accretion and Retention of Omega-3 Fatty Acids by the Retina

Given the significant function of membranes in the photoreceptor outer segments (Section VII), it is not surprising that the retinal membrane lipids are unique in their constituents and metabolism. The retina features unique mechanisms for DHA uptake, conservation and recycling in an effort to maintain adequate levels, even during prolonged dietary deficiency (Rodriguez de Turco et al., 1990; Stinson et al., 1991b; Anderson et al., 1992; Bazan et al., 1992).

Of the PUFAs in mammalian retinae, between 38% and 92% are DHA (Fliesler and Anderson, 1983; Salem et al., 1986; Martinez et al., 1988; Salem, 1989; Hrboticky et al., 1991; Wiegand et al., 1991; Gulcan et al., 1993; Makrides et al., 1994). Thirty percent of rod outer segment fatty acids incorporate DHA and 54% of retinal phosphatidylethanolamine (PE) acyl chains contain DHA (Benolken et al., 1973; Fliesler and Anderson, 1983; Bazan et al., 1990; Wiegand et al., 1991). The rod outer segment disc membrane contains 42%–45% each of phosphatidylcholine (PC) and PE and 10%–12% phosphatidylserine (PS) (Salem et al., 2001). Optimal photochemical function of rhodopsin has been reported to depend on both the surrounding phospholipid head group and the fatty acyl chain content (Wiedmann et al., 1988). Rhodopsin activation can also be modulated by rhodopsin concentration with elevated concentrations inhibiting activation (Niu and Mitchell, 2005).

Retinal DHA is concentrated through the formation of di-DHA species or by coupling with other unsaturated fats (Bell and Tocher, 1989; Stinson et al., 1991a; Polozova and Litman, 2000). DHA is commonly positioned at the *sn*-2 carbon although, uniquely within the retina, it may also occupy the *sn*-1 position (Lin et al., 1994). The DHA content of PE and PS is highest in the retina but is also found in high concentrations in synaptic vesicles and synaptic plasma membranes (Cotman et al., 1969; Anderson, 1970; Anderson and Sperling, 1971; Breckenridge et al., 1972; Sun and Sun, 1972; Breckenridge et al., 1973). Of the aminophospholipids, PS and PE have the highest concentrations of DHA (Yabuuchi and O'Brien, 1968).

Photoreceptors synthesize a small amount of DHA (Wetzel et al., 1991; Wang and Anderson, 1993a) and further production occurs in the pigmented epithelium (Wang and Anderson, 1993a) and vascular endothelium (Delton-Vandenbroucke et al., 1997). Most retinal DHA is transported from hepatic stores across the RPE and is incorporated into ellipsoids to form new photoreceptor outer segment disks (Bazan et al., 1992; Wang and Anderson, 1992, 1993b; Gordon and Bazan, 1993).

Despite the specialized processing of DHA within the retina, there is well-founded evidence for retinal omega-3 fatty acid depletion (Leat et al., 1986; Neuringer et al., 1986; Connor and Neuringer, 1988; Chen and Anderson, 1993; Weisinger et al., 1998; Abedin et al., 1999; Moriguchi et al., 2001) and subsequent functional losses following lengthy dietary deprivation of α-linolenic acid (LNA) or DHA (Benolken et al., 1973; Wheeler et al., 1975; Neuringer et al., 1986; Connor and Neuringer, 1988; Birch et al., 1992; Weisinger et al., 1996a; Armitage et al., 1999; Jeffrey et al., 2002a).

IV. METHODS OF ACHIEVING OMEGA-3 DEFICIENCY

Investigating the neural dysfunction that arises from omega-3 deficiency is dependent on the level of deficiency that can be attained. In animal models, this is achieved by the extent, nature and timing of dietary intervention. In humans, studies of omega-3 deficiency are limited to perinatal nutritional modifications or epidemiological assays of dietary habits, with the latter providing less precision than the former. However, achieving substantial omega-3 depletion within the nervous system, and particularly the retina, by dietary modification can be hindered by the tenacious retention and recycling mechanisms of this tissue, described in the previous section.

An important issue in diet modification trials is the timing of the intervention, given species-dependent variations in retinal growth and DHA accretion. Whereas there is substantial postnatal growth and differentiation of the retinal neurons of rodents (Bonting et al., 1961; Fulton et al., 1995), cellular differentiation and functional development in precocial species (guinea pigs, primates and humans) is completed *in utero* (Huang et al., 1990; Hendrickson, 1993; Greiner et al., 1996, 1997; Bui and Vingrys, 1999; Su et al., 1999; Vingrys and Bui, 2001). In precocial mammals, 35% of DHA accretion occurs in the last phase of gestation (Locke et al., 1998). Not surprisingly, maximum dietary modifications in humans are realized in premature, low birth weight infants (Uauy et al., 1990; Birch et al., 1992; Hoffman et al., 1993; Faldella et al., 1996; Leaf et al., 1996; Neuringer, 2000; Uauy and Hoffman, 2000; Innis, 2003). This implies that early manipulations of omega-3 intake either before or during pregnancy will have greatest effect on the fatty acid profiles of subsequent generations, an issue that will be developed in the following section.

However, human trials are limited by the modest capacity to which DHA profiles can be modulated by diet to induce changes within neonatal tissues. For example, the average breast milk DHA content of Western women (0.45% w/w) is about half that of non-Western women (0.88%) (Jensen, 1999), resulting in a small absolute dietary deficiency compared to that which can be realized in animals (~40:1) (Figure 43.2). Despite this relatively small modification, studies in human infants report benefits to visual and cognitive function by supplementing milk formulae with long-chain omega-3 PUFA (Uauy et al., 1990; Werkman and Carlson, 1996; Birch et al., 1998; Makrides et al., 2000).

As detailed in earlier editions of this book, human infants nourished with milk supplemented with DHA and EPA had larger electroretinogram (ERG) amplitudes than did infants fed the short-chain omega-3 precursor, ALA (Uauy et al., 1990, 1992; Birch et al., 1992; Hoffman et al., 1993, 2003). These findings contrast with a study in monkeys that revealed no differences in ERG amplitudes

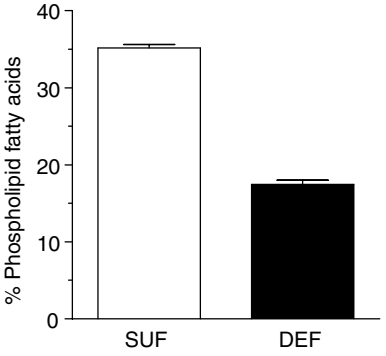

FIGURE 43.2 Retinal phospholipid DHA content of 20-week-old rats raised on an omega-3 deficient diet from conception. Mean (± SEM, n = 12/group) retinal DHA content expressed as a percentage of total retinal phospholipid fatty acids. Omega-3 sufficient rats (Suf, unfilled bar) were raised on a diet comprising 7% lipid by way of a mixture of safflower, flaxseed and tuna oils (5%:1.5%:0.5% ω-6/ω-3 = 5.5:1). Omega-3 deficient rats (Def, filled bar) were raised on a diet where the 7% lipid was provided by safflower oil alone (ω-6/ω-3 = 235:1).

following either ALA or DHA and arachidonic acid (AA) supplementation (Jeffrey et al., 2002b). Given the previous discussion of the importance that a mother's diet has on neonatal development, it is possible that the disparity between the human and monkey studies reflects differences in maternal omega-3 status. The mothers in the human trials were all of Western origin, presumably with relatively modest intakes of omega-3 lipids (Simopoulos, 2001), whereas the monkey chow may have contained higher levels of omega-3 fats. It is possible that the different maternal omega-3 status may have influenced neural development. Alternatively, the monkey study (n = 10/diet) (Jeffrey et al., 2002b) may have been of insufficient power to detect the modest ERG anomalies reported in human trials (n = 30/diet) (Hoffman et al., 2003).

Understanding the mechanisms involved in omega-3 modulation of neural physiology is facilitated by using animal models, where greater levels of dietary deprivation are possible. Larger magnitudes of tissue depletion increase the likelihood of functional deficits. Moreover, the changes to lipid profiles of specific neural tissues in animals can be directly assayed and correlated to the level of dysfunction, and the extent of deficiency can be amplified in animals by dietary manipulation across generations. For example, retinal DHA content can be reduced by 88% in guinea pigs after three generations (Leat et al., 1986; Weisinger et al., 1996b). In rats, the same period of intervention elicits a retinal DHA deficit of up to 62% (Leat et al., 1986; Weisinger et al., 2002), although most of the depletion appears to occur in the first generation (Leat et al., 1986). These substantial dietary manipulations can be considered extreme in terms of omega-3 intake; however, they are necessary for investigating the mechanisms through which omega-3 PUFA act in neural tissues. The consequences of small omega-3 insufficiencies over a period of many years, as is typical in humans, are unknown.

Single generation deficits are amplified by initiating maternal dietary intervention either during or preceding pregnancy. Weisinger et al. (1998) quantified the effect that maternal omega-3 status has in depleting guinea pig offspring. Prefeeding pregnant monkeys with omega-3 deficient diets achieved a retinal DHA deficit in their offspring of 80% by 20 weeks of age (Neuringer et al., 1986, 1991). Our laboratory has adopted this prefeeding protocol with rats and finds a 52% decrement in the retinal DHA of 20-week-old offspring (Figure 43.2). Artificial feeding in newborn rats (Moriguchi et al., 2004) gives rise to a greater DHA deficit, similar to that reported in monkeys, at the expense of more complex technical demands. This indicates that appropriate dietary intervention elicits extensive retinal DHA deficiency in rat pups, although natural, maternal prefeeding has a lesser capacity for depletion than artificial feeding.

V. FUNCTIONAL ASSESSMENT OF VISION

Assessment of the visual pathway is dependent on the species involved and the region of the visual pathway under study. Subjective measures that test visual thresholds using preferential looking can be adopted in humans and monkeys (Neuringer et al., 1984; Uauy et al., 1992; Birch et al., 1998). An objective correlate is the visual evoked potential (VEP), which is a measure of the local electroencephalogram of the visual cortex in response to a specific stimulus modality (e.g., contrast) and is particularly suited to the examination of nonverbal infants (Faldella et al., 1996; Birch et al., 1998; Makrides et al., 2000; Hoffman et al., 2003; Gillingham et al., 2005).

Objective measures can also be applied to animal models of omega-3 deficiency. The ERG is a sensitive method for evaluating the massed retinal response to light stimulation. Although focal or multifocal techniques are useful in localizing lesions across a diseased retina, the full-field flash ERG is appropriate for assessing the general deficits induced by systemic omega-3 deficiency in nonfoveate mammals such as rats. The ERG has been commonly used to assess retinal dysfunction following deprivation of omega-3 PUFA in animal models. The following sections provide an overview of retinal structure and how this relates to components of the ERG response. This is followed by specific consideration of the effects that omega-3 deprivation can have on these processes.

VI. RETINAL STRUCTURE

The retina is a multilaminar cellular structure composed of specialized neurons (Figure 43.3). The photoreceptors transduce visual stimuli into electrical signals which are transmitted to the brain via the optic nerve. The functional efficacy of this tissue is a reflection of the specific organization of its different neuronal classes. Several classes of retinal neurons have been identified (Kolb, 1991) and the mammalian retina may be divided into ten layers according to histological cellular arrangements (Kolb, 1991; Hendrickson, 1993).

The outermost retinal layer lies adjacent to the choroidal blood supply and consists of a single row of cells, forming the RPE (Figure 43.3). The RPE stratum, and its intercellular junctional occlusions, form the blood-retinal barrier and an element of electrical resistance called the "R membrane" (Brindley, 1970). This component of resistance has the capacity to alter the magnitude of electrophysiological recordings of retinal activity (Tomita et al., 1960). The outer segments of photoreceptors lie between the apical processes of RPE cells (McLaughlin and Boykins, 1981). The RPE has common embryological origins with other pigmented cells of the uvea (Hendrickson, 1993). They are cuboidal cells that support retinal metabolism (Boulton and Dayhaw-Barker, 2001), sustain photoreceptor outer segment turnover and renewal (Young, 1967; Gordon and Bazan, 1990) and mediate visual pigment regeneration (Bok, 1993; McBee et al., 2001).

Photoreceptors are elongated cells responsible for light capture and the transduction of light energy into a graded electrical potential that is passed onto other cells (Baylor and Fettiplace, 1976; Yau, 1994). Photoreceptors have an outer segment near the RPE connected to an inner segment via a ciliary stalk (Besharse et al., 1985). Two classes of photoreceptors are found in mammalian

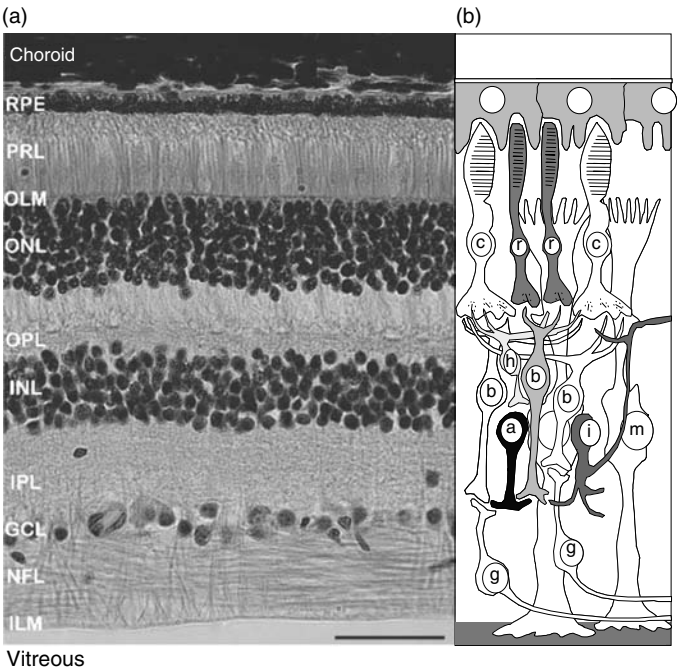

FIGURE 43.3 Cross-sectional retinal structure. (a) Vertical section of primate retina stained with hematoxylin and eosin showing cell bodies and synaptic stratification of the retina. Scale bar: 50 μm. (Photo courtesy of Dr. T. Puthussery.) (b) Schematic of the retinal structure shown to the left identifying the key layers as: RPE: retinal pigmented epithelium; PRL: photoreceptor layer; OLM: outer limiting membrane; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; NFL: nerve fiber layer; ILM: inner limiting membrane. The cells within these layers are: r: rod; c: cone; b: bipolar cell; h: horizontal cell; a: amacrine cell; i: interplexiform cell; g: ganglion cell; m: Müller cell.

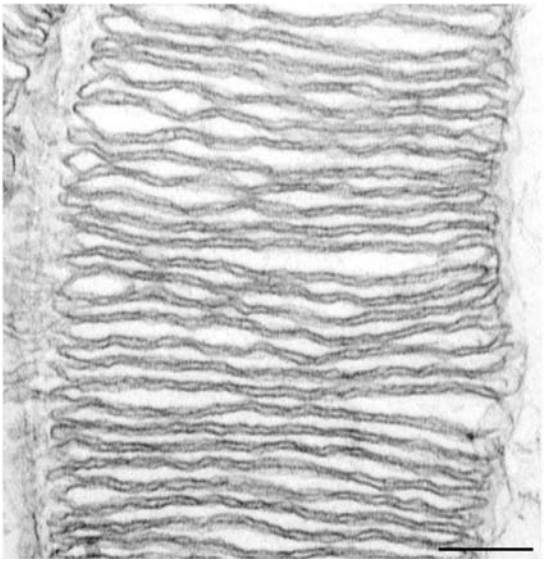

FIGURE 43.4 Transmission electron microscopy of a rod outer segment section. Membranous disks are stacked in a laminar arrangement. These carry the proteins involved in phototransduction. Scale bar: 200 nm. (Photo courtesy of Dr. T. Puthussery.)

retinae, rods (Figure 43.4) and cones (Szel et al., 2000), which differ in morphology and photopigment structure (Baylor et al., 1984b; Applebury and Hargrave, 1986). Cones are morphologically distinguished from rods in that the cone inner segment is of greater diameter than the outer segment and the outer segment can have a conical shape (Laties and Enoch, 1971; Borwein, 1983). The inner segments of both contain the nucleus and organelles needed for metabolism (Hoang et al., 2002; Lluch et al., 2003; Perkins et al., 2003). The outer limiting membrane of the retina is created by Müller cell processes which envelop the photoreceptors.

The structure of the photoreceptoral outer segment is significant in its role of capturing light (Figure 43.4). The outer segment membranes of both rods and cones invaginate and pairs of adjacent invaginations form a stack of double-membrane disks (Daemen, 1973). These membranes contain the highest concentrations of DHA in the body (Anderson, 1970; Anderson and Maude, 1970). In rods, these disks are contained within—but separated from—the outer plasma membrane, whereas those of cones remain continuous with the outer plasma membrane (Daemen, 1973). There is continuous regeneration of the photoreceptor outer segment disks as aged membranes are shed to be phagocytosed by the RPE (Young, 1967; Gordon and Bazan, 1993). It is within these disk membranes that the light-absorbing proteins, photopigments, are incorporated (Applebury and Hargrave, 1986; Szel et al., 2000).

Differences in retinal function between species can be, in part, attributed to variations in retinal structure. In contrast to the rat retina which has few cone photoreceptors (0.85%–1.5%, La Vail, 1976; Carter-Dawson and LaVail, 1979; Szel and Rohlich, 1992), 5% of the retinal photoreceptors in primates are cones (Wikler et al., 1990). The differences in cell structure, spatial organization (Laties and Enoch, 1971; Wikler et al., 1990) and neural wiring (Sassoe-Pognetto et al., 1994; Brandstatter et al., 1995) between these two classes of receptors provide cones with better temporal, color and spatial resolution (acuity) whereas rods possess greater contrast gain at low light levels (Rieke and Baylor, 1998). Particular to human and primate retinae (Szel et al., 2000), the close packing of cones at the fovea affords high visual acuity in this region (Wikler et al., 1990). The rodent retina is, therefore, useful for analyzing rod function, as it comprises a receptoral landscape typical of that found in the human peripheral retina.

Several specialized neurons are involved in signal transmission and modification within the retina (Brindley, 1970; Baylor and Fettiplace, 1976; Tomita, 1986; Ghosh et al., 2001). These include bipolar cells, horizontal cells, amacrine cells and interplexiform cells in the outer nuclear layer and mainly ganglion cells in the ganglion cell layer (Figure 43.3). The through pathway carries receptoral responses to the inner retina via two subtypes (ON- and OFF-) of bipolar cells which transmit graded potentials to ganglion cells (Euler and Wassle, 1995; Morgans, 2000; Berntson et al., 2003; Ichinose et al., 2005). Lateral processing via horizontal cells can modify the signal at the receptor-bipolar cell synapse in the outer plexiform layer whereas amacrine and interplexiform cells may alter the signal transmitted to the ganglion cell in the inner synaptic layer (Wu, 1991; Brandstatter et al., 1995; Hartveit, 1999; Trexler et al., 2005). In response to the input from the other retinal neurons, ganglion cells produce action potentials (Diamond and Copenhagen, 1995) that are transmitted to the brain along the optic nerve (Enroth-Cugell and Robson, 1984; Lipton and Tauck, 1987; Fitzgibbon and Taylor, 1996). Recently, a unique ganglion cell containing a photosensitive pigment, melanospin, has been described (Provencio et al., 2000; Berson et al., 2002; Dacey et al., 2005; Peirson and Foster, 2006). These cells appear to be involved in regulating the pupillary light response as well as circadian rhythms (He et al., 2003; Hannibal et al., 2005; Hattar et al., 2006). Müller cells are large glia whose somata extend throughout the thickness of the retina (Reichenbach, 1989). They provide metabolic support to retinal neurons and are involved in buffering extracellular ion concentrations (Oakley et al., 1992; Reichelt et al., 1993; Newman, 1999; Kofuji et al., 2002; Connors and Kofuji, 2006).

Axons leave the ganglion cell to extend across the retina and form the nerve fiber layer (Fitzgibbon and Taylor, 1996). Expanded Müller cell processes occupy the inner retinal face to form the inner limiting membrane that contains the nerve fiber layer (Shaw and Weber, 1983; Lundkvist et al., 2004). This separates the sensory retina from the vitreous body. Retinal nerve fibers bundle together at the optic disc to exit the globe as the optic nerve and terminate in midbrain centers.

VII. RETINAL SIGNAL PROCESSING

This section outlines the processes involved in visual signal transduction and visual pigment regeneration. These topics have been well covered in the following reviews (Stryer, 1986; Pugh and Lamb, 2000; McBee et al., 2001; Lamb and Pugh, 2004).

A. The Dark Current

The ability of the photoreceptors to respond to light arises from the maintenance of ionic gradients across the inner to outer segments (Schnapf and McBurney, 1980; Woodruff et al., 1982). These gradients are modulated by the state of membrane-bound cation channels (Figure 43.5).

Sodium/potassium-ATPases actively transport potassium ions into and sodium ions out of the inner segments (Berman et al., 1977; Baylor et al., 1984a; Stirling and Sarthy, 1985; Schneider and Kraig, 1990). This creates a longitudinal concentration gradient for sodium. Sodium ions extruded from the inner segment pass down their concentration gradient to enter outer segment pores that are held in an open state in the dark by cyclic-GMP (cGMP, Hagins and Yoshikami, 1975; Pinto and Brown, 1984; Gray and Attwell, 1985; Zimmerman et al., 1985; Matthews, 1986, 1987). The sodium ions that enter the outer segment are shunted to the inner segment via the connecting cilium, completing the circuit known as the "dark current" (Figure 43.5) (Hagins et al., 1975). A sodium/calcium:potassium ion exchanger maintains intracellular calcium and potassium ion concentrations in the outer segment (Bauer and Schauf, 2002). Innexr segment potassium concentrations are sustained by voltage-sensitive potassium channels (Barnes, 1994; Wollmuth, 1994).

This ionic flow is reliant on the Na^+/K^+-ATPase maintaining a concentration gradient for sodium ions across the receptoral inner and outer segments. The change in dark current brought about following light absorption (Schnapf and McBurney, 1980; Woodruff et al., 1982) results from hydrolysis of cGMP, reducing binding to the nonspecific cation channels located in the outer segments (Gray

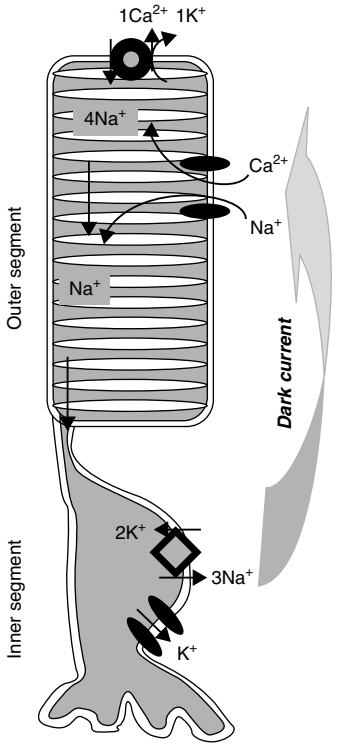

FIGURE 43.5 The inner segment Na^+/K^+-ATPase maintains the dark current. (Adapted from Saari, J.C., 1992. The biochemistry of sensory transduction in vertebrate photoreceptors. *Adler's Physiology of the Eye*. 9th ed., W.M. Jr. Hart, ed., St. Louis: Mosby-Year Book, Inc., p. 486.)

and Attwell, 1985; Matthews, 1987). This closes the channels (Figure 43.7), preventing sodium and calcium influx into the outer segment (Miller and Korenbrot, 1993). However, calcium extrusion via the 4 Na^+/Ca^{2+}:K^+ ion exchanger continues, resulting in decreased cytosolic Ca^{2+} concentration (Yau, 1994), a process important for adaptation mechanisms, as detailed later.

In the dark, the circulating "dark current" is maximal and maintains the photoreceptor membrane in a depolarized state (Moriondo et al., 2001) with continuous release of glutamate from the synaptic terminal of the photoreceptor. The light-induced closure of cation channels in the outer segment inhibits cation influx causing hyperpolarization (Baylor et al., 1984a,b). This is passively transmitted from the outer segment to the inner synaptic terminal causing a graded decrease in glutamate release (Kaneko and Shimazaki, 1976), proportional to the intensity of the light stimulus (Penn and Hagins, 1972; Baylor et al., 1979, 1984b; Gomez and Nasi, 1994).

B. Photon Absorption

Photoreceptor photopigments are responsible for light capture and are incorporated into the disk membranes (Dratz et al., 1979; Fotiadis et al., 2003; Liang et al., 2003). There are two components to each photopigment molecule: an opsin to which is attached a chromophore. The opsin is a seven-α-helix transmembrane protein (Applebury and Hargrave, 1986), a good template for studying G-protein-coupled receptors (Hargrave and McDowell, 1992; Lamb and Pugh, 1992). The chromophore is a vitamin A aldehyde (11-*cis*-retinal) and is directly responsible for light absorption. The spectral distribution of light absorption of each photoreceptor is determined by the structure of the opsin (Baylor et al., 1984b; Applebury and Hargrave, 1986) and its dielectric interactions

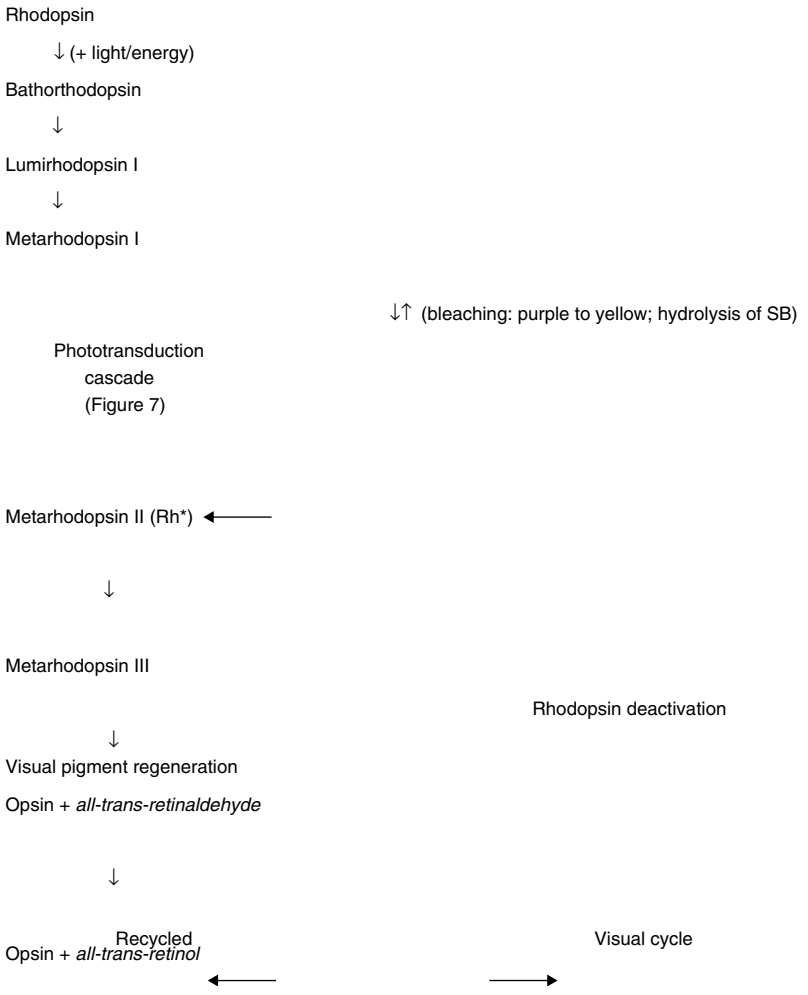

FIGURE 43.6 Steps involved in rhodopsin activation and processing. Rhodopsin is activated to metarhodopsin II by absorption of a photon of light (hv). The active form of rhodopsin (Rh* or metarhodopsin II) is formed on hydrolysis of the Schiff base (SB) linkage between the visual pigment (chromophore) and the opsin. The deactivation processes and rhodopsin regeneration are detailed in later sections.

with the chromophore (Kochendoerfer et al., 1999). The photopigment found in rods is known as rhodopsin (Fotiadis et al., 2003; Liang et al., 2003).

Chromophore activity is affected by the location of the photopigment molecule within the membrane (Piscitelli et al., 2006). A significant proportion of the rhodopsin molecule lies within the hydrophobic portion of the membrane bilayer, thus exposing it to interactions with other membrane phospholipids (Albert and Yeagle, 1983). The helical structure of rhodopsin also creates three cytoplasmic loops and three intradiscal loops (Liu and Northup, 1998; Bailey et al., 2003), with the cytoplasmic loops being important to the process of phototransduction (Figure 43.7). The chromophore is buried in the centre of the membrane bilayer, surrounded by the opsin helices (Hsu et al., 1993).

Absorption of a photon by the chromophore effects an alteration in its secondary structure called bleaching (Figure 43.6). This process involves the conversion of 11-*cis*-retinal to all-*trans*-retinol (Wald, 1968). A number of structural intermediaries are formed before hydrolysis of the Schiff base linkage (Longstaff et al., 1986) between the chromophore and the opsin (Straume et al., 1990).

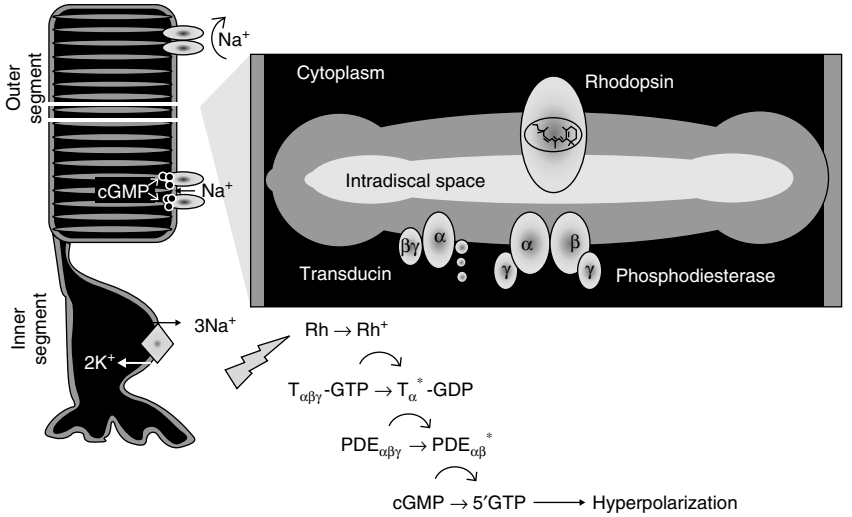

FIGURE 43.7 Phototransduction cascade. Schematic showing the orientation of the membrane-bound proteins that mediate phototransduction and the cascade of protein activations leading to hyperpolarization of the photoreceptor. Proteins diffuse within the outer segment disk membrane bilayer leading to sequential activation. Hydrolysis of cGMP results in a reduction in the cytoplasmic concentration of cGMP, releasing this molecule from the pore-binding site and leading to closure of the outer segment cation channels. Asterisks indicate active forms Rh: rhodopsin; GTP: guanosine triphosphate; GDP: guanosine diphosphate; PDE: phosphodiesterase; cGMP: cylic guanosine monophosphate. See text for details.

The formation of these intermediaries is temperature dependent (Lewis et al., 1981). One of the key products, metarhodopsin II, catalyzes the initial reaction of the transduction cascade (Figure 43.7, Kibelbek et al., 1991), and is often described as the active form of rhodopsin (Rh*).

C. Phototransduction Cascade

Phototransduction is achieved through (Pugh and Lamb, 2000) a cascade of sequential protein activations following light capture by rhodopsin (Figure 43.7). Cyclic GMP maintains the nonspecific cation channels in the rod outer segment open in the dark (Figure 43.5) (Matthews, 1987). Activated rhodopsin (Matthews et al., 1963) initiates a cascade of sequential interactions with the peripheral membrane proteins, transducin and cGMP phosphodiesterase (PDE) (Deterre et al., 1986; Stryer, 1986; Wensel and Stryer, 1986; Watanabe and Murakami, 1992). Transducin is a G-protein composed of three subunits: $T_{\alpha}GDP_{\beta\gamma}$ (Tanabe et al., 1985; Fong et al., 1986; Umbarger et al., 1992; Matsuda et al., 1994; Liu and Northup, 1998). Rod PDE is a tetrameric protein with an alpha, beta and two gamma subunits (Artemyev et al., 1996; Kameni Tcheudji et al., 2001; Kajimura et al., 2002; Cote, 2004; Guo et al., 2005). The gamma subunits in both these molecules are inhibitory and their cleavage activates the respective proteins (Kisselev et al., 1994; Granovsky and Artemyev, 2001; Kajimura et al., 2002).

Active PDE (Figure 43.7) catalyzes the hydrolysis of many cGMP molecules (Takizawa et al., 1998) allowing closure of sodium channels. The subsequent reduction in positive ionic influx results in hyperpolarization of the photoreceptor (Matthews, 1986; Koch and Stryer, 1988; Dumke et al., 1994). As detailed later, the multiple binding and activation of transducin and PDE and the subsequent multiple cGMP hydrolyses lead to amplification ($\times 10^5$) in the response. The lateral diffusion dependence of the activation of each of these proteins (Dumke et al., 1994; Koutalos et al., 1995; Calvert et al., 2001; Niu and Mitchell, 2005) introduces a delay to the various steps involved in phototransduction (Lamb and Pugh, 1992b).

The photoreceptor G-protein cascade, triggered by photopigment light capture, is considered a good model for the G-protein cascades throughout the rest of the body (Lamb and Pugh, 1992a).

D. Rhodopsin Deactivation and Regeneration of the Dark Current

The activated proteins formed during the phototransduction cascade continue in their role of activation until deactivated. The closure of the outer segment cation channels prevents calcium influx without altering the calcium efflux by the Na^+/Ca^{2+}:K^+ exchanger (Figure 43.5). This decrease in the cytosolic calcium concentration (Albani et al., 1980) allows calcium to dissociate from recoverin (Figure 43.8), decreasing the concentration of the inactive, membrane-bound form (Calvert et al., 1995; Sanada et al., 1996; Valentine et al., 2003). Recoverin promotes activation of rhodopsin kinase (Kuhn and Wilden, 1987; Palczewski et al., 1991; Hurley and Chen, 2001; Komolov et al., 2005) which then phosphorylates metarhodopsin II (Ohguro et al., 1996; Hurley et al., 1998; Mendez et al., 2000); the first step of rhodopsin deactivation. Subsequent "capping" of the phosphorylated metarhodopsin II complex by arrestin (Figure 43.8) inhibits further activity (Kuhn and Wilden, 1987; Hofmann et al., 1992; Pugh et al., 1999). Given the inactivation of metarhodopsin II, regeneration of the dark current requires the concurrent deactivation of transducin and PDE. The guanosine triphosphate (GTP) subunit of the activated T-GTP-PDE complex is hydrolyzed to form inactive T-GDP-PDE (Miller et al., 1989). The beta and gamma subunits of transducin reassociate with transducin and the inactive T-GDP complex dissociates from the inactive PDE. Cytoplasmic concentrations of cGMP are replenished by the enzymatic activity of transmembrane guanylate cyclases (Yang and Garbers, 1997).

E. Visual Pigment Regeneration

Deactivation of metarhodopsin II requires the chromophore to separate from the opsin and enter the visual cycle (McBee et al., 2001; Lamb and Pugh, 2004). Chromophore recycling involves a series of isomerization reactions that take place in the RPE (Figure 43.9). All-*trans*-retinal is transferred across rod and cone outer segment membranes and into the cytosol. A specific, transmembrane ATP-binding cassette transporter (ABCR) mediates this process for the membrane-bound fraction of all-*trans*-retinal (Sun et al., 1999; Weng et al., 1999; Schmitz and Kaminski, 2002; Beharry

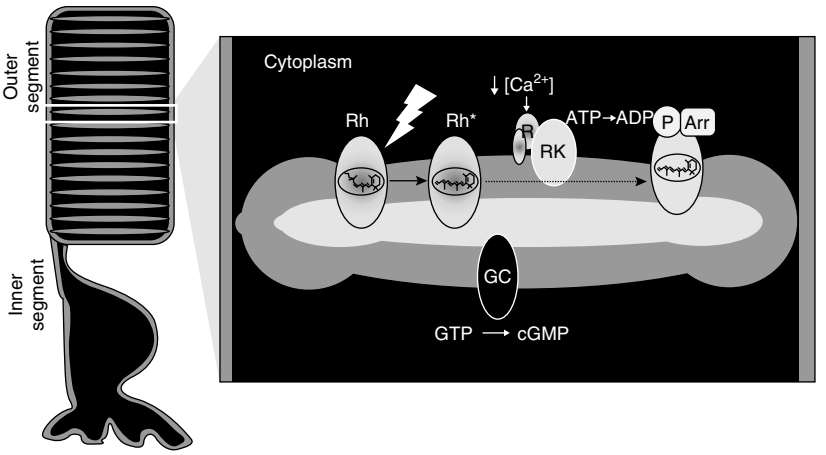

FIGURE 43.8 Schematic of rhodopsin activation by light (arrow) and subsequent deactivation (dotted arrow). Sequential recruitment of proteins involved in the deactivation of rhodopsin in the outer segment disks. Rh: rhodopsin; Rh* activated form of rhodopsin; R: recoverin; RK: rhodopsin kinase; P: phosphate; Arr: arrestin; GC: guanylate cyclase; GTP: guanosine triphosphate; cGMP: cyclic guanosine monophosphate. See text for details.

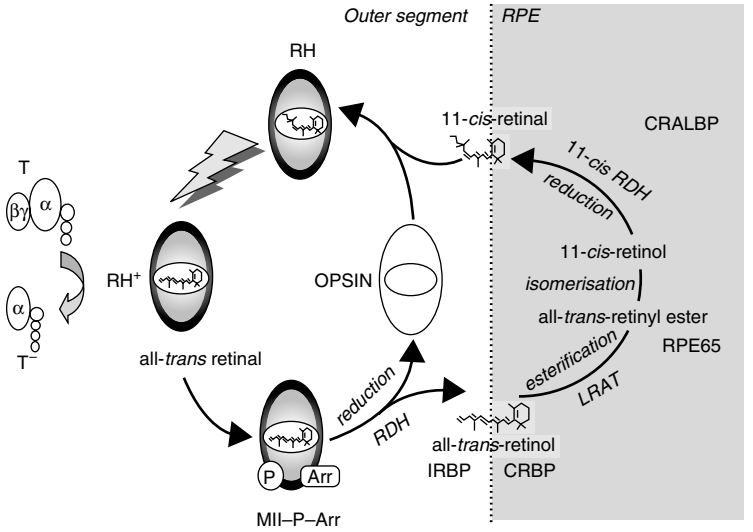

FIGURE 43.9 The visual cycle: pigment regeneration. Schematic of visual pigment regeneration in the RPE. Events leading to the transduction cascade are shown in Figure 43.6 and the formation of the MII–P–Arr complex are shown in Figure 43.8. Arr: arrestin; CRALBP: cellular retinaldehyde-binding protein; CRBP: cellular retinol-binding protein; IRBP: interphotoreceptor retinoid-binding protein; LRAT: lecithin retinol acyl transferase; MII: metarhodopsin II; P: phosphate; RDH: retinol dehydrogenase; Rh: rhodopsin; RPE: retinal pigmented epithelium; T: transducin. (Adapted from Leibrock, C.S., et al., 1998. *Eye.* 12 (Pt 3b), 511–520.)

et al., 2004). All-*trans*-retinol dehydrogenase (RDH) catalyzes the reduction of all-*trans*-retinal to all-*trans*-retinol in the cytosol (Zimmerman et al., 1975; Chen et al., 2005a). All-*trans*-retinol is then transported to the RPE, esterified by lecithin retinol acyl transferase (LRAT, Canada et al., 1990; Ruiz et al., 1999) and converted to 11-*cis*-retinol by an isomerohydrolase (Bernstein and Rando, 1986; Bridges and Alvarez, 1987; Rando, 1991; Moiseyev et al., 2003). 11-*cis*-retinol is reduced by 11-*cis*-RDH and returned to the photoreceptor outer segment where it binds opsin to form a new rhodopsin molecule (Zimmerman et al., 1975).

Retinoid-binding proteins modulate the rates of retinoid transport between the photoreceptors and the RPE and are DHA-dependent (Bazan et al., 1985). All-*trans*-retinol is transported to and from the RPE by interphotoreceptor retinoid-binding protein (IRBP, Noy and Xu, 1990; Noy and Blaner, 1991; Pepperberg et al., 1993; Chen and Noy, 1994) whereas cellular retinaldehyde-binding protein (CRALBP) mediates transport within the RPE (Stecher et al., 1999; McBee et al., 2000). A membrane-dependent protein, RPE 65 (Tsilou et al., 1997), has also been implicated in presenting the retinoid to the isomerase (Mata et al., 2004). Fluorescence assays indicate that IRBP affinity for DHA is twice that of AA and three times that of LNA (Chen et al., 1993). DHA also appears to alter the IRBP affinity for other retinoids in the visual cycle (Chen et al., 1996). Chen et al. proposed that this effect arises from the concentration gradient of DHA between the photoreceptor outer segments and the RPE, and increases the efficacy of the transport of retinoids across the interphotoreceptor matrix.

Although it has been asserted that the rate-limiting component of visual pigment regeneration is the reduction of all-*trans*-retinal to all-*trans*-retinol in mice (Saari et al., 1998; Chen et al., 2005a), more recent data from human studies suggests that the delivery of 11-*cis*-retinal to the opsin limits the regeneration of rhodopsin (Schadel et al., 2003; Lamb and Pugh, 2004).

VIII. ASSAYING RETINAL BIOCHEMICAL RESPONSES

The ERG has been used as an objective measure of retinal dysfunction in omega-3 deficiency (Birch et al., 1992; Hood and Birch, 1994; Weisinger et al., 1996b; Jeffrey et al., 2002a; Malcolm et al., 2003;

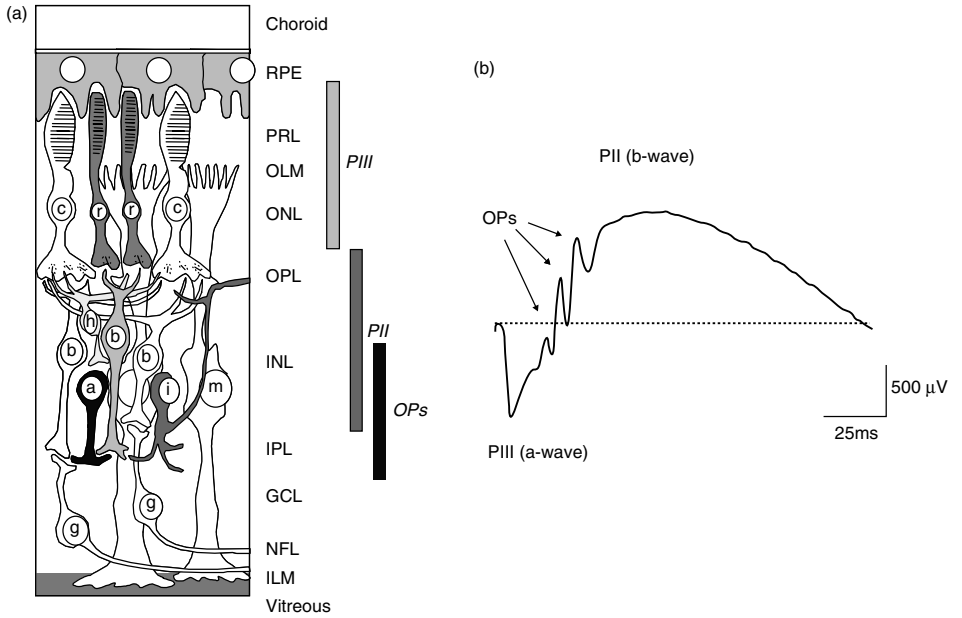

FIGURE 43.10 Neuronal derivations of ERG waveform components. (a) Schematic of retinal structure with the components defined in Figure 43.3. (b) Representative rat dark-adapted ERG waveform collected with a bright flash. Vertical bars identify layers of the retina that are thought to contribute to different waveforms of the ERG. See text for details.

Anderson et al., 2005). It quantifies the electrical activity of retinal neurons by recording changes to ionic gradients within the retina following light activation (Granit, 1933, 1968; Hood and Birch, 1990; Weisinger et al., 1996c). Unlike other objective (e.g., VEP) (Faldella et al., 1996; Birch et al., 1998; Makrides et al., 2000; Jeffrey et al., 2002b; Hoffman et al., 2003; Gillingham et al., 2005) and subjective (e.g., visual acuity) (Neuringer et al., 1984; Uauy et al., 1992; Auestad et al., 1997; Anderson et al., 2005) methods that have been used to assess the visual pathway following omega-3 deprivation, the ERG has the capacity to resolve retinal function independently of higher neural processing. This section details the extent to which component analysis of the ERG waveform affords localization of neuronal function within the retina. As for most studies, this review includes the early components (<500 ms) of the ERG, given that the later components are difficult to measure and reflect glial- or RPE-derived ionic fluxes.

Figure 43.10b shows a representative ERG from a dark-adapted rat. The major components have been labeled according to their order of appearance.

A. The a-Wave

Hyperpolarization of the photoreceptors consequent to the light-induced change in dark current (Figure 43.5) is detected as the corneal-negative a-wave of the ERG (Murakami and Kaneko, 1966; Penn and Hagins, 1969). It has a latency of 3–4 ms and reaches its maximum amplitude at an implicit time of 6–15 ms depending on stimulus intensity. The a-wave is only recordable at relatively bright light intensities when enough photoreceptors have been activated to produce an adequate signal. Due to relative rod-cone cell populations (Szel and Rohlich, 1992) as well as lower phototransduction amplification in cones (Tachibanaki et al., 2001), most of its magnitude is derived from the activity of rod photoreceptors (Fulton et al., 1995; Nixon et al., 2001). The leading edge of the a-wave, called the PIII, was so-named because it is the last (third) potential of the ERG to be suppressed under ether anesthesia (Granit, 1933). Later it became known as the fast PIII to differentiate

it from the slower, negative-going glial response, which is known as the slow PIII. The magnitude and gain of the fast PIII are dependent on stimulus intensity and the efficacy of the phototransduction cascade (Hood and Birch, 1990). This ultimately depends on the closure of cGMP-gated channels in the outer segment following phototransduction (Figure 43.5). The Hill equation describes the relationship between cGMP (cG) concentration and the fraction of open channels (Yau and Baylor, 1989), as shown in Equation 43.1.

$$F(t) = \left[\frac{\mathrm{cG}(t)}{\mathrm{cG}_{\mathrm{dark}}}\right]^{n} \tag{43.1}$$

Thus the circulating current at any time is normalized to the maximal dark current such that $F(t)$ is equivalent to the proportion of open cGMP-gated channels (n, the Hill coefficient, usually = 3). Lamb and Pugh (1992b) showed that this circulating dark current is a linear function of the rate of cGMP hydrolysis, which is the rate-limiting step in the phototransduction cascade. This can be used to specify the rising phase of the a-wave, $R(t)$, as a delayed Gaussian function of time (Equation 43.2).

$$R(t) = 1 - \exp\left[-\frac{1}{2}\Phi \mathrm{A}\left(t - t_{\mathrm{EFF}}\right)^{2}\right] \tag{43.2}$$

This equation models the rising phase of the a-wave as a function of the number of rhodopsin photoisomerizations, Φ, an amplification constant, A, and an effective time delay, t_{EFF} (Section VII C). The sources of amplification and some of the delay arise from the protein–protein interactions (Figure 43.7) during the cascade. The major sources of delay are associated with recording latencies, including the electrical time constant of the photoreceptor, the finite flash duration and electrical delays in the recording equipment (Lamb and Pugh, 1992b; Breton et al., 1994; Cideciyan and Jacobson, 1996).

The equivalent model of Hood and Birch (1990, Equation 43.3) expresses rod PIII amplitude as a function of intensity, i, rather than photoisomerizations, Φ, providing a more user-friendly formulary.

$$\mathrm{PIII}\ (i,t) \approx [1 - \exp\{-i \cdot S \cdot (t - t_{\mathrm{d}})^{2}\}] \cdot R_{\mathrm{m}_{\mathrm{PIII}}} \tag{43.3}$$

The summed rod photocurrent, PIII (μV), at any given time, t (seconds), is expressed as a fraction of the saturated response, $\mathrm{Rm}_{\mathrm{PIII}}$ (μV), stimulus luminous energy, i (log scot cd·s·m^{-2}), and a delay, t_{d} (seconds), scaled by sensitivity, S. Equations 43.2 and 43.3 are equivalent formulations that can be used to describe the rod a-wave.

At rod-saturating stimulus intensities, inaccuracies occur in describing the leading edge of the a-wave (Equation 43.2) due to the photoreceptor's capacitance having a greater influence on the recorded response (Cideciyan and Jacobson, 1996; Smith and Lamb, 1997). However, the application of the capacitive-corrected functions to dietary studies is limited by uncertainties in approximating their time constants across species and experimental manipulations.

Although the saturating exponential (Equation 43.2) has also been shown to describe the single cell recordings of cone photocurrents (Schnapf et al., 1990; Pugh and Lamb, 1993), Hood and Birch (1993) have proposed that cone phototransduction is better modeled by a Michelis–Menten function. They demonstrate that the waveform can be described by a modification of the Michelis–Menten that takes into account the longer capacitive time constant of the cone outer segment membrane (Hood and Birch, 1995) compared with the rod (Penn and Hagins, 1972). This finding is supported by others who have also incorporated terms for cone membrane capacitance in their models (Bush and Sieving, 1994; Cideciyan and Jacobson, 1996), although determining cone capacitance introduces a component of uncertainty, similar to that noted previously for the rod. Moreover, unlike the model

for rod phototransduction (Lamb and Pugh, 1992b), the Michelis-Menten has not been related to the processes of cone phototransduction, rendering its application less compelling for this purpose.

The relationship defined by Equation 43.3 has limited application. Increasing flash intensity increases a-wave amplitude where the maximum rate of rise (dR/dt_{MAX}) is a square-root function of intensity (Φ, e = 2.718, Equation 43.4, Lamb and Pugh, 1992b).

$$\frac{dR}{dt_{MAX}}(\Phi) = \sqrt{\frac{A\Phi}{e}} \tag{43.4}$$

This ceiling in a-wave saturation is caused by decreasing concentrations of cGMP being available for hydrolysis (Lamb and Pugh, 1992b). Decreased amplification might also produce a finite output of the phototransduction cascade at very high intensities. On the other hand, low stimulus intensities promote other processes that force the relation described in Equation 43.3 to deviate from the physiological response (Jamison et al., 2001). This involves intrusion from inner retinal neurons summating with photoreceptoral responses at the leading edge of the a-wave (Frishman and Steinberg, 1989).

It has been reported that postreceptoral intrusion of the photoreceptoral response is eliminated through the use of intensities eliciting a maximal PIII response within an implicit time of 14 ms (Jamison et al., 2001). Intraretinal recordings in the cat retina indicate that for intensities which saturate the amplitude of the a-wave, the implicit time for maximal photoreceptor activity is 8 ms, with partial recovery occurring before any postreceptoral intrusion (Kang Derwent and Linsenmeier, 2001). Consequently, some investigators quantify a-waves at a criterion time to avoid the effects of intrusion by these factors. Here the time is chosen such that it occurs before any postreceptoral intrusion and before the maximal response (~8 ms) is achieved.

B. The b-Wave

This corneal-positive component can be detected in response to low stimulus intensities due to amplification of the photoreceptoral response (see previous section). As its implicit time (60–120 ms) is later than that of the a-wave, it has been called the b-wave (Figure 43.10). Its neural generator is called the PII and reflects bipolar cell depolarization (Popova, 2003; Bramblett et al., 2004; Hanitzsch et al., 2004). Interaction between the fast PIII (a-wave), slow PIII and the PII forms the corneal-positive b-wave of the ERG (Robson and Frishman, 1995; Hood and Birch, 1996).

Traditionally, the b-wave was thought to arise from potassium fluxes across Müller cells (Hanitzsch, 1981; Yanagida, 1982; Dick and Miller, 1985; Frishman et al., 1988; Wen and Oakley, 1990; Katz et al., 1992), however a recent study found elimination of Müller cell potassium channels had no effect on the recorded b-wave (Kofuji et al., 2000) indicating that this signal arose directly from bipolar cells. Müller cells were shown to be involved in generating the slow PIII. Hyperpolarization of photoreceptors leads to a slow reduction of potassium ions in the extracellular space due to the continued activity of the Na^+/K^+-ATPase (Wurziger et al., 2001; Dhingra et al., 2002). This reduction in potassium is known as the "distal decrease" and it creates Müller cell currents that produce a corneal-negative slow PIII of the ERG.

C. Oscillatory Potentials

Under mesopic and photopic conditions, rapid oscillations (Figure 43.10) may be recorded on the ascending limb of the b-wave (Wachtmeister and Dowling, 1978; Dong et al., 2004). It has been proposed that oscillatory potentials (OPs) arise from inhibitory feedback currents generated in the inner retinal layers by amacrine, interplexiform, and ganglion cells (Wachtmeister and Dowling, 1978; Heynen et al., 1985). They have been isolated by band-pass filtering the conditioned waveform, where the a- and b-wave rising slope are removed using direct subtraction (Bui et al., 2002) or windowing in the time domain (Derr et al., 2002).

IX. RETINAL DYSFUNCTION IN OMEGA-3 DEFICIENCY

Defining the nature of retinal dysfunction following omega-3 deficiency is imperative for determining the mechanisms through which these lipids modulate neural function. The following sections outline evidence for omega-3 deficiency leading to functional deficits of specific retinal neurons.

A. Rhodopsin Activation

Studies in isolated membranes provide evidence for rhodopsin activation being dependent on the surrounding membrane composition (Mitchell et al., 1992; Litman and Mitchell, 1996; Niu and Mitchell, 2005). Brown (1994) reported that metarhodopsin II formation was facilitated by smaller phospholipid head groups and bulky membrane acyl chains. More recently, the rate and extent of metarhodopsin II formation has been directly related to membrane DHA content (Litman et al., 2001; Mitchell et al., 2003; Niu et al., 2004).

The earliest subcomponent of an ERG waveform in response to a bright stimulus is the early receptor potential (ERP) (not shown in Figure 43.10b). It was first reported by Brown and Murakami (1964) and requires a bright stimulus of short (<1 ms) duration and high sampling rates for detection (Cone, 1964; Sieving et al., 1978). It has been proposed that, on photon capture, the conformational changes involved in the thermal conversions of lumirhodopsin to metarhodopsin I, and metarhodopsin I to metarhodopsin II (Figure 43.6) result in charge displacement about the photopigment in the photoreceptor outer segment disks that gives rise to the corneal-positive (R_1) and corneal-negative (R_2) waveforms of the ERP (Brindley and Gardner-Medwin, 1966; Wald, 1968; Brown and Coles, 1979).

Although there has been little physiological investigation of the potential effects of omega-3 deficiency on the ERP, it is likely that the conformational change leading to charge displacement will be affected by altered membrane acyl chain packing. Vingrys et al. (2001) have reported a diet effect on the ERP on a limited sample of rats. One of the difficulties with interpreting the findings of the data given by Vingrys et al. (2001) is that they were undersampled and fail to properly define the ERP. This is a challenge for ERP recordings, as is the intrusion of the photovoltaic artefact; nevertheless, this limited dataset suggests that this waveform can be severely affected by omega-3 deficiency.

B. The Phototransduction Cascade

Benolken et al. (1973) were the first to report that omega-3 fatty acids could alter the ERG. Since then, other groups have evaluated the effect with divergent outcomes as to the nature of the functional changes. Although much of this work has involved relatively short term (<1 year), substantial dietary manipulation, it is taken as proof of the importance of omega-3 deficiency in humans. Other mechanisms that may be relevant to the chronic, low level of deprivation typical of human nutrition will be described later.

Evidence that omega-3 deficiency leads to a-wave changes differs across studies and possibly reflects the low experimental power of the small samples used in most of these works, an issue to be detailed later. For example, although a 58% decrement in retinal DHA induced reductions in Rm_{PIII} in guinea pigs (Weisinger et al., 1996a,b, 1999) no changes in Rm_{PIII} have been noted in adult monkeys following either deprivation (–10% red blood cell DHA, Jeffrey et al., 2002a) or supplementation (3 times higher plasma DHA, Jeffrey et al., 2002b) of omega-3 PUFA. Alternatively, this discrepancy might reflect the (adult) age of dietary intervention in these aforementioned studies. Intervention commenced at weaning that induced a 50% reduction in retinal DHA did lead to diminished rod and cone a-wave amplitudes in 3–4 month-old monkeys (Neuringer et al., 1991). Nevertheless, these functional anomalies were noted to resolve by 2 years of age.

The age of initiation of dietary intervention is obviously important in eliciting changes, with the largest and most consistent effects being reported in those studies that commenced dietary modification at weaning or during pregnancy. Weisinger et al. (1999) report a dose–response relationship

between retinal DHA content and PIII sensitivity in 16-week-old guinea pigs when the dietary modification commenced at weaning. In this study, sensitivity increased as a saturating function of retinal DHA. This contrasted with the finding for Rm_{PIII}, where a relative reduction in retinal DHA of –26% elicited maximal Rm_{PIII} reduction (–27%) and further DHA depletion did not cause any additional functional loss. It is also interesting to note that, whereas Rm_{PIII} was reduced at 6 weeks of age, sensitivity was normal at this time. The fact that the pattern and age of the loss due to retinal DHA depletion was different for PIII sensitivity and amplitude implies the presence of more than one mechanism underlying the functional anomalies measured by these two parameters. As detailed earlier, this is not surprising given that the Rm_{PIII} reflects the total dark current circulating about the rod outer segments and it can be affected by anatomical factors as well as the function of ionic pumps, such as the Na^+/K^+-ATPase (Figure 43.5), whereas the sensitivity parameter is independent of these factors and reflects the efficacy of the transduction cascade (Figure 43.7).

Rod sensitivity was reduced in rats that had been deprived of omega-3 over three generations (–55% retinal DHA, Weisinger et al., 2002), although this has not been replicated in monkeys with dietary intervention implemented from birth (–71% plasma DHA, Jeffrey et al., 2002b) or from maternal prefeeding (–97% plasma DHA, Jeffrey et al., 2002a).

There is no *in vivo* evidence for the *in vitro* prediction that omega-3 PUFA deficiency increases a-wave latency. In many cases, previous analyses were not appropriate for detecting such change as the investigators adopted the standard approach of fixing the delay parameter of phototransduction (t_d, Equation 43.3) to normal values (Weisinger et al., 1996a, 1999; Christensen et al., 1998). Nevertheless, studies in monkeys specifically considered the possibility of changes to the delay in omega-3 deficiency, with none being found (Jeffrey et al., 2002a). Instead, the membrane capacitance for rods was found to be decreased in PUFA- (DHA and AA) supplemented monkeys (Jeffrey et al., 2002b). Given that a change in capacitance would affect signal timing, this finding is difficult to interpret in the presence of normal implicit times for the later ERG components. However, a-wave implicit times were delayed in cats (Pawlosky et al., 1997) and monkeys (Jeffrey et al., 2002a) that had been deprived of omega-3 through maternal prefeeding.

Weisinger et al. (1999) report reduced activity within the cone pathway in omega-3 deprived guinea pigs although they did not specifically consider the phototransduction cascade of the cone photoreceptors. Conversely, Jeffrey et al. (2002b) report no effect on cone phototransduction in omega-3 supplemented monkeys. These findings lead to several possible interpretations. Given that the cone a-wave is small, a negative finding might reflect the reduced power of small sample studies, an issue to be developed later. Alternatively, the disparate results between studies might arise from a species-specific effect. A third possibility is that photoreceptor function is indeed normal, and that the effect that Weisinger et al. (1999) observed reflects postreceptoral anomalies.

Many of the discrepancies between the outcomes of studies restricting dietary omega-3 PUFA intake may reflect insufficient experimental power for detecting functional anomalies *in vivo*. Studies cited earlier included up to 10 monkeys, 5 cats, 12 guinea pigs and 6 rats per diet group. Later, it will be shown that these numbers are too small to provide an experimental power that will reliably detect the levels of change predicted from *in vitro* studies.

X. OMEGA-3 DEFICIENCY REDUCES BIPOLAR CELL ACTIVITY

A reduction in PII amplitude has been reported in omega-3 deficient guinea pigs (Weisinger et al., 1999) although diet does not appear to affect b-wave amplitude in monkeys (Jeffrey et al., 2002a,b; Anderson et al., 2005) or rats (Leat et al., 1986; Weisinger et al., 2002). Interestingly, Acar et al. (2002) report that dietary intake of *trans* omega-3 decreases b-wave amplitude in rats. Studies in rhesus monkeys revealed delayed b-wave implicit times in animals fed LNA compared with those on diets supplemented with DHA (Neuringer and Connor, 1986; Neuringer et al., 1991; Jeffrey et al., 2002b). Similar findings have been reported in rats (Weisinger et al., 2002) and humans (Birch et al., 1992; Leaf et al., 1996) but not in guinea pigs (Weisinger et al., 1999). Given the serial nature of the

ERG generators, a loss of a-wave amplitude should produce a reduced b-wave, whereas decreased a-wave sensitivity should result in delayed b-wave timing (Hood and Birch, 1992).

Cone bipolar cell activity has not been thoroughly assessed in omega-3 deficiency. Reduced cone trough-to-peak amplitudes have been reported in guinea pigs (Weisinger et al., 1999). Human infant studies suggest that omega-3 deficiency leads to delays in cone b-wave implicit time (Birch et al., 1992) in the presence of normal amplitudes (Uauy et al., 1990), consistent with effects sequential to a-wave sensitivity losses (Hood and Birch, 1992). Herein lies one of the shortcomings of much of this early work in that many investigators have not considered to what extent postreceptoral changes directly reflect downstream a-wave deficits, given the serial processing of the ERG signal. This is particularly germane for b-wave implicit times as Hood and Birch (1992) have demonstrated that abnormal a-wave sensitivity can delay b-waves. The literature has interpreted b-wave changes following dietary manipulation as either being serial to photoreceptoral losses in humans (Birch et al., 1992) and monkeys (Jeffrey et al., 2002b) or as being additive for the b-wave and OP amplitudes of guinea pigs (Weisinger et al., 1999). Delayed OPs were found in human infants fed a formula of comparatively lower omega-3 content from 10 days of age than in infants fed breast milk (Birch et al., 1992). Smaller OPs were also reported in omega-3 deficient guinea pigs (Weisinger et al., 1999) although a detailed evaluation of the nature of diet-mediated deficits to OPs has never been conducted.

Apart from these limited studies, there has been little other consideration of the effect that omega-3 PUFA has in modulating the activity of inner retinal neurons. This lack of information is rather surprising, as it could be argued that the inner retinal response would more closely mirror that of the cortex or of other neurons, in contrast to the specialized response of the photoreceptor.

XI. ADAPTATION AND SLOWED ROD RECOVERY

Given the role that DHA can have in modulating deactivation of the photoresponse (Section VII E), it is, perhaps, not surprising that Neuringer et al. (1986, 1991) and Jeffrey et al. (2002a) have reported that rod recovery from a saturated flash takes longer in omega-3 deficient monkeys. Monkeys raised on an omega-3 deficient diet reveal delays in both rod recovery (Jeffrey et al., 2002a) and dark adaptation (Neuringer et al., 1986). Analysis of the kinetics of rod recovery ($n = 7$) found abnormal rates of recovery in the presence of normal delays to onset (Jeffrey et al., 2002a). Similar findings have been reported in rats (Weymouth et al., 2005).

The delays in recovery are consistent with the decreased number of RPE phagosomes noted in omega-3 deficient rodents (Watanabe et al., 1987; Bush et al., 1991). Retinoid transport (Chen and Anderson, 1993; Chen et al., 1996) and isomerization (Rando, 1991) are also delayed with reduced DHA concentration. In particular, chromophore isomerization in the RPE is dependent on membrane lipid constituents (Canada et al., 1990). These factors have implications for chromophore recycling and would be expected to delay the dark adaptation of omega-3 deficient animals, a contention supported by the finding of slower regeneration of rhodopsin in DHA deficiency (Bush et al., 1991, 1994).

XII. PROPOSED MECHANISMS UNDERLYING FUNCTIONAL LOSSES

The nature of retinal dysfunction following omega-3 deficiency presented in the previous section reveals discrepancies between and within species, sometimes yielding divergent outcomes from the same laboratory. These *in vivo* findings have hindered the ability to identify potential mechanisms that underlie omega-3-mediated changes in neural physiology. In addition, the variety of outcomes does not concur with the systematic, directed predictions of *in vitro* methods, as detailed elsewhere in this book. The following sections summarize the proposed mechanism/s underlying retinal protein dysfunction determined from *in vitro* studies, and discuss possible reasons why *in vivo* findings do not fully support these predictions.

A. Membrane Biophysical Properties Modulate Retinal Protein Function

Biophysical properties of membranes are modulated by omega-3 content. Membrane lipid constituents are known to affect retinal protein activity (Litman and Mitchell, 1996; Litman et al., 2001; Mitchell et al., 2001; Niu et al., 2001). Studies in isolated membrane preparations propose that the changes to protein function in omega-3 deficiency directly reflect altered membrane biophysical properties (Wiedmann et al., 1988; Brown, 1994; Mitchell et al., 2001; Wu et al., 2001; Turner et al., 2003, 2005b; Niu et al., 2004).

The extent of formation of metarhodopsin II is modulated by the degree of unsaturation of retinal phospholipid (PC) fatty acids such that DHA-rich bilayers support the highest levels of MII formation (Litman and Mitchell, 1996; Litman et al., 2001). Furthermore, DHA-rich membranes reduce the inhibitory effect of cholesterol on rhodopsin activation (Litman and Mitchell, 1996). The degree of PC unsaturation is related in a similar way to the kinetics of formation of the activated rhodopsin–transducin complex (Mitchell et al., 2001; Niu et al., 2001). PDE activity reflects the combined result of both the extent of production of MII and the rate and extent of its subsequent formation of a complex with transducin. PDE activity is greater in DHA-rich bilayers than in more saturated membranes (Litman et al., 2001; Mitchell et al., 2003).

The unique ability of the retinal lipids to form dipolyenes is thought to result in the increased rate of rhodopsin activation (formation of metarhodopsin II) compared with a monoene structure (Mitchell et al., 1992; Brown, 1994; Litman and Mitchell, 1996). This may be a direct consequence of higher membrane concentrations of dipolyenes resulting in more rapid protein diffusion within a more fluid membrane (Mitchell et al., 1992, 2001; Brown, 1994; Litman and Mitchell, 1996; Booth et al., 1997; Litman et al., 2001; Niu et al., 2001). Alternatively, membrane incorporation of dipolyenes may alter the lipid microdomain surrounding rhodopsin, affecting the activity of this protein within its local environment (Albert and Yeagle, 1983; Wiedmann et al., 1988; Polozova and Litman, 2000; Simons and Toomre, 2000). Studies in reconstituted membranes reveal that rhodopsin is surrounded by di22:6-PC whereas the lipid composition surrounding cholesterol is rich in di16:0-PC (Polozova and Litman, 2000). This specificity in local lipid distribution may lead to increased efficacy in protein activation and interaction.

It is possible that DHA affects the protein–lipid interactions associated with receptor-mediated intercellular communication. DHA is highly enriched in aminophospholipids, especially PS, in neuronal membranes (Salem et al., 1980; Stinson et al., 1991a; Farquharson et al., 1995; Suh et al., 1996). PS content may affect cellular function through its influence on the activity of many signaling proteins, including the protein kinases (Mosior and Newton, 1998), modulation of gene transcription proteins and modification of hormone receptor activity within the cell nucleus (Lin et al., 1999; Kim et al., 2000, 2001). Although there is no direct evidence, it may also affect the G-protein cascade of phototransduction. The following section considers the membrane-dependent aspects of DHA modification.

Niu et al. (2001) propose that the lag and decreased amplitude and sensitivity of the leading edge of the a-wave in DHA deficiency is a consequence of the slower kinetics and reduced amplification associated with transducin activation during phototransduction. They also assert that other functional losses related to DHA deficiency may have a common underlying mechanism: decreased efficacy in G-protein signaling. Such a prospect is inconsistent with the observation made by Weisinger et al. (1999) for a different time course in the onset of rod sensitivity and Rm_{PIII} losses, suggesting multiple etiologies.

At a gross, anatomical level, the rate of rod outer segment disk synthesis (Wiegand et al., 1991), the number and morphology of photoreceptors (Benolken et al., 1973; Bush et al., 1991; Wiegand et al., 1991) and the width of the outer nuclear layer (Wiegand et al., 1991) remain unaltered in omega-3 deficient rats. Given these observations, it is unclear why the *in vivo* studies fail to support the findings of isolated membranes. It has been shown that the negative finding of Leat et al. (1986)

arose from the inadequate experimental power of that study (Weisinger et al., 1996a; Sinclair et al., 1997). The following section will consider in detail the importance of experimental power in designing *in vivo* studies.

B. Relating *In vitro* Predictions To *In vivo* Findings: Experimental Power

The advantage of *in vitro* studies compared with equivalent *in vivo* experiments is that noise tends to be lower and easier to control. Whereas *in vivo* studies incorporate variation between animals as well as within animal noise, intersample variation *in vitro* tends to be relatively lower and accounted for more readily. The following discussion defines the expected *effect size* from *in vitro* observations in order to determine appropriate sample sizes given the noise associated with signal acquisition *in vivo*.

The study by Niu et al. (2004) in isolated rod outer segment membranes, proposes that the changes in activation of proteins involved in phototransduction are a direct consequence of membrane biophysical properties being altered by DHA concentrations. In that study, weaning female rats were maintained on omega-3 sufficient or deficient diets then mated at 11 weeks of age. Second generation pups were sacrificed at 3 weeks of age to assess phototransduction protein activity and phospholipid acyl chain content in isolated rod outer segment membranes. There was no effect of diet on the ratio of rhodopsin to membrane phospholipids, and equivalent membrane protein concentration was ensured by removing all transducin and PDE and returning known aliquots of bovine counterparts to the isolated membranes of both diet groups. An average relative reduction of 78% in the retinal phospholipid DHA of omega-3 deprived rats was achieved. Niu et al. (2004) report that this induced a 23% reduction in the concentration of activated transducin relative to the omega-3 sufficient group. They also report timing delays by way of a 38% decrease in the rate of formation of activated transducin, a 16% delay in coupling of activated rhodopsin with transducin, and a 38% increase in the time constant of formation of activated transducin.

The magnitudes of the diet effect reported by Niu et al. (2004) *in vitro* predict a 23% reduction in Rm_{PIII}, a 16%–38% decrease in sensitivity and a 38% delay in t_d due to omega-3 deficiency. Given these expected changes, Table 43.1 lists the sample sizes required for an experimental power of 80% for different levels of experimental noise and separations between group means. The range of coefficients of variation (% variability) shown in Table 43.1 that encompass those typical of ERG recordings are shown in bold typeface.

The variability of ERG parameters is dependent on the test method, the parameter in question and the number of repeated observations. Average (± SEM, $n = 10$) interanimal variability for individual measures of trough-to-peak amplitude is 14% ± 6% and taking 10 signal repeats per animal reduces this coefficient of variation to 4% ± 1%. The smaller magnitudes of the rod a-wave response

TABLE 43.1
Sample Sizes for Two-Tailed *t*-Test with 80% Power

	Difference Between Means (%)				
% Variability	**18**	**23**	28	38	48
15	12	8	6	<5	<5
20	**20**	14	9	6	<5
25	30	**19**	13	8	6
30	45	28	19	11	8
40	>50	50	33	19	12
50	>50	>50	50	28	18

at 6 ms criterion time (A_6) demonstrate 19% ± 6% variability for $n = 10$ and taking 10 repeats per animal reduces this to 5% ± 1%. Table 43.1 shows that a sample size of 20 rats is sufficient to detect either an 18% difference between means given 20% variability in the data, or a 23% change in means (as predicted by Niu et al., 2004) with a more conservative estimate of 25% variability in the data. It is evident that large sample sizes and many signal repeats are both required to reduce variability in order to detect the magnitudes of diet effect predicted by *in vitro* membrane observations; neither of which has been achieved by past *in vivo* studies.

This analysis indicates that previous *in vivo* studies have had insufficient experimental power to expose the functional anomalies predicted from *in vitro* experiments (Table 43.1). Not surprisingly, *in vivo* studies of retinal function in omega-3 deprived animals do not fully concur with the findings of Niu et al. (2004). Whereas Weisinger et al. reported reductions in phototransduction amplitude in guinea pigs ($n = 12$ per diet, 1996a,b) and sensitivity in rats ($n = 6$ per diet, 2002), Jeffrey et al. (2002b) found a decrease in the membrane capacitance time constant for phototransduction in rods but no change in rod amplitude, sensitivity or cone phototransduction in long-chain PUFA-(DHA and AA) supplemented monkeys ($n = 10$ per diet, Jeffrey et al., 2002b). Elsewhere, these authors report no difference in any rod phototransduction parameters between omega-3 sufficient and deficient monkeys ($n = 7$ per diet, Jeffrey et al., 2002a).

Some discrepancies between *in vivo* and *in vitro* studies of omega-3 deficiency may reflect factors other than insufficient experimental power, especially considering the chronic nature of dietary insufficiency in humans. As discussed in the following sections, these possibilities might also explain why some species or ages are more susceptible to deprivation compared with others. In combination with altered membrane biophysical properties, they may prove sufficient to account for the age-related (Weisinger et al., 1999) and development-dependent (Armitage et al., 1999, 2005) anomalies in retinal function observed *in vivo*.

C. Does Omega-3 Modulate Retinal Protein Content?

Studies that have considered the relationship between total retinal rhodopsin and DHA content are inconclusive. When compared with rats fed LNA as the sole omega-3 fatty acid, rats fed omega-3 deficient diets were found to have higher levels of rhodopsin (Bush et al., 1991, 1994). However, the more recent work of Niu et al. (2004) failed to detect any diet-mediated difference in rhodopsin content in the presence of a 78% reduction in DHA.

Niu and Mitchell (2005) report that rhodopsin is most active in membranes with lower concentrations of rhodopsin, reinforcing the importance of protein motility within the membrane. Reduced local membrane DHA content has also been associated with decreased sensitivity for rhodopsin for light capture (Wiedmann et al., 1988; Brown, 1994; Litman and Mitchell, 1996).

The effect that omega-3 PUFA have on the concentration of retinal proteins requires further investigation and should provide insight into understanding the etiology of functional anomalies in omega-3 deficiency.

D. Does Omega-3 Modulate Protein Expression?

The variable nature of retinal dysfunction reported in *in vivo* studies may reflect multiple lesions underlying the attenuation of retinal protein function following omega-3 deficiency. The complex etiology of functional losses following omega-3 deprivation is also demonstrated in repletion studies where dysfunction is sustained despite normalization of retinal DHA profiles (Armitage et al., 1999; Anderson et al., 2005). It is possible that the increased rhodopsin concentration reported in omega-3 deficient rats (Bush et al., 1991, 1994) may reflect upregulation of protein expression. DHA has been implicated in modulating the extent of expression of genes regulating hepatic (Clarke and Jump, 1994; Price et al., 2000; Arachchige et al., 2006) and retinal fatty acid metabolism (Puskas et al., 2004). Rojas et al. (2003) reported a tendency for general retinal gene expression to be upregulated by omega-3 polyunsaturates and downregulated by omega-6 PUFA. The prospect that DHA

modulates the extent or nature of subunit expression of phototransduction proteins requires evaluation in order to define the nature of the deficit.

E. Does Omega-3 Modulate Ion Channel Proteins?

In response to a stimulus, membrane ion channels undergo conformational modifications within the membrane bilayer in order to increase or decrease their affinity for their substrate ion, hereby transporting the charged molecule across the hydrophobic barrier. This conformational change may be susceptible to the nature of the lipid species within the membrane bilayer. Alternatively, the membrane lipid constituents may alter the affinity of the ion pore for its substrate ion.

DHA has been shown to affect the potassium (Poling et al., 1995, 1996), sodium (Xiao et al., 1995; Kang and Leaf, 1996; Vreugdenhil et al., 1996; Young et al., 2000) and calcium (Kang and Leaf, 1996; Vreugdenhil et al., 1996) ion channels in neural membranes. Ehringer et al. (1990) demonstrated that DHA is more effective than LNA in enhancing membrane permeability. DHA and EPA reduce electrical excitability in single cell recordings of hippocampal neurons (Xiao and Li, 1999).

Ionic gradients across the rod outer segment membrane are mediated by the states of voltage- and cGMP-gated ion channels (Figure 43.5). Any change in structure or activity of these channels alters the dark current. The subsequent effects on phototransduction would manifest as a change in outer segment ion gradients as measured using electroretinography.

It has been well-documented that the activity of the Na^+/K^+-ATPase pump is dependent on the composition and biophysical properties of its surrounding membrane (Else et al., 1996, 2003; Else and Wu, 1999; Wu et al., 2001; Turner et al., 2003, 2005a–c). Given the ubiquity of these transporters throughout the body, this discussion has widespread implications. Dietary deficiency of long-chain omega-3 PUFA leads to decreased Na^+/K^+-ATPase activity in hepatic (Sennoune et al., 1999) and neural membranes (Bourre et al., 1989; Gerbi et al., 1993, 1994, 1997, 1998, 1999; Bowen and Clandinin, 2002). Gerbi et al. (1997, 1998, 1999) assert that the extent of Na^+/K^+-ATPase activity may reflect omega-3-mediated changes subsequent to preferential expression of the alpha subunits. These findings have implications for the photoreceptoral Na^+/K^+-ATPase in terms of its capacity to maintain the dark current. Despite the multiplicity of studies in other tissues, it is unknown whether or not retinal Na^+/K^+-ATPase activity is modulated by dietary omega-3 intake.

A role for the interaction of native DHA not associated with the membrane has also been reported. The activity of voltage-gated sodium and calcium ion channels in rat cardiomyocytes and hippocampal neurons is suppressed by DHA in its free fatty acid form (Leaf, 1995; Vreugdenhil et al., 1996). If this were to extend to the retina, it would suggest the potential for a complex functional outcome in terms of ERG waveforms.

XIII. SUMMARY

A balance of dietary intake of essential fatty acids is vital in maintaining healthy neural function. Omega-3 PUFA have been linked with many ocular disorders including dry eye, age-related macular degeneration, glaucoma and diabetic retinopathy. In many of these cases, it is possible that the dysfunction reflects complex changes in eicosanoid metabolism. However, omega-3 PUFA can also modulate the biophysical properties of membranes and are particularly concentrated in and retained by retinal membranes. Past work has identified anomalies within the phototransduction cascade and visual pigment processing as a consequence of dietary deprivation of omega-3 fatty acids. Studies assessing protein activity in isolated membranes have led to the assertion that lipid-mediated changes in the biophysical properties of the membrane give rise to the attenuation of protein function. It has been proposed that insufficient levels of omega-3 PUFA lead to greater membrane rigidity, hindering the function of membrane-bound proteins. If changes to membrane acyl chain packing were the sole reason for the protein dysfunction, a generalized susceptibility of all membrane-bound proteins to their lipid environment should ensue. This predicted effect is not consistent with the variety of aspects

of aberrant phototransduction that has been reported *in vivo* in humans and animals. This might reflect insufficient experimental power, or factors other than changes to membrane biophysical properties, that are responsible for the functional deficits. Future studies that address these possibilities will further our understanding of the multifaceted modulation of retinal function by omega-3 PUFA.

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44 Fatty Acids and Cardiovascular Disease

Geza Bruckner

CONTENTS

I. INTRODUCTION

Cardiovascular disease (CVD) remains one of the most significant diet-related health problems in the United States and is also the leading cause of death in the United States. Heart disease and stroke, the principal components of CVD, are the first and third leading causes of death in the United States, accounting for nearly 40% of all deaths. Nearly 930,000 Americans die of CVDs each year, which amounts to one death every 33 s. About 70 million Americans (almost one-fourth of the population) have some form of CVD, which is responsible for more than 6 million hospitalizations each year (American Heart Association, 2005). Much of the burden of heart disease and stroke could be eliminated by reducing major risk factors associated with the disease: high blood pressure, high blood cholesterol, tobacco use, diabetes, physical inactivity, and poor nutrition. About 90% of middle-aged Americans will develop high blood pressure in their lifetime, and nearly 70% of those who have it now do not have it under control (American Heart Association, 2003). In 2002, more than 106 million people were told that they had total blood cholesterol levels that were above normal or high (≥200 mg/dL or higher) (American Heart Association, 2005).

Estimated cost for health care related to CVD increased from $128 billion in 1994 to $151 billion in 1996 (Stone, 1996). However, the cost has increased exponentially and in 2006 is estimated to be $403 billion, according to the American Heart Association and the National Heart, Lung, and

Blood Institute (NHLBI). This amount includes both direct and indirect costs. Direct costs include the cost of physicians and other professionals, hospital and nursing home services, the cost of medications, home health care, and other medical durables. Indirect costs include lost productivity that results from illness and death. During the past decade a great deal of progress has been made toward defining the role of dietary fats and hypercholesterolemia in the pathogenesis of atherosclerosis and other vascular disease. Our enhanced knowledge along with more public nutrition education may be related to the observed steady decline in morbidity and mortality from CVD during recent decades (Steyn et al., 1997) (see Figures 44.1 and 44.2). Since 1964, coronary heart disease mortality has declined steadily, by more than 32% overall (Higgins and Lefant, 1989; Ginter, 1997). However, not all populations have experienced declines, for example, there has been a significant increase in CVD in American Indians (Ellis and Campos-Outcalt, 1994).

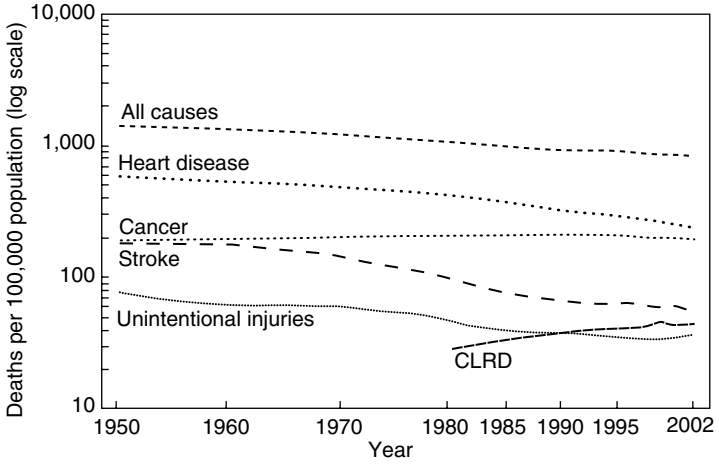

FIGURE 44.1 Leading causes of death for all ages. (*Source:* Centers for disease control and prevention, national center for health statistics, *Health, United States, 2005*, Figure 29.)

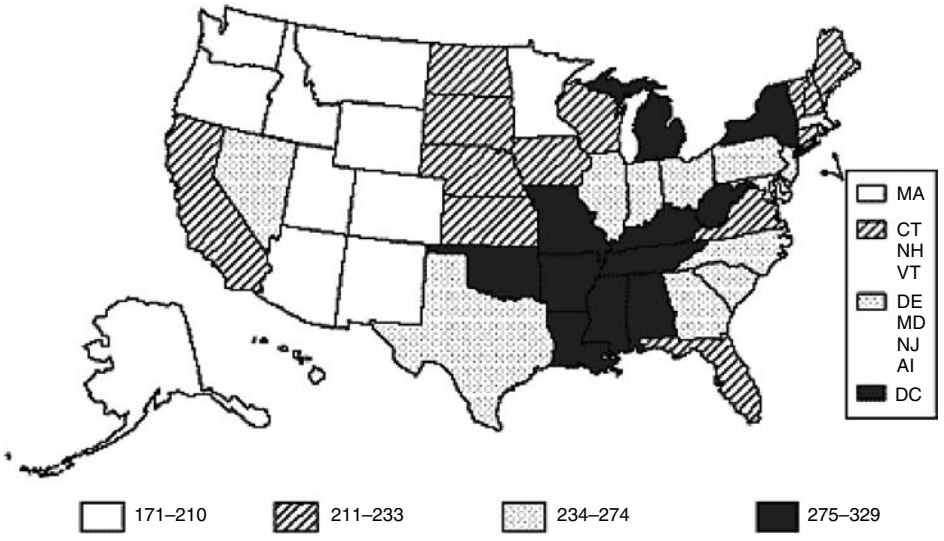

FIGURE 44.2 Rates of death due to diseases of the heart, 2001. Deaths per 100,000, age adjusted to 2000 total U.S. population. From the CDC, 2006.

CVD is multifaceted, and the course of its pathology is influenced by physiology, genetics, environment, and psychosocial stresses. However, the multifaceted nature of CVD has recently been questioned and it has been suggested that the true cause may be solely due to various lipid peroxidation reactions (Spiteller, 2005).

Major identified risk factors for CVD include cigarette smoking, elevated blood pressure, elevated plasma cholesterol, obesity, diabetes, and elevated plasma triglycerides. The single risk factor that has received the most attention is cholesterol; however, the mechanisms involved and the interactions between serum cholesterol and other factors such as sources of dietary protein, amounts and types of dietary fatty acids, and antioxidants are not clearly understood (Gaziano and Manson, 1996). During recent years, we have made great progress toward understanding the interrelationship between these variables; however, to define the exact mechanisms will require further investigative efforts. For example, it is known that different fatty acid isomers, n6 vs. n3, 20-carbon fatty acids, can elicit completely different vascular responses as evidenced by the Greenland Eskimo studies and subsequent reports by Bang and Dyerberg and others (Dyerberg and Bang, 1978; Bang and Dyerberg, 1981; Hornstra, 1989a).

Work by Grundy (1986), Daumerie (1992), and Woollet (1994) and epidemiological studies (Aravanis and Ionnidis, 1984; Caggiula and Mustad, 1997) also suggest that specific saturated and monounsaturated fatty acids (MUFAs), which contain *cis* or *trans* isomers (Aro, 1997), can have different serum cholesterol-lowering effects. Although it is well documented that serum cholesterol levels in a given population are closely correlated with increased risk for atherogenesis, there still remains skepticism regarding the relationship between dietary cholesterol intake and serum cholesterol concentrations (James et al., 1989; Hayes, 1995; McNamara, 1995). Analysis of the results of 30 years of studies related to cholesterol feeding indicate that for the majority of individuals modest changes in dietary cholesterol have little if any effect on plasma cholesterol levels (McNamara, 1995). Other factors such as the type and amount of fat consumed play a more regulatory role in influencing serum cholesterol (McNamara, 1987; Kris-Etherton and Yu, 1997; Mozaffarian et al., 2006).

Although there is general agreement that reducing the intake of cholesterol, saturated fat, and *trans* fatty acids would be beneficial, there still remains controversy as to what constitutes more beneficial dietary fats. For example, it has been demonstrated in both epidemiological and controlled studies that the incidence of CVD can be quite varied given the same serum cholesterol concentrations (Keys et al., 1980). Furthermore, a study by Wenxun et al. (1990) indicates that geographical differences in CVD mortality within China are caused by factors other than dietary or plasma cholesterol. A number of studies have shown that n3 fatty acids when consumed with equal, or even greater, amounts of dietary cholesterol, compared to an n6 diet, resulted in decreased platelet aggregability and a lower incidence of atherogenesis (Hornstra, 1989a). Other findings have generated even greater controversy because of clear contradictions to long-standing American Heart Association dietary recommendations. Notably the indication that lipid oxidation products may play an important role in the pathogenesis of CVD has raised many questions regarding the exact role of dietary lipids in the disease process (Spiteller, 2005). Animal, human, and epidemiological studies make it clear that the linkage between diet and serum cholesterol is still poorly understood. Questions still remain on issues such as (1) why is there a greater risk for CVD among men than among women?; (2) why is there an enhanced CVD risk in women after menopause?; (3) why is there lower CVD mortality in some countries, for example, France, Greenland, Greece, compared to the United States despite substantial cholesterol and fat intakes?; and (4) why do male U.S. railroad workers compared to a Mediterranean male population have twice the number of deaths given the same serum cholesterol levels? (Keys et al., 1980). What are the underlying causes that result in these intriguing exceptions to the long-held dogma that elevated serum lipids is the most significant statistically associated risk factor for CVD? Could lipid peroxidation and oxidative stress be the common link? The majority of CVD deaths in the total population occur in people with low serum lipids, and not all individuals with high serum lipids develop CVD. Although knowing the relationship between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) ratios has

increased our understanding of risk factors, it is clear that these lipoproteins do not fully explain how atherogenesis truly develops. Could lipid peroxidation and oxidative stress be the common link?

The purpose of this chapter is to give an overview of how dietary fatty acids may influence the development of CVD through multifaceted and "lipid peroxidation" mechanisms.

II. DIET-RELATED CVD RISK FACTORS

Risk is a continuous gradient that is used in epidemiology to identify high-risk populations. Although epidemiological studies cannot demonstrate cause-and-effect relationships, they provide targeted research areas for investigators to better understand possible pathogenic mechanisms associated with a disease process (Caggiula and Mustad, 1997; Khor, 2004).

Hyperlipidemia has been identified as a major risk factor for atherosclerosis and is associated with elevated plasma levels of cholesterol, cholesteryl esters, and triglycerides. These lipid components are transported into the circulatory system in association with proteins, phospholipids, and carbohydrates in various micelle forms-chylomicrons (CM), very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), LDL, HDL, lipoprotein (a), and various micelle remnants. Although the mechanisms are not completely understood, individuals with premature CVD generally exhibit some of the following: (1) increased LDL levels; (2) increased cholesterol and triglycerides, primarily in VLDL; and (3) increased triglycerides with normal cholesterol primarily as VLDL. A positive correlation with serum LDL and atherosclerotic events has been found, and this appears to be inversely related to serum HDL levels (Kris-Etherton et al., 1988; Kris-Etherton and Yu, 1997).

Other diet-related factors that may increase CVD risk are (1) total calories consumed; (2) amount of carbohydrate consumed (low-carb vs. High-carb diets); (3) amount of alcohol imbibed; (4) type of fat in the diet; (5) oxidative status of the diet and the individual; (6) levels of dietary trace minerals and vitamins, phytochemicals, type and the amount of these in the diet; and (7) type of dietary protein consumed. Diets high in simple carbohydrates are generally hypertriglyceridemic in humans and hypertriglyceridemia is considered to be an independent risk factor for CVD (Albrink et al., 1961; Austin, 2000). However, it is primarily in middle-aged women that elevated plasma triglycerides have been shown to be an important risk factor (Castelli, 1986). Therefore, it is questionable what role, if any, high-dietary carbohydrates play with respect to lipid metabolism in the overall onset of CVD. Interpopulation studies, in fact, indicate that diets high in complex CHOs are associated with a lower risk for CVD (Kagan et al., 1974; Connor et al., 1978; Shintani and Hughes, 1994; Seal, 2006). It is possible that some forms of hyperlipidemia, per se, are not detrimental when the amount of antioxidant components in the diet are high; the studies reported by Kushi et al. (1985), Aravanis and Ioannidis (1984), and Formica and Regelson (1995) suggest that fruit, vegetables, and grains might be protective; these foods contain ascorbic acid, β-carotene, isoflavones, bioflavonoids, and vitamin E, which might decrease lipid oxidation products and thereby reduce CVD risk.

Excessive calories, whether from carbohydrates, fats, or proteins, will result in obesity. Data from the Framingham Heart Study showed that increased relative body weight is an independent risk factor for CVD in addition to its association with low concentrations of HDL, decreased glucose tolerance, elevated plasma triglycerides and cholesterol, and hypertension (Hubert et al., 1983). The body type of the obese has also been associated with CVD risk; increased waist-to-hip circumference ratios, androgenic obesity, represent greater risk than lower body obesity (Bjontorp, 1988). The abdominal pattern of adiposity, and specifically visceral adiposity, appears to carry the highest risk. Abdominal adiposity has been identified as promoting insulin resistance, hypertension, and dyslipidemia (Garrison et al., 1996). Many studies have re-emphasized the notion put forward by Vague (1947) that obesity "android or male-type obesity" is not a homogeneous condition and that the regional distribution of adipose tissue is closely correlated to disturbances in glucose and lipid metabolism (Ohlson et al., 1985; Donahue, 1987; Bouchard et al., 1993). Some early studies used skinfold and waist-to-hip ratios to define visceral adiposity and therefore did not truly distinguish

between subcutaneous vs. visceral intra abdominal fat. Computed tomography (CT) clearly distinguishes fat from other tissues and allows the measurement of visceral and subcutaneous abdominal fat. Visceral fat area has been shown to closely correlate with glucose intolerance in the presence of hyperinsulinemia (Despres et al., 1989; Park et al., 1991; Pouliot et al., 1992) and other metabolic syndrome risk factors, for example, hypertriglyceridemia (Despres, 1991; Bjorntorp, 1997) and hypertension (Blair et al., 1984). Genetic susceptibility may modulate the risk associated with a given excess of visceral adipose tissue (Vague, 1947). There is general agreement that visceral fat is closely correlated with dyslipidemia and hyperinsulinemia, but controversy exists regarding the importance of subcutaneaous vs. visceral adipose stores on insulin sensitivity (Abate et al., 1995; Goodpaster et al., 1997). It was suggested that individuals with greater visceral fat mass, lose more visceral fat regardless of the intervention applied (Smith and Zachwieja, 1999). An increased amount of deep abdominal visceral fat has also been generally accepted as an important cardiovascular risk factor, and disturbances in hemostasis and fibrinolysis have been suggested to play a role (Mertens and Van Gaal, 2005). The mechanism(s) suggested for increased visceral fat loss appear to be related to a higher lipolytic rate for visceral fat in the steady state and this greater turnover and delivery to the liver have been implicated in various pathways to disease.

Endogenous cholesterol synthesis is apparently increased by 20 mg/day for every excess kilogram of body weight (McNamara, 1987). It is estimated that one-third of the population is sensitive to dietary cholesterol whereas two-thirds are resistant to plasma cholesterol changes. An average change in cholesterol intake of 100 mg/dL will potentially bring about a 2.5 mg/dL change in plasma cholesterol. However, a 1% decrease in energy intake from SFAs decreases plasma cholesterol by 3 mg/dL (McNamara, 1995).

Dietary fatty acids, as mentioned previously, have disparate results with respect to the risk for CVD. It is generally agreed that high-fat intakes (excess calories) are associated with increased risk, but it is not clear whether the risk is associated directly with the amount of fat consumed or with the resultant adiposity of the individual or the location of the adiposity, visceral vs. subcutaneous. Most studies indicate that increasing the ratio of polyunsaturated fatty acids to saturated fat (PUFA/SFA or P/S) decreases plasma cholesterol and in some studies plasma triglycerides (McNamara, 1987; Kris-Etherton and Yu, 1997). However, it is very evident that not all PUFAs and SFAs elicit the same cholesterolemic and/or triglyceridemic response.

Most unsaturated fatty acids in nature are found in the *cis* double bond configuration, but during hydrogenation of vegetable oils, inversions of the double bonds result in *trans* fatty acid isomers. These *trans* fatty acids also have double bonds in other than the normal n6, n7, n9, or n3 position from the methyl end of the fatty acid molecule. Many of the *trans* fatty acid isomers behave as saturated fats in most metabolic processes and therefore cannot be used in calculating P/S ratios based simply on the number of unsaturated double bonds. As mentioned, *trans* fatty acids appear to be as, or more, hypercholesterolemic than palmitic or myristic fatty acids and Mazaffarin et al. have recently covered this topic in an excellent review (Kris-Etherton and Yu, 1997; Mozaffarian et al., 2006).

Total dietary protein is considered to be of minor importance in the etiology of CVD, although epidemiological studies have shown that consumption of animal protein is positively correlated and vegetable protein negatively correlated with CVD mortality rates (Connor et al., 1978). Although a number of animal studies have shown that animal-derived protein is more atherogenic than vegetable protein, it is difficult to factor out these effects in human studies. It is also important to remember that animal protein and fat (particularly saturated fat) increases concomitantly in the diet (Kris-Etherton et al., 1988). Although soy protein has been found to have a modest hypocholesterolemic effect, the mechanisms by which it elicits these effects are unclear (Potter, 1995; Hecker, 2001).

In a number of epidemiological studies, moderate alcohol consumption has been associated with a reduced incidence of CVD (Yano et al., 1972; Klatsky et al., 1974; Garziano and Manson, 1996; Kannel and Ellison, 1996; Goldfinger, 2003). It appears that moderate alcohol consumption (7–14 oz weeks) increases the HDL cholesterol concentration and decreases LDL oxidation and

thus may offer protection against CVD. Most wines (red wines in particular) contain reserveratrol and piceid that function as antioxidants; these compounds functioning as antioxidants may protect against membrane damage by preventing lipid and lipoprotein oxidation (Kannel and Ellison, 1996; Sato et al., 1997; Singh, 2006). It is evident that the development of atherosclerosis and the dietary factors that constitute increased risk are far more complex than previously assumed.

III. ESSENTIAL AND NONESSENTIAL FATTY ACID METABOLISM

The requirements for essential fatty acids were first described by Burr and Burr (1930); however, almost 40 years elapsed before the connection between essential fatty acids and physiologically bioactive lipids (prostanoids and eicosanoids) was recognized (Bergstrom et al., 1964).

Polyunsaturated fatty acids are defined as fatty acids containing two or more unsaturated double bonds. Two broad categories of PUFAs exist that are of concern with respect to cardiovascular homeostasis, "essential" and "nonessential" fatty acids. Essential PUFAs (E-PUFAs) are considered to include linoleic and α-linolenic acids (all-*cis*-18:2n6 and 18:3n3, respectively). These essential fatty acids must be provided in the diet because they cannot be synthesized from simple carbon precursors in mammalian organisms. However, nonessential PUFAs (NE-PUFAs) can be synthesized from simple acetate units. If E-PUFAs are provided in the diet, the synthesis of nonessential highly unsaturated fatty acids, that is, 20:3n9 and 20:4n7 is minimal and only trace amounts can be detected in cellular storage lipids and membrane phospholipids. The n9 and n7 fatty acids cannot substitute for the membrane or biochemical functions that are associated with the n3 and n6 fatty acids (Vergroesen, 1988). The composition of PUFAs in membranes can be modified by diet and by alteration of cellular desaturation and acylation reactions (see Figure 44.3). Most cellular membranes appear to reflect the dietary fatty acid constituents, and as PUFAs are increased in the membrane there is an increase in membrane fluidity and membrane associated functions can be altered. As reported by Lands et al. (1990), there exists a quantitative relationship among PUFAs for specific esterification sites, primarily the *sn*-2 position of phospholipids. Although dietary fat can influence

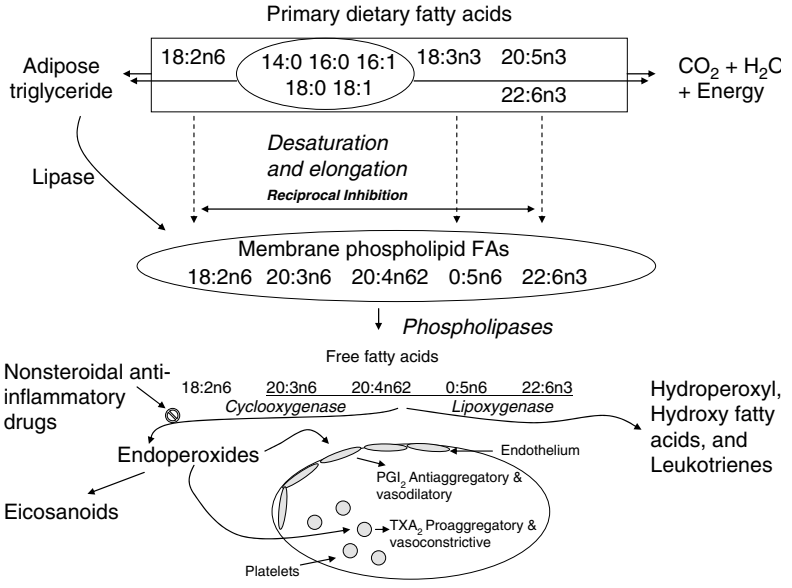

FIGURE 44.3 Effect of various dietary fatty acids on membrane fatty acid composition and eicosanoid production.

the levels of membrane fatty acids, there is an overall similarity in organ fatty acid composition between animal species. As the regulation of these membrane lipids appears to be tightly controlled, cellular function is closely associated with membrane composition.

Both the dietary essential and nonessential fatty acids can be desaturated and elongated to yield more unsaturated, longer chain fatty acid derivatives. For the nonessential saturated fatty acids (16:0 and 18:0), the first double bond that is introduced by Δ9 desaturase establishes the n7 and n9 fatty acid families (16:ln7 and 18:ln9, respectively). The next desaturation step (Δ6 desaturase) can apparently introduce a double bond in n7, n9, n6, or n3 fatty acids; Δ6 desaturase is the rate limiting enzyme and is regulated by feedback inhibitors (Brenner, 1981). In this key regulatory role, Δ6 desaturase can influence the PUFA composition of the membrane and the subsequent bioactive lipid end products formed (see Figure 44.2). By feeding long-chain PUFAs, that is, 20:4n6, 20:5n3, 22:6n3, the conversion of 18:2n6, 18:3n3, 18:ln9, and 16:ln7 to more unsaturated fatty acids can be inhibited. It has repeatedly been demonstrated that high-dietary levels of 20:5n3 and/or 22:6n3 will decrease the phospholipid and neutral lipid arachidonic acid (AA) concentrations; this occurs through inhibition of the Δ6 desaturase as well as competition for the phospholipid acylation sites. However, it is not the ratio of 18 carbon n3 to n6 fatty acids but rather the amount of each that appears to alter the end products produced (Goyens et al., 2006), suggesting that these fatty acids may be associated with specific Δ6 desaturase enzymes.

When animals are fed fat-free or completely saturated fat diets, their tissue lipids have decreased amounts of n3 and n6 PUFAs, and they accumulate the elongated, desaturated products of the n7 and n9 series, 20:3n9 and 20:4n7. As one includes n3 and n6 fatty acids in the diets of these essential fatty acid-deficient animals, the endogenous NE-PUFAs, are rapidly replaced by E-PUFAs at the *sn*-2 position of phospholipids and in storage lipids. The E-PUFAs, once released from the membrane, can serve as substrates for subsequent eicosanoid formation; eicosanoids are a group of compounds derived from PUFAs by the initial action of cyclooxygenase, p450, and/or lipoxygenase enzymes. With the discovery that AA could be converted by cyclooxygenase, p450, and/or lipoxygenase to bioactive lipid end products, many of the unexplained phenomena regarding the physiological effects of E-PUFAs became better understood.

Many of the eicosanoids produced via cyclooxygenase act in a "yin-yang" relationship and thereby modulate short-term localized responses to help maintain cardiovascular homeostasis. The pathways involved are detailed in the chapter by Dr. Hwang in this volume. As with the Δ6 desaturase, there is competition between the fatty acids for acylation into the membrane phospholipids as well as for the cyclooxygenase and lipoxygenase enzymes.

The fatty acids derived from marine animal fats differ from most oils and fats of terrestrial origin in that the marine oils generally contain longer carbon chains and higher degrees of unsaturation; the cold water marine oils are particularly rich in n3 fatty acids. Because the long-chain n3 fatty acids effectively compete for the desaturation and acylation enzyme sites with other fatty acid families, the changes in tissue fatty acid composition are greater than what the actual percent of the diet might predict. Moderate intakes of long-chain n3 fatty acids can markedly increase the concentration of these fatty acids in plasma, platelets, red blood cells, lung, kidney, liver, heart, adipose tissue, brain, macrophages, retina, nerve, and blood vessels; for a detailed review, see Herold and Kinsella (1986), Hornstra (1989a,b), and Vanden Heuvel (2004).

Since the n3 fatty acids replace primarily the n6 fatty acids from membrane lipids, the membrane content of AA is decreased (Hwang and Carroll, 1980; Bruckner et al., 1984; Harris, 1997a,b; Harris et al., 2003). The decreased availability of AA for eicosanoid formation and competition with the n3 fatty acid for the cyclooxygenase and lipoxygenase enzyme sites generally results in a decrease in the production of 2-series prostanoids, for example, thromboxane A_2 (TXA_2), PGE_2. TXA_2 is a potent stimulus for platelet aggregation and arteriole vasoconstriction. The release of this eicosanoid from the platelet membrane is primarily stimulated by thrombin, epinephrine, AA, and collagen. The vascular endothelium, via cyclooxygenase, produces prostacyclin (PGI_2) from AA, which is a potent antiaggregatory agent and vasodilator of blood vessels. Therefore, the "yin-yang"

relationship of these two eicosanoids appears to play a major role in normal vascular homeostasis as well as in vascular pathologies.

IV. EFFECTS OF SFAs, MUFAs, AND PUFAs ON THROMBOSIS–PLATELET–ENDOTHELIUM INTERACTIONS

Many of the experimentally induced forms of atherosclerosis are dependent on endothelial injury and subsequent platelet–macrophage–endothelium interactions. Ross (1985) proposed the "response to injury hypothesis" as the primary cause for the development of atherosclerosis. The endothelial injury may be subtle or intermediate or ultimately result in cell death. It has been demonstrated that platelet and macrophage interactions with the endothelium are required for lesion formation. These lesions may manifest themselves as endothelial cell separation from connective tissue, endothelial cell death, and/or increased endothelial cell permeability; any of these may result in endothelial retraction and endothelial cell desquamation. Many of these damaging events involve ROS. The exposure of the sub-endothelial connective tissue will propagate further platelet adherence and aggregation and possibly mural thrombus formation. Injury to the endothelial cells can result from exposure to endotoxins, vasoactive amines, viral infection, lipid peroxides, hypercholesterolemia, mechanical injury (hypertension), carbon monoxide, and/or hyperlipidemia. Once the endothelium or platelets are modified and platelet–endothelium interactions are initiated, a potentially pathogenic cascade may be triggered.

Platelets contain vasoactive amines, ADP, platelet-derived growth factor (PDGF), diacylglycerol (DAG), and platelet-activating factor (PAF) and are capable of synthesizing TXA_2, which is a proaggregatory and vasoconstrictive eicosanoid. Once the platelet is stimulated by an agonist, for example, collagen, the release of these bioactive substances can be triggered. PDGF is a principal mitogen responsible for the proliferation of smooth muscle cells, and it has been demonstrated that increased growth of intimal smooth muscle cells is a key factor in atherogenesis. In addition, the release of AA from the platelet membrane yields TXA_2, which potentiates the aggregatory response. During injury, such as disruption of the vessel wall, these positive feedback stimulatory responses lead to thrombus formation, vasoconstricition, and clot formation and thus prevent loss of vascular fluids. However, if these pathways are repeatedly stimulated, with migration of monocytes and macrophages to the site of injury, lipid accumulation and smooth muscle cell proliferation will lead to the formation of vascular plaque (see Figure 44.5). Under ideal conditions the "yin-yang" relationship between platelets and endothelial cells is maintained via production by the platelets of TXA_2 and PDGF and endothelial cell synthesis of PGI_2 and endothelium-derived relaxing factor (EDRF); EDRF has been identified as nitric oxide (NO), one of the most potent vasodilating substances known. Physiological regulation of platelet adhesion is mediated by both NO and superoxide, with the former inhibiting and the latter promoting platelet adhesion. The interactions between platelets and the vascular endothelium appear to have important implications in the initiation and/or progression of tissue injury associated with different experimental models of human disease (Tailor et al., 2005). Both PGI_2 and NO inhibit platelet aggregation and arteriole vasoconstriction. In addition, PGI_2 may stimulate the enzymes that metabolize cholesteryl esters that are known to accumulate in atherosclerotic plaque (Moncada, 1987). It appears that dietary lipids can modify the platelet–endothelial interactions via their cholesterol content (mostly oxidized cholesterol) and/or the type and amount of fatty acids consumed.

A. SFA/*Trans* Fatty Acid Effects

The Seven Countries Study (Keys et al., 1980) gave rise to the idea that dietary saturated fats contribute to high plasma cholesterol concentrations and atherogenesis, but skepticism remains about the degree of such an effect (Mann, 1977; Gurr, 1992). Renaud et al. (1981, 1986) and others (Mustard and

Murphy, 1962) have studied in a variety of ways the effects of diets high in saturated fats on both coagulation and platelet function. Merskey and Marcus (1963), in their comprehensive review, concluded that there was no evidence that the type of saturated dietary fat had any influence on coagulation and this opinion has been echoed regularly since then (Hoak, 1997). Factor VII activation by saturated fatty acids was reported and it was suggested that stearic acid may be responsible for this activation. However, the concentration used *in vitro* is not likely to be attained *in vivo*. As stated in the excellent recent review by Knapp (1997), others have reported that stearic acid interacted more favorably with the coagulation system (factor VII activity) than did palmitic, myristic, or lauric acids, whereas others found no effect of dietary saturated fat on platelet function or thromboxane generation or on the excretion of thromboxane and 6-keto-prostaglandin $F_{1\alpha}$. Although there is still ongoing debate, it appears that similar prothrombotic changes in platelet function and homeostatic variables are elicited by postprandial test meals of saturated or n6 unsaturated fatty acids or of the latter and saturated medium-chain triacylglycerols (Knapp, 1997). Also it has been noted, that stearic and *trans*-FA diets had similar effects on platelet activation and endothelial PGI_2 production (Turpeinen et al., 1998) and that stearic acid is not more thrombogenic than oleic or linoleic fatty acids (Thijssen et al., 2005). Furthermore, Sanders et al. (2003) demonstrated that *trans* fatty acids (*t*18:1) do not have any specific effects on known haemostatic risk markers for CVD in healthy young men in the short-term. *Trans* fatty acids may influence other risk factors for CVD. Consumption of *trans* fat reduced the activity of serum paraoxonase (de Roos et al., 2002) and impaired the postprandial activity of tissue plasminogen activator (Muller et al., 2001). The development of better techniques for assessing the many interactive components of coagulation and hemostasis *in vivo* will help to resolve these issues.

B. MUFA Effects

Oleic acid (18:1n9) is usually considered to be the neutral reference point for studying the potential antiaggregatory effects of polyunsaturated fats and proaggregatory effects of saturated fats (Knapp, 1997). However, it is not clear whether oleic acid is truly devoid of biological effects in humans. Olive oil and other oleic acid enriched oils certainly impact on cholesterol and lipoprotein homeostasis as discussed in the following section. A recent report indicated increased plasmin activity following 18:1n9 and 18:2n6 addition *in vitro* whereas saturated fatty acids were ineffective. As recently reported, factor VIIc was found to be lower on a MUFA diet vs. a saturated fat diet, but fibrinogen and insulin concentrations and plasminogen activator inhibitor-1 activity did not differ between diets (Allman-Farinelli et al., 2005). Certainly, the paucity of studies in this area contributes to our lack of fully understanding the effects of MUFAs on platelet–endothelial cell interactions and their involvement in the process of coagulation and atherogenesis.

C. PUFA Effects

Sinclair (1956) postulated that a deficiency of AA might lead to atherosclerosis; stated from a slightly different viewpoint, it appears that a diet high in saturated fat and consequently low in essential fatty acids contributes to CVD. Several epidemiological and experimental studies suggest that low tissue levels of linoleic acid might be an independent risk factor for CVD (Oliver et al., 1990). However, increased tissue levels of AA have also been postulated to increase risk for CVD. The mechanisms by which dietary linoleic acid compared to *trans* and more saturated fat diets might influence intravascular thrombosis are by (1) decreasing plasma cholesterol and LDL; (2) lowering blood pressure; (3) altering blood viscosity; (4) decreasing membrane fluidity; (5) altering signal transduction pathways; and (6) decreasing membrane cholesterol. Although a dietary linoleic acid deficiency would be expected to lower both TXA_2 and PGI_2 concentrations, owing to the synthesis of less 20:4n6, there may be differences between platelet and endothelial effects. For example, it has been shown that increased incorporation of cholesterol into platelet membranes will enhance TXA_2 production; endothelial membrane eicosanoid production under these conditions has not been studied.

It has been noted that Greenland Eskimos and Japanese fishermen have platelets that are less reactive *in vitro* than those of Caucasian Danes and Japanese farmers, respectively (Bang and Dyerberg, 1981). In addition, as summarized by Hornstra (1989a,b) and more recently by Knapp (1997) in a number of studies, there was a significant decrease in the number of circulating platelets after dietary fish oil (rich in 20:5n3 and 22:6n3) supplementation. Although the results are equivocal, it appears that diets high in fish oils or n3 fatty acids result in decreased synthesis of the 2-series eicosanoids by platelets, for example, TXA_2 measured as its TXB_2 metabolite (see Figures 44.3 and 44.5). After consumption of n3 fatty acids, not only the platelet TXA_2 synthesis but also the endothelial synthesis of PGI_2 is decreased. Therefore, it appears that fish oil fatty acids reduce the concentration of both the pro- and antiaggregatory eicosanoids. However, the n3 fatty acids have been shown not only to decrease the synthesis of 2-series eicosanoids but also to serve as substrates for the 3-series eicosanoids (see Chapter 28).

Eicosapentaenoic acid (EPA) (20:5n3) can be converted by platelets to TXA_3 and by endothelial cells to PGI_3. TXA_3 is a weak aggregatory and vasoconstrictive eicosanoid. However, the PGI_3 produced is equipotent to PGI_2 in its antiaggregatory and vasodilatory properties. Therefore, it is hypothesized that fish oils may decrease the overall potency of the proaggregatory and vasoconstrictive eicosanoids (TXA_2 and TXA_3) and maintains or increases the levels of the antiaggregatory and vasodilatory compounds (PGI_2 and PGI_3). Several laboratories have demonstrated significant *in vivo* synthesis of PGI_3 in humans after fish oil feeding, measured as the 2,3-dinor-Δ17-6 keto-PGFlα urinary metabolite (Hornstra, 1989a). As reviewed by Knapp (1997), at least 23 papers dealing with n3 fatty acid supplementation have demonstrated decreased TXA_2 production by platelets *in vitro*. The noninvasive assessment of endogenous thromboxane synthesis via measurement of its urinary metabolites provides a clinically useful index of *in vivo* biosynthesis. There have been few studies on this important point. Two groups found that in either patients with atherosclerosis who were supplemented with 10 g/day of 20:5n3 (Knapp et al., 1986) or apparently healthy individuals (Von Schacky and Weber, 1985) who excrete large amounts of dinorthromboxane B_2, there is a reduction toward the normal range. Relatively small amounts of dinorthromboxane B_3, formed from the biologically less-active thromboxane A_3, were noted and total thromboxane production was reduced. In healthy subjects with normal dinorthromboxane production, fish oil only changed the ratio of B_3/B_2 metabolites but did not decrease total production. The complexity of the effects of 20:5n3 on *in vitro* and *in vivo* processes in humans is apparent; certainly more direct *in vivo* measurements are needed to determine the effects of n3 fatty acids on not only thromboxane modulation but also on prostacylin biosynthetic rates as they relate to hemostasis.

In a series of elegant experiments utilizing an abdominal aortic loop model to measure obstruction time (OT, *in vivo* clotting time) of rats fed different dietary fats, it was clearly demonstrated that OT had a highly significant negative relationship with total saturated fatty acids and a positive relationship with PUFAs (Hornstra, 1989a; Hoak, 1997). The PUFAs had different effects on OT depending on the fatty acid families involved. Marine oils that contain high concentrations of n3 PUFAs and low amounts of saturated fatty acids proved to increase the OT, examples are cod liver and sperm whale oils. Other marine oils that are rich in n3 fatty acids also contain significant amounts of saturated fatty acids; whale and Peruvian fish oil were not effective in prolonging OT. PUFAs in whale oil are found at the 1- and 3-positions of the TGs, unlike those of fish oils, which contain PUFAs primarily in the 2-position (Bottino et al., 1967). These differences may occur because of differences in digestion and absorption of the monglycerides formed; more *sn*-2 monoglycerides are formed and therefore absorption may be higher than for the *sn*-1 or *sn*-3 monoglycerides. It should also be noted that not all saturated fatty acids elicit thrombogenic responses. Palm oil, which contains significant amounts of palmtic acid (16:0), does not appear to be as thrombogenic or as hypercholesterolemic as the shorter chain saturated fatty acids, 12:0 and 14:0 (Hornstra, 1989a; Hayes et al., 1990; Kris-Etherton and Yu, 1997).

Eicosanoid ratio changes, after fish oil ingestion, may not be the only explanation for the increased bleeding time and decreased platelet responsiveness *in vivo* and *in vitro*. Certainly, the thrombocytopenia noted in individuals after fish oil consumption may contribute to the observed

increases in bleeding and clotting time. Other mechanisms related to hemostasis may be due to membrane alterations as n3 fatty acids are incorporated into phospholipids. The increased n3 fatty acids in the membrane phospholipids have been reported to alter membrane fluidity, blood viscosity, receptor-agonist affinity, platelet–platelet and/or platelet–endothelial interactions, clotting factors and fibrinolysis (Knapp, 1997; Breslow, 2006).

Much has been publicized regarding COX-2 inhibitors and increased risk of cardiovascular events. Selective COX-2 inhibitors increase the risk of myocardial infarction and stroke that is attributed to their ability to inhibit PGI_2, resolvins, lipoxins, and endothelial nitric oxide (eNO) without affecting platelet COX-1 derived TXA_2. In contrast, aspirin blocks both COX-1 and COX-2 enzymes that results in increase in the intracellular concentrations of dihomo-gamma-linolenic acid (DGLA), AA, EPA, and docosahexaenoic acid (DHA), and reduced formation of eicosanoids. DGLA, AA, and EPA form precursors to PGE_1, PGI_2, and PGI_3, respectively, which are potent vasodilators and platelet antiaggregators. EPA has been shown to have antiarrhythmic action, and EPA, DHA, DGLA, and PGE_1 have anti-inflammatory actions as well. EPA, DHA, and AA augment eNO formation that has antiatherosclerotic action. Hence, combining EFAs with COX-2 inhibitors may prevent thrombotic cardiovascular events (DAS UN, 2005) and diminish the untoward effects of COX-2 inhibitors.

V. FATTY ACIDS AND LIPOPROTEIN METABOLISM

A. Lipoprotein Homeostasis

Lipoproteins are the key transport vehicles for cholesterol, cholesteryl esters, and triglycerides. Lipoproteins are in a constant state of flux as lipids are absorbed, packaged, and transported throughout the body. In the intestine, absorbed lipids are packaged into CM containing apoproteins B48, A-1, and A-V. These CM are transported via the thoracic lymph duct into the systemic circulation, where they acquire apo E and apo C from the circulating HDL. As the chylomicron comes into contact with the capillary endothelium, the enzyme lipoprotein lipase (LPL) hydrolyzes the triglycerides (TG) from the chylomicron. Thus the TG core of the chylomicron is decreased until only a cholesterol-enriched CM remnant remains. These CM remnants are catabolized by the liver (see Figure 44.4).

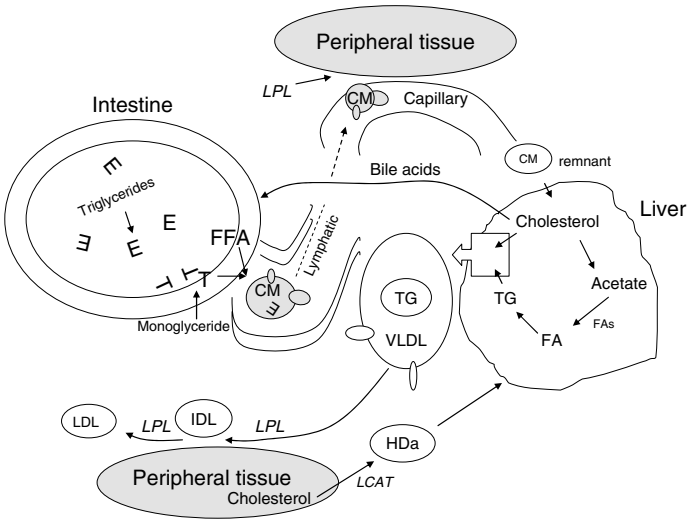

FIGURE 44.4 Lipoprotein homeostasis. LPL = lipoprotein lipase; CM = chylomicron; HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LCAT = lecithin cholesterol acyl transferase.

If there is an excess in caloric intake from carbohydrates, fats, and/or proteins, there is an increase in acetate units available for fat or cholesterol biosynthesis. In the liver, the synthesized fatty acids and cholesterol are processed into VLDL containing apoproteins B-100, C, and E and secreted into the circulation. Once again lipoprotein lipase removes the triglycerides from these VLDL particles. As the TG content is decreased and the relative cholesterol portion increased, the micelle particle is designated as IDL and ultimately as LDL. Constant exchange occurs between the various lipoprotein fractions and HDLs as they are transported to and from the liver.

The LDL is a cholesterol-rich particle (LDL-C) and can be removed from the circulation by the liver, endothelial cells, and/or the macrophage scavenger pathway.

In all species studied to date, the liver is the primary site for the clearance of LDL-C from the plasma; 70%–85% of LDL-C removal from the plasma takes place in liver (Dietschy, 1997; Turley, 2004). Thus, while most cholesterol synthesis takes place in extrahepatic tissues, most plasma LDL-C is returned directly to liver.

Two separate transport processes have been described with regard to uptake of LDL particles from plasma, (1) receptor-dependent uptake that manifests saturation kinetics and almost certainly is mediated by the LDL receptor and (2) LDL receptor independent transport that manifests linear uptake kinetics with respect to the plasma LDL-C concentration. Of the total clearance of LDL-C from plasma, under normal dietary conditions, the receptor-dependent process accounts for approximately 60%–90% of LDL-C degradation. If hypercholesterolemic conditions prevail then more transport occurs via the receptor independent pathway; however, the mechanism(s) is unknown. As elegantly explained by Dietschy (1997) and Turley (2004), the control of cholesterol homeostasis can be influenced not only by the up-down regulation of LDL receptors and HMG CoA-reductase, but also by the rate of cholesteryl ester formation within the cell. Excess sterol is esterified by the enzyme sterol *O*-acyltransferase, the activity of which appears to be driven by the availability of the two substrates for the esterification reaction, cholesterol and the acyl-CoA derivative of *cis* 18:1n9 (Goodman, et al., 1964; Suckling and Strange, 1983; Dietschy, 1997). Thus, when free cholesterol in the liver is increased, there is presumably expansion of the pool of cholesteryl esters. According to the model detailed by Dietschy (1997), regulation of hepatic receptor activity by specific fatty acids is in part via an effect of these fatty acids on the sterol *O*-acyltransferase reaction. When the liver becomes enriched with a fatty acid that is not an ideal substrate for the sterol *O*-acyltransferase reaction, such as 16:0, the esterification reaction is partially inhibited, and the size of the free cholesterol pool is increased. As a consequence of this expansion of the free cholesterol pool, receptor messenger RNA concentration is reduced and LDL receptor activity in the liver is down regulated. However, if the preferred substrate, *cis* 18:1n9, for sterol *O*-acyltransferase is provided then cholesterol is apparently shifted into the cholesteryl ester pool and LDL receptor activity is upregulated. According to this model, the regulatory effects of both dietary cholesterol and fatty acids operate by altering the size of the free sterol regulatory pool. Therefore, this model better explains the regulatory effect of different dietary fatty acids when cholesterol is also present in the diet.

As mentioned previously, elevated levels of circulating LDL are positively correlated with increased risk for CVD, and HDL levels are negatively correlated with CVD risk (Kris-Etherton et al., 1988; Kris-Etherton and Yu, 1997; Kratz, 2005). However, it has recently been questioned whether it is the LDL or its oxidized, modified lipid, and/or protein constituents that are the primary CVD-risk-related factors. Lipid oxidation products include cholesterol oxides, fatty acid hydroperoxides, malonaldehyde, and other secondary breakdown products of lipid oxidation, such as cyclooxygenase and lipoxygenase lipid intermediates. In addition, the apoprotein moiety may also be modified (oxidized, glycosylated) and thus be taken up by the scavenger pathway in macrophages. The role of oxidized lipoproteins in atherogenesis has been reviewed by Berliner and Heinecke (1996) and Frostegard (2002) and it is evident that liporotein oxidation is promoted by several diffferent systems *in vitro*, including free and protein-bound metals, thiols, reactive oxygen species, lipoxygenase, and cyclooxygenase-derived active lipid intermediates, peroxynitrite and myloperoxidase. Although these have been shown to contribute to lipoprotein oxidation *in vitro*, the *in vivo* effects are not

clearly understood. Therefore, it is apparent that the type of dietary lipids ingested, the degree of lipid oxidation, the susceptibility of the lipid to peroxidation, and the subsequent composition of the membrane may alter the risk of CVD.

B. n6 PUFAs and Lipoprotein

Generally, it has been observed that dietary n6 PUFAs decrease plasma total cholesterol (Kris-Etherton, 1997), as predicted by the equations of Hegstead et al. (1965, 1993) and Keys et al. (1965). These equations predict that the plasma cholesterol-elevating effects of dietary SFAs are approximately twice the cholesterol-lowering effect of the n6 fatty acids. Dietary recommendations have therefore included decreasing the SFAs, increasing the n6 PUFAs, and reducing dietary cholesterol. The n6 PUFAs may lower plasma cholesterol by (1) increasing fecal bile acid secretion; (2) decreasing hepatic VLDL synthesis; and/or (3) altering VLDL, IDL, and LDL lipoprotein composition, and thereby influencing the metabolism of these circulating particles. Although most studies have shown that total plasma cholesterol is lowered by increasing the dietary content of n6 PUFAs, HDL cholesterol also appears to be lowered by n6 PUFAs; high-HDL cholesterol is associated with decreased CVD risk (Goldberg and Schonfeld, 1985). However, there is still ongoing debate as to whether high-LDL cholesterol or low HDL cholesterol is the best predictor of CVD risk. It appears that vegetarians who have a lower incidence for CVD have decreased plasma levels of both HDL and LDL cholesterols, suggesting that LDL cholesterol might be a more reliable index or that other vegetable constituents may provide a protective effect. It seems that dietary n6 PUFAs reduce plasma LDL cholesterol without altering VLDL cholesterol.

C. MUFAs and Lipoproteins

Monounsaturated fatty acids, such as 18:l n9, have been shown to lower plasma total cholesterol and LDL cholesterol when substituted for SFA in the diet. It is not clear whether the MUFAs are directly responsible for the decrease in plasma cholesterol or whether it is due to decreased SFA intake. However, it has recently been noted that MUFAs were as hypocholesterolemic as n6 fatty acids relative to SFAs when administered as liquid formulas. These cholesterol changes were primarily due to decreased LDL cholesterol because the HDL cholesterol remained unchanged (Grundy, 1986). Other more recent studies have noted that *cis* n9 fatty acids actually increase HDL while lowering LDL (Dietschy, 1997; Kris-Etherton, 1997; Sanders, 2003). Changes in HDL/LDL ratios by n9 fatty acids may, in part, explain the lower incidence of CVD noted in the Greek Island Studies, since the consumption of olive oil (rich in n9 fatty acids) in the Mediterranean diets is high (Keys et al., 1980).

D. Saturated Fatty Acids

Well-controlled dietary studies carried out in the 1950s and 1960s found that relative to an isoenergetic amount of carbohydrate, a mixture of saturated fatty acids increased serum total cholesterol concentrations (Hegsted et al., 1965; Keys et al., 1965). However, these earlier studies did not examine the effects of fatty acids on specific lipoproteins. Based on the recent meta-analysis, it can be stated that replacement of carbohydrates with saturated fatty acids not only increased serum total cholesterol concentration but also LDL and HDL. The total HDL cholesterol ratio however was not affected (Mensink et al., 2003).

E. *Trans* FAs and Lipoproteins

Naturally occurring MUFAs and PUFAs contain primarily fatty acids in the *cis* configuration; however, most processed foods also contain *trans* fatty acid isomers. These *trans* isomers are formed when animal fats or vegetable and marine oils are hydrogenated to alter their plasticity

and melting points. It was estimated in 1984 that 6%–8% of the total fat consumption is derived from these *trans* isomers (Emken, 1984); however, this amount has decreased to 2%–3% in 2006 (Mozaffarian et al., 2006). Elaidic acid (the *trans* isomer of *cis* 18:1n9) is most abundant in the diet. Although it was generally accepted that the *trans* isomers of MUFAs and PUFAs, as present in normal Western diets, do not precipitate in any known pathophysiologies, it has recently been shown that elaidic acid alters serum lipoproteins in a manner similar to 14:0 and 12:0 SFAs (Aro et al., 1997; Kris-Etherton, 1997). Mensink and Katan (1990) concluded that *trans* fatty acids are at least as unfavorable as the cholesterol-raising SFAs, because they not only raise LDL cholesterol but also decrease HDL cholesterol levels. However, the *trans* fatty acids appear to be less hypercholesterolemic than 14:0 (the most hypercholesterolemic) and 12:0 SFAs. Aro et al. (1997) compared the effects of a high *trans* diet to a high stearic acid diet and found that similar lipoprotein changes occurred with the two diets; however, Lp (a), a strong independent risk factor for CVD (Gries et al., 1990), was increased significantly more by the *trans* diet than by the stearic acid enriched diet. The Food and Drug Administration (FDA) ruled that, effective January 1, 2006, the nutrition labels for all conventional foods and supplements must indicate the content of *trans* fatty acids (Food and Drug Administration, 2005) and is the first substantive change to food labeling since 1990. The Department of Agriculture made a limited intake of *trans* fatty acids, a key recommendation of the new food-pyramid guidelines (Department of Health and Human Services, 2005) subsequent to the recommendations of the Dietary Guidelines Advisory Committee that the consumption of *trans* fatty acids be kept below 1% of total energy intake (Dietary Guidelines Advisory Committee, 2005). Each of these actions was prompted by evidence that consumption of *trans* fatty acids increases the risk of coronary heart disease (CHD). It seems evident that *trans* fatty acids contribute to risk associated with CVD.

F. n3 FAs and Lipoproteins

The discovery of the unique characteristics of n3 fatty acids, their role in lipoprotein regulation and possible role in preventing CHD, has sparked a flurry of research activity over the past two decades. Fish oils (rich in long-chain n3 fatty acids; 20:5n3 and 22:6n3) have been found to effectively lower VLDL levels in normal and hyperlipidemic subjects (Nestel, 1990; Harris, 1997a,b). The average reduction in VLDL is greater in hypertriglyceridemic subjects than in normal individuals. Decreased serum TGs (VLDL) are not due to changes in lipoprotein lipase or hepatic triglyceride lipase activities, which indicates that the n3 fatty acid effects are most likely due to altered synthesis rates. As shown by Nestel et al. (1984), the net synthesis of liver triglyceride as VLDL is greatly reduced. The reduced output of liver VLDL is dose-dependent and appears to be due to (1) increased mitochondrial and peroxisomal oxidation of fatty acids; (2) decreased fatty acid synthesis; (3) increased phospholipid vs. TG synthesis; and (4) decreased activity of the esterifying enzymes. It is speculated that lipogenesis is reduced owing to feedback inhibition of the acetyl CoA carboxylase, the rate-limiting enzyme. Apoprotein B100, which is an integral part of VLDL synthesis, is also suppressed by the n3 fatty acids.

Harris (1989, 1997a,b) summarized the lipoprotein changes that result from n3 fatty acid dietary supplementation. Linolenic acid (18:3n3), unlike the longer chain PUFAs found in fish oils, does not reduce VLDL in most studies but does exhibit hypocholesterolemic effects similar to the n6 fatty acids. Total cholesterol and LDL cholesterol are not changed markedly in normolipidemic individuals following fish oil supplementation. However, in hyperlipidemics the n3 fatty acids tend to elevate LDL-C (5%–10%) and HDL-C (1%–3%); serum triglyceride concentrations are decreased by 25%–30%. Only in cases where very high levels of n3 fatty acids (20+ g/day) were consumed have total cholesterol and LDL cholesterol serum levels decreased. In addition, it has been shown that up to 6 g, n3 fatty acids did not alter plasma Lp(a) levels; Lp(a) is positively correlated with CVD (Gries et al., 1990). As mentioned, n-3 PUFAs reduce plasma triacylglycerols and improve the lipoprotein profile by decreasing the fraction of atherogenic small, dense LDL. These effects are

likely mediated through the activity of transcription factors related to expression of genes involved in lipid synthesis and oxidation. It has been hypothesized that other pleiotropic effects of n-3 PUFAs may contribute to decreasing the burden of the metabolic syndrome, such as modulating inflammation, platelet activation, endothelial function, and blood pressure. Although studies comparing the effect of both major n-3 PUFAs are limited, DHA appears at least as effective as EPA in correcting several risk factors (Carpentier et al., 2006). It should be cautioned that although the overall effects of n3 fatty acids on serum lipoproteins appear to be beneficial, particularly with respect to VLDL, their susceptibility to oxidation may induce peroxidative damage to LDL, and thus increase the atherogenicity of this lipoprotein fraction (Hau et al., 1996; Tsai and Lu, 1997).

VI. CARDIOVASCULAR DISEASE AND LIPIDS

Hypertension is a primary risk factor for CVD and it has been noted that many populations with low mean blood pressure eat a diet low in total fat and saturated fatty acids. Specifically, linoleic acid levels in adipose tissue, a reflection of dietary intake, were inversely correlated to blood pressure in populations in Germany, Scotland, and eastern Finland; however, these correlations were not observed in other studies. These discordant findings may be caused by lack of multivariate analysis, which might include variables such as other fatty acids, alcohol intake, salt intake, and cigarette smoking (Sacks, 1989). The low blood pressure, observed in societies with a low intake of fat, may be due to other nutrients in vegetable products, such as the bioflavanoids.

In more controlled animal studies, the dietary intake of n3 PUFAs attenuate the development of hypertension in salt-induced, one-clip, DOCA-salt-induced (Bond et al., 1989), and subtotal nephrectomized rat models. Conversely, hypertension in the rat is accelerated by diets deficient in PUFAs.

The n3 fatty acids have been reported to exhibit hypotensive properties in humans; however, these effects are inconsistent. As the n3 fatty acids can alter the biosynthesis of eicosanoids, which in turn regulate physiological functions involved with blood pressure regulation, it is speculated that vascular reactivity, salt and water balance, renin release, and blood rheology may be altered by ingestion of these fatty acids. As summarized by Knapp (1989), pharmacological doses of n3 fatty acids appear to slightly lower blood pressure in humans, but this effect is most likely not mediated via altered production of eicosanoids.

Several mechanisms have been proposed to explain how EPA plus DHA might beneficially influence CVD (Breslow, 2006). These include preventing arrhythmias (Connor, 2000; Leaf et al., 2003), lowering plasma triacylglycerols (Harris, 1997b; Sacks and Katan, 2002), decreasing blood pressure (Geleijnse et al., 2002), decreasing platelet aggregation (Knapp, 1997; Hornstra, 2001), improving vascular reactivity (Goodfellow et al., 2000), and decreasing inflammation (Calder, 2001).

Other risk factors for the development of atherosclerosis include increased plasma concentrations of clotting factors, such as fibrinogen and platelet aggregation. In addition to the Greenland Eskimo studies, a number of investigators have reported that 3–5 g of n3 fatty acids results in prolonged bleeding time (Goodnight, 1986) and in some studies decreased platelet aggregation. However, n3 fatty acids do not appear to affect the plasma concentration of fibrinogen. In addition, an increased plasma plasminogen activator inhibitor (PPAI) has been associated with CVD; the effects of n3 fatty acids on PPAI are varied, and no correlations can be drawn (Hornstra, 1989a; Knapp, 1997).

Peripheral vascular disease is associated with vasoconstriction and venous and arteriole occlusions. Although the number of studies related to peripheral vascular disease is limited, there have been marked clinical improvements demonstrated in patients with chronic arterial occlusion following the administration of EPA-ethyl esters (Sakuri et al., 1987). Changes in peripheral capillary blood cell velocities were noted by Bruckner et al. (1987) in normal subjects after n3 fatty acid supplementation. Furthermore, the increased flow is apparently dependent on the oxidative status of the individual. Ware et al. (1992) found that n3 fatty acids given as supplements to elderly subjects compromised the peripheral blood flow, and increased flow was observed only with the concomitant

supplementation of vitamin E. It is possible that vascular tone is influenced not only by changes in eicosanoid ratios of TXA_2 and TXA_3 to PGI_2 and PGI_3 but also by increased synthesis of NO and decreased PAF. Changes in microviscosity might be another factor that contributes to increased blood flow. It has been reported that red blood cell deformability is increased and whole blood viscosity decreased after fish oil supplementation (Hornstra, 1989a; Knapp, 1997).

As stated by Schwalfenberg (2006) in an excellent recent review article, "omega-3 fatty acids are cardioprotective mainly due to beneficial effects on arrhythmias, atherosclerosis, inflammation, and thrombosis. There is also evidence that they improve endothelial function, lower blood pressure, and significantly lower triglycerides. *Physicians need to integrate dietary recommendations for consumption of omega-3 fatty acids into their usual cardiovascular care*."

VII. A UNIFYING HYPOTHESIS FOR VASCULAR EVENTS LEADING TO ATHEROGENESIS

CHD can be viewed as a three-phase process. During the first phase, initiation, an initial endothelial injury is produced, which may be due to lipid oxidation products, smoking (carbon monoxide), hypertension (shear stress), endotoxins, toxic oxygen metabolites, ROS, and/or viral infections. The second phase, progression, entails plaque formation due to cholesteryl ester deposition, oxidized LDL cholesterol macrophage uptake, proliferation of smooth muscle cells, and continued platelet activation. Phase three involves thrombosis, vascular spasm, and/or vascular occlusion leading to tissue damage, and ultimately tissue death (termination).

Ross (1985) postulated that atherosclerosis is a response to injury, which we can now expand to include injury via various ROS; however, the mechanisms by which specific initial injurious agents elicit endothelial damage are still not well understood. Lipid oxidation products can induce endothelial cell injury and may be one of the initial cytotoxic agents starting the atherosclerotic process. Assuming that lipid oxides are involved in the initiation process, the effects of dietary fatty acid isomers on atherogenesis can be related to their impact on the "oxidative status" of the organism.

The pathological process initiated by lipid oxides includes arterial endothelial injury, activation of platelets, monocyte recruitment, monocyte adherence, conversion of moncytes to macrophage scavenger cells, macrophage uptake of lipid peroxides, and transformation to foam cells; these events lead to the evolution of fatty streaks, intimal thickening, and ultimately to vessel occlusion. If one combines the Ross injury hypothesis, the lipid hypothesis, and the lipid peroxidation hypothesis, many of the discrepancies involving lipids and atherogenesis can be explained. To understand how dietary lipids are involved in initiation, propagation, and termination of vascular events, the long-standing dogma that cholesterol and saturated fatty acids are, in and of themselves, detrimental needs to be reconsidered (see Figure 44.5). As previously stated, not all SFAs are hypercholesterolemic and the role of MUFAs with respect to sterol *O*-acyltransferase specificity appears to play a significant role in cholesterol homeostasis. Cholesterol and most fatty acids in pure bound form do not appear to precipitate injurious vascular events; however, more work is needed to clarify this area since linoleic acid may be more injurious to endothelial cells than other unsaturated or saturated fatty acids (Hennig et al., 1993, 2006).

Evolution has provided for tight control mechanisms to regulate the amounts of free fatty acids and cholesterol as well as the specific types and amounts of these lipids found in membranes. Furthermore, due to the now apparent toxicity of oxidized lipids, nature has provided an elaborate antioxidant defense mechanism. These lipid protective compounds enter into the food chain as dietary vitamins E, C, β-carotene, bioactive flavonoids, alpha-lipoic acid (Packer et al., 1995), butylated hydroxytoluene (BHT), and other synthetic antioxidants added during processing; these compounds can protect foods (lipids) from autoxidation. Fruits and vegetables are rich sources of phenolic and other antioxidants and their consumption is correlated with decreased CVD. In addition, as pointed out by James et al. (1989), the decline in CVD in the United States, while paralleling the increased consumption of more PUFAs, is also paralleled by the increased consumption of antioxidants found or added to these vegetable oils and fats.

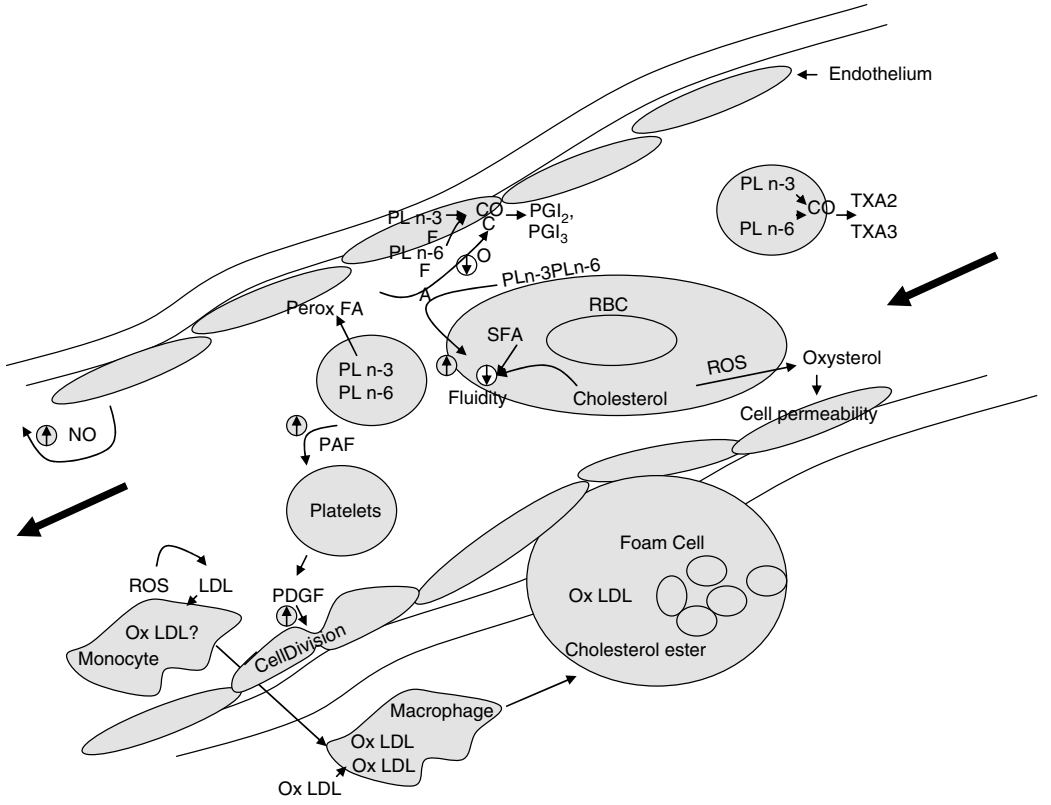

FIGURE 44.5 Pathways influenced by n-6 and n-3 fatty acids that may alter blood flow and atherogenesis. Possible antiatherogenic effects of n-3 fatty acids. 1: Decrease membrane 20:4n-6 (acylation and Δ6 desaturase inhibition). 2: Increase membrane 20:5n-3 (alter membrane fluidity). 3: Increase membrane 20:5n-3 (alter agonist-receptor sensitivity). 4: Increase synthesis of 3-series eicosanoids, TXA3 and PGI3, which favors vasodilatory-antiaggregatory environment. 5: Compete for cycloxygenase and lipoxygenase enzyme sites with n-6 fatty acids; thereby, (a) decrease 2-series and increase 3-series eicosanoids and (b) decrease production of unstable endoperoxide and hydroperoxide intermediates. 6: Decrease VLDL synthesis. 7: Increase HDL synthesis. 8: Increase blood flow (vasodilation-fluidity). 9: Increase EDRF, decrease PAF and PDGF. (↑) Vasoconstrictive-proaggregatory; (↓) vasodilatory-antiaggregatory; TXA_2, thromboxane A_2; TXA_3, thromboxane A_3; EPI, epinephrine; PGI_2, prostacycline I_2; PGI_3, Prostacycline I_3; Chol, Cholesterol; CO, cyclooxygenase; LO, lipoxygenase; PL, phospholipids; SFA, saturated fatty acid; FL, fluidity; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; and EDRF, endothelium-derived relaxing factor (nitric acid).

The findings that oleic and stearic acids also decrease serum cholesterol levels challenges many long-standing assertions. By focusing on lipids and their effects on systemic and cellular peroxidation reactions, many discrepancies related to the "lipid hypothesis" can be explained. It has been shown that cholestane-3β,5α,6β-triol and 25-hydroxycholesterol produce endothelial damage both *in vivo* and *in vitro* (Peng et al., 1985; Hennig and Boissonneault, 1987; Liu et al., 2004). Linoleic hydroperoxides have also been shown to have potent endothelial cytotoxic effects *in vitro* (Yagi, 1988). Numerous studies have been published that clearly indicate that the lipid and protein peroxidation products and not the pure compounds possess the atherogenic properties (Addis, 1990; Hennig et al., 1993; Liu et al., 2004).

These lipids oxidation products exhibit very different physiological responses compared to the pure compounds. For example, (1) cholesterol oxides and not pure cholesterol are scavenged by macrophages; (2) linoleic hydroperoxides inhibit PGI_2 synthesis whereas the pure fatty acid does not; (3) cholesterol oxides and not pure cholesterol increase endothelial cell permeability; and

(4) LDL containing oxidized vs. pure cholesterol is more chemotactic toward recruitment of monocytes (Berliner and Heinecke, 1996).

What are the sources of these oxidized lipid products? In the human diet, cholesterol oxides and fatty acid hydroperoxides are most likely formed during deep frying, dehydration, and powdering processes. During deep frying of french fries using animal fat, both cholesterol oxides and fatty acid hydroperoxides, aldehyde epoxides, and/or hydroxy acids can be formed; using vegetable oils, only the cholesterol products would be eliminated. To date, we do not know the biopotencies of these compounds relative to each other and therefore cannot accurately predict their biological effects.

Once pure lipids are absorbed, there are a multitude of enzymatic cellular reactions that may form lipid oxidation end products, for example, cyclooxygenase, lipoxygenase, P-450, and other heme-containing enzymes. These enzymes, which introduce O_2 into lipid molecules, are protected by an elaborate network of antioxidant molecules and enzymes capable of inactivating free radical and peroxidation reactions; examples are vitamins E, C, β-carotene, superoxide dismutase, catalase, and glutathione peroxidase. If the balance is upset between these prooxidant and antioxidant reactions, deleterious lipid oxidation products may be formed. During injury, such as a skin incision, the release of lipid oxides may protect against pathogenic organisms via their cytotoxicity; however, if the injury and release of these lipid oxides is due to lack of antioxidant defense mechanisms or due to sustained endothelial injury, atherogenesis may be precipitated.

These concepts indicate that the n3 fatty acids may be most protective with respect to atherogenesis only if the antioxidant dietary constituents and cellular defense mechanisms are intact. Many of the physiological effects of n3 fatty acids can be explained as they relate to membrane function, changes in eicosanoid biosynthesis, and their influence on other bioactive compounds, such as PAF, NO, PDGF, TNF, and interleukin-1. However, since these n3 fatty acids are not readily converted to the 3-series eicosanoids, they can also be viewed as monooxygenase competitive inhibitors. It is possible that the overall effect of n3 fatty acids binding to these monooxygenases results in a decrease in lipid peroxide intermediates; in addition, the eicosanoids that are produced appear to be beneficial.

Perhaps the primary effect of dietary fatty acids, as they relate to atherogenesis, is via their influence on membrane composition and the subsequent increase or decrease in monooxygenase activities; excess cholesterol in the platelet membrane has been shown to increase TXA_2 production. TXA_2 biosynthesis is accompanied by equimolar amounts of malonyldialdehyde, a lipid oxidation product.

Since the first publication of this chapter in 1991, there has been a flurry of research activity related to oxidative stress (injury), lipid oxidation, and atherogenesis. Our understanding of how atherogenesis may be initiated by oxidative stress and the contribution of specific fatty acids and antioxidants, to the regulation of lipid and lipoprotein metabolism via these mechanisms has given us greater insight toward helping to ameliorate CVD. There is also a concomitant flurry of research activity looking at the interactions of nutraceuticals (e.g., plant bioflavanoids) and lipid peroxidation reactions that hold great promise in ameliorating CVD.

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45 Dietary Fatty Acids and Cancer

Howard Perry Glauert

CONTENTS

I. INTRODUCTION

The amount and type of fat consumed in the diet may be important in the development of human cancer. Several organizations have advocated decreasing the fat content of the diet as a means of preventing the development of cancer. For example, the American Institute for Cancer Research states to "limit consumption of fatty foods, particularly those of animal origin" (World Cancer Research Fund/American Institute for Cancer Research, 1997). On the other hand, the American Cancer Society no longer specifically recommends lowering fat intake, and instead advises individuals to "eat a variety of healthy foods, with an emphasis on plant sources" (American Cancer Society, 2006).

In this review, the role of dietary fat on the development of human and experimental cancer will be discussed. Because of the large number of studies published, reviews will be cited where possible. Cancer clearly is a disease of alterations both in genetic structure and in genetic expression, both of which can be affected by dietary fat.

II. EPIDEMIOLOGICAL STUDIES

Numerous epidemiological studies have examined the effect of dietary fat on human cancer. Several correlational studies have noted an increase in the rates of colon, breast, and other cancers in areas

where dietary fat consumption is high (Fraser, 1996). Additionally, studies with immigrant populations have identified dietary fat intake as a causative factor in the development of these cancers (Fraser, 1996).

For colon cancer, epidemiological studies have not reached a clear consensus about the influence of dietary fat. Case–control studies overall have not found a positive association with dietary fat, although many have observed a positive association with meat intake (Kushi and Giovannucci, 2002). Prospective epidemiological studies have conflicting results: some studies show a positive association, others see no effect, and others see an actual protective effect of high-fat intakes (Table 45.1). In several of these studies, the consumption of red meat was found to be significantly correlated with colon cancer risk, but independently of fat intake. The Women's Health Initiative intervention study was recently published (Beresford et al., 2006). In this study 19,500 women lowered their fat intake by about 10% compared to 29,000 women who did not alter their diet, for a follow-up period that averaged 8 years. The intervention group had a relative risk of 1.08, which was not statistically significant, indicating that a diet lower in fat did not inhibit the development of colon cancer in this study.

Numerous epidemiological studies have attempted to identify factors which influence breast cancer risk in humans. Established breast cancer risk factors include age of first pregnancy, body build, age at menarche or menopause, and the amount of radiation received by the chest (Kelsey and Berkowitz, 1988). The effect of dietary fat has been studied in correlational, case–control, and prospective epidemiological studies. Studies examining international correlations between dietary fat intake and breast cancer risk, and migrant studies have reported a positive association between dietary fat intake and breast cancer risk (Kelsey and Berkowitz, 1988). Case–control studies generally have also supported a connection between total fat intake and breast cancer risk (Kushi and Giovannucci, 2002). A combined analysis of 12 case–control studies found a significant positive association between breast cancer risk and saturated fat intake (Howe et al., 1990). Most prospective studies, however, did not find any link between dietary fat intake and the development of breast cancer (Table 45.2). Furthermore, a combined analysis of seven of these prospective studies did not find any evidence of a link between dietary fat intake and breast cancer risk, even for women consuming less than 20% of their calories as dietary fat (Hunter and Willett, 1996). The Women's Health Initiative intervention study (described in the preceding paragraph) examined the effect of low-fat diets on the development of breast cancer (Prentice et al., 2006). Although dietary fat did not significantly affect the development of breast cancer, there was a relative risk of 0.91 in the low-fat intervention group.

For the pancreas, international comparisons do not show as strong a trend as with colon or breast cancer (Howe and Burch, 1996). Case–control studies which have examined total or saturated fat intakes do not show a clear trend; studies examining the consumption of meat or cholesterol tend to show a positive correlation, however (Howe and Burch, 1996). In prospective studies, Nothlings et al. (2005) and Michaud et al. (2003, 2005) found that dietary fat did not influence the development of pancreatic cancer. However, Stolzenberg-Solomon et al. (2002) found that dietary fat enhanced the development of pancreatic cancer. Five prospective studies have examined the relationship of meat consumption with pancreatic cancer; four of the five reported a positive correlation (Hirayama, 1989; Michaud et al., 2003; Mills et al., 1988; Nothlings et al., 2005; Zheng et al., 1993).

A number of case–control and prospective studies have examined the role of dietary fat in prostate cancer. Several case–control studies observed a positive correlation between the intake of total and saturated fat and the development of prostate cancer, although others, particularly the more recent studies, did not see an effect (Kushi and Giovannucci, 2002; Wu et al., 2006). Four prospective studies have been performed, with two of these observing a positive association between dietary fat and prostate cancer incidence or mortality and two observing no effect (Kushi and Giovannucci, 2002; Wu et al., 2006).

Fewer studies have been conducted for other major forms of human cancer. For endometrial cancer, several but not all case–control studies have noted an association with dietary fat (Hill and

TABLE 45.1
Dietary Fat and Colorectal Cancer: Prospective Studies

Investigators	Subjects	Years of Follow-Up	Effect of Dietary Fat
Hirayama (1981)	265,118 subjects in Japan	13	Significant negative effect for meat
Stemmermann et al. (1984)	7,074 Hawaiian—Japanese men	15	Significant negative effect
Garland et al. (1985)	1,954 men in Chicago	19	No significant effect
Phillips and Snowdon (1985)	25,493 Seventh Day Adventists in California	21	No significant effect
Willett et al. (1990)	88,751 female nurses in United States	6	Significant positive effect for total, animal, monounsaturated, and saturated fat, and for red meat
Giovannucci et al. (1992)	7,284 male health professionals	2	Significant positive effect for total, animal, monounsaturated, and saturated fat, and for red meat
Thun et al. (1992)	764,343 men and women	6	No significant effect of fat or red meat
Goldbohm et al. (1994)	58,279 men and 62,573 women in Netherlands	3.3	No significant effect
Giovannucci et al. (1994)	47,949 male health professionals	6	No significant effect of fat, but significant positive correlation with red meat
Bostick et al. (1994)	35,215 women in Iowa	4	No significant effect
Gaard et al. (1996)	50,535 men and women in Norway	11	No significant effect of fat, but significant positive correlation with sausage intake
Chyou et al. (1996)	7,945 Japanese–American men in Hawaii	27–30	Significant negative correlation with total and monounsaturated fat for colon but not rectal cancer
Kato et al. (1997)	14,727 women in New York and Florida	7	No significant effect of fat or meat
Singh and Fraser (1998)	32,051 Seventh Day Adventist men and women in California	6	Significantly increased risk with red, white or total meat intake
Pietinen et al. (1999)	27,111 male smokers in Finland	8	No significant effect of fat or meat
Jarvinen et al. (2001)	9,959 Finnish men and women	27–32	High-cholesterol intake was associated with increased risk, but not consumption of total, saturated, monounsaturated or polyunsaturated fat
Terry et al. (2001a,b)	61,463 women in Sweden	9.6	No significant effect of intake of fat or a “Western” diet
Flood et al. (2003)	45,496 women in United States	8.5	No significant effect of fat or meat consumption
Chao et al. (2005)	148,610 men and women in United States	9, 19	Significant increase with red and processed meat consumption; poultry and fish consumption protective

TABLE 45.2
Dietary Fat and Breast Cancer: Prospective Studies

Investigator	Subjects	Years of Follow-Up	Effect of Dietary Fat
Jones et al. (1987)	5,485 women in United States	10	No significant effect
Willett et al. (1987)	89,538 nurses in United States	4	No significant effect
Mills et al. (1989)	20,341 Seventh Day Adventist women in California	6	No significant effect
Knekt et al. (1990)	3,988 women in Finland	20	No significant effect
Howe et al. (1991)	56,837 Canadian women	5	Slightly elevated risk
Graham et al. (1992)	18,586 women in New York State	7	No significant effect
Kushi et al. (1992)	34,388 women in Iowa	4	No significant effect
Willett et al. (1992)	89,494 nurses in United States	8	No significant effect
van den Brandt et al. (1993)	62,573 women in Netherlands	3.3	No significant effect
Toniolo et al. (1994)	14,291 women in New York City	6	No significant effect; but significant positive correlation with red meat
Gaard et al. (1995)	31,209 women in Norway	7–13	No significant effect for fat or saturated fat; but significant positive correlation with meat and monounsaturated fat
Holmes et al. (1999)	88,795 nurses in Unite States	14	No significant effect of total fat or specific fatty acids
Velie et al. (2000)	40,022 women in 29 centers throughout United States	5.3	No overall association; but among women with no history of benign breast disease, positive association between total and unsaturated fat intake and breast cancer risk
Thiebaut et al. (2001)	65,879 women in Europe	3.4	Small positive association between fat intake and breast cancer risk
Terry et al. (2001c)	61,463 women in Sweden	9.6	No association between “Western” dietary pattern and breast cancer risk
Byrne et al. (2002)	44,697 nurses in United States	14	No effect of fat in women with no history of benign breast disease

Austin, 1996; McCann et al., 2000; Salazar-Martinez et al., 2005). Case–control studies examining dietary fat and bladder cancer showed an association in some but not all studies; a prospective study did not observe a correlation between dietary fat intake and the development of bladder cancer (La Vecchia and Negri, 1996; Radosavljevic et al., 2005). Dietary fat has been found to be a risk factor for ovarian cancer in some epidemiological studies but not in others (Bertone et al., 2002; Kushi et al., 1999; Pan et al., 2004; Risch et al., 1994; Zhang et al., 2002). Lung cancer risk was not found to be significantly affected by dietary fat in two prospective studies, but several case–control studies have observed an association (Alavanja et al., 2001; Mohr et al., 1999; Ziegler et al., 1996), although several investigators indicated that their results may have been affected by confounding from smoking. For testicular cancer, case–control studies have observed an association between high-fat diets and increased incidence (Bonner et al., 2002; Sigurdson et al., 1999). Using case–control and cohort study designs, Granger et al. (2006) found that increased dietary fat consumption protected against the development of skin cancer. Davies et al. (2002), however, found that dietary fat did not influence basal cell carcinoma development. The development of esophageal cancer was found to be increased by dietary fat in a case–control study (Wolfgarten et al., 2001).

III. EXPERIMENTAL CARCINOGENESIS STUDIES

A. Nutritional Considerations

In most experiments examining the effect of dietary fat, fat is substituted for carbohydrate isocalorically in order to ensure that fat is the only experimental variable. In some studies, however, fat was added to a nutritionally complete diet, such as a ground unrefined diet. Since animals consume approximately the same number of calories, animals on such a high-fat diet would consume less of all other nutrients in the diet. Therefore, any changes seen in the high-fat group could not strictly be attributed to a higher consumption of fat. In other studies, fat was substituted for a carbohydrate (such as corn starch or dextrose) on a gram-for-gram basis. Since fat contains 9 kcal/g and carbohydrate contains only 4 kcal/g (National Research Council, 1989), such a protocol would also result in lower consumption of other nutrients in the diet.

Another issue in studies examining dietary fat and carcinogenesis is whether the enhancing effect of dietary fat, when it is seen, is due to dietary fat per se (e.g., by an effect on gene expression) or rather to a more efficient utilization of calories. Even when the ratio of calories to nutrients remains the same when the dietary fat content of the diet is raised, animals on high-fat diets frequently gain more weight than animals on a low-fat diet. When fat is substituted for carbohydrate isocalorically, the substitution is done on the basis of metabolizable energy rather than net energy. Donato and Hegsted (1985), however, have demonstrated that fat is used more efficiently than carbohydrate, so that equating their metabolizable energy values may be inaccurate.

B. Skin Carcinogenesis

Mouse skin is one of the oldest and most widely used systems for studying chemical carcinogenesis, including multistage carcinogenesis. Two-stage carcinogenesis (initiation–promotion) was first observed in mouse skin and involves initiation by a subcarcinogenic dose of radiation or of a chemical such as a polycyclic aromatic hydrocarbon (PAH) followed by the long-term administration of croton oil or its active ingredient 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Berenblum and Shubik, 1947). More recently, transgenic skin carcinogenesis models have been developed (Greenhalgh et al., 1996; Humble et al., 2005).

Most studies examining dietary fat have studied complete carcinogenesis by PAH or ultraviolet (UV) light. Early studies demonstrated that high-fat diets enhanced skin carcinogenesis induced by tar (Watson and Mellanby, 1930) or PAH (Baumann et al., 1939; Boutwell et al., 1949; Jacobi and Baumann, 1940; Lavik and Baumann, 1941, 1943; Tannenbaum, 1942, 1944). In studies where

skin tumors were induced by UV light, Mathews-Roth and Krinsky (1984) found that high-fat diets increased skin carcinogenesis, whereas Black et al. (1983) found that high-fat diets did not increase skin carcinogenesis, but that feeding a saturated fat inhibited tumorigenesis.

The effect of fatty acids on the initiation and promotion of skin carcinogenesis has also been studied. Certain fatty acids—oleic and lauric acids—were found to have promoting activity when applied daily to mouse skin after a single application of 7,12-dimethylbenz(a)anthracene (DMBA); stearic acid and palmitic acid did not have any effect (Holsti, 1959). When diets varying in their fat content were fed during the promotion stages of DMBA-initiated, TPA-promoted mouse skin carcinogenesis, high-fat diets were found to enhance the promotion of skin carcinogenesis in some studies (Birt et al., 1989b,c) but not in others (Lo et al., 1994; Locniskar et al., 1991b). High-fat diets also partially offset the tumor inhibitory effects of caloric restriction (Birt et al., 1996). Locniskar et al. (1990) found that substituting menhaden oil for corn oil or coconut oil did not affect skin tumor promotion by TPA. When benzoyl peroxide was used as the promoting agent, mice fed mainly coconut oil had the highest tumor incidence and mice fed corn oil had the lowest tumor incidence, with those fed mainly menhaden oil having an intermediate tumor incidence (Locniskar et al., 1991a). In a study using mezerein as the promoting agent, high-fat diets did not increase the skin carcinogenesis (Birt et al., 1994). High fat diets were found to not affect or slightly inhibit initiation (Birt et al., 1989c; Locniskar et al., 1991c), and substituting coconut oil for corn oil did not influence UV-induced skin carcinogenesis (Berton et al., 1996).

C. Hepatocarcinogenesis

Many early studies of dietary fat and cancer used the liver as the target organ. In these studies, aromatic amines and azo dyes were frequently used to induce hepatocellular carcinomas. In later studies, effects of dietary fat on initiation and promotion in the liver were examined. In initiation–promotion protocols, the administration of a single subcarcinogenic dose of a carcinogen (such as diethylnitrosamine (DEN) or DMBA) along with a proliferative stimulus (such as partial hepatectomy) followed by the long-term feeding of chemicals such as phenobarbital, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or polyhalogenated biphenyls leads to a high incidence of hepatocellular adenomas and carcinomas (Glauert et al., 2001; Pitot and Dragan, 1994). Transgenic mouse models of liver carcinogenesis have also been developed (Calvisi and Thorgeirsson, 2005). In addition, foci of putative preneoplastic hepatocytes appear before the development of gross tumors. These foci, known as altered hepatic foci or enzyme-altered foci, contain cells which exhibit qualitatively altered enzyme activities or alterations in one or more cell functions (Pitot and Dragan, 1994). The enzymes most frequently studied include γ-glutamyl transpeptidase (GGT) and placental glutathione-*S*-transferase (PGST), which are normally not present in adult liver but which are often present in foci; and ATPase and glucose-6-phosphatase, which are normally present but which are frequently missing from foci (Hendrich et al., 1987; Pitot et al., 1985). Altered hepatic foci can also be identified on hematoxylin and eosin stained tissue (Bannasch et al., 1989; Harada et al., 1989). The appearance of foci has been correlated with the later development of malignant neoplasms (Emmelot and Scherer, 1980; Kunz et al., 1983).

The first studies examined the effect of dietary fat on the induction of hepatocellular carcinomas by complete hepatocarcinogens. In the liver, increasing the fat content of the diet enhances the development of 2-acetylaminofluorene- (AAF), *p*-dimethylaminoazobenzene- (DAB), and aflatoxin B_1 (AFB)-induced tumors and GGT-positive foci in rats (Baldwin and Parker, 1986; Hietanen et al., 1991; Kline et al., 1946; McCay et al., 1980; Sugai et al., 1962). Furthermore, hepatocarcinogenesis by DAB is enhanced by feeding a diet which contains a greater proportion of polyunsaturated fatty acids (Miller et al., 1944a,b). In these studies, however, the diets were administered at the same time as the carcinogen injections, so that the stage of carcinogenesis which was affected could not be determined.

More recent studies have examined whether this enhancement of hepatocarcinogenesis is caused by an effect on the initiation of carcinogenesis, the promotion of carcinogenesis, or both.

Misslbeck et al. (1984) found that increasing the corn oil content of the diet after the administration of ten doses of aflatoxin increased the number and size of GGT-positive foci, but Baldwin and Parker (1987), using a similar protocol, found no effect of dietary corn oil. Glauert and Pitot (1986) similarly found that increasing the safflower oil or palm oil content of the diet did not promote DEN-induced GGT-positive foci or greatly affect phenobarbital promotion of GGT-positive foci. The promotion of GGT-positive foci by dietary tryptophan also is not affected by dietary fat (Sidransky et al., 1986). Newberne et al. (1979) found that increasing dietary corn oil (but not beef fat) during and after the administration of AFB increased the incidence of hepatic tumors, but not when the diets were fed only after AFB administration. Baldwin and Parker (1987) also found that increasing the corn oil content of the diet before and during AFB administration increased the number and volume of GGT-positive foci. When rats are fed diets high in polyunsaturated fatty acids (but not in saturated fatty acids) before receiving the hepatocarcinogen DEN, they develop more GGT-positive and ATPase-negative foci than rats fed low-fat diets (Glauert et al., 1991). Finally, the feeding of diets high in corn oil but not lard enhanced the initiation of PGST-positive foci induced by azoxymethane (AOM) (Rahman et al., 2001). The results of these studies suggest that the enhancement of hepatocarcinogenesis by dietary fat is primarily due to an effect on initiation, and that polyunsaturated fats have a greater effect than do saturated fats.

D. Colon Carcinogenesis

Studies in experimental animals have produced differing results. A variety of chemicals have been used to induce colon tumors, usually in rats or mice. These include 1,2-dimethylhydrazine (DMH) and its metabolites AOM and methylazoxymethanol (MAM); 3,2'-dimethyl-4-aminobiphenyl (DMAB); methylnitrosourea (MNU); and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) (Druckrey, 1970; Nauss et al., 1984; Reddy and Ohmori, 1981; Rogers and Nauss, 1985). DMH and AOM have been used most frequently to study nutritional effects. Both can induce colon tumors by single (Decaens et al., 1989; Glauert and Weeks, 1989; Karkare et al., 1991; Schiller et al., 1980; Ward, 1975) or multiple (Bull et al., 1979; Glauert et al., 1981; Nauss et al., 1983; Reddy et al., 1974; Sakaguchi et al., 1986) injections. The Min mouse, which has a mutation in the mouse homolog of the adenomatous polyposis coli (APC) gene, develops colon tumors spontaneously and is used as a model of colon carcinogenesis (Thompson, 1997). In addition to tumors, putative preneoplastic lesions, aberrant crypt foci (ACF), are induced by colon carcinogens (Bird, 1987). ACF, which are identified by fixing the colon in formalin and then staining with methylene blue, are stained darker and are larger than normal crypts (Bird, 1987). Some but not all studies have shown that ACF correlate well with the later appearance of adenocarcinomas (Alrawi et al., 2006; Hardman et al., 1991; Pereira et al., 1994; Wargovich et al., 1996).

Animal studies examining the effect of dietary fat have used a variety of protocols, and the results obtained often have been dependent on the investigator's protocol. In these studies, rats or mice were subjected to multiple doses of a colon carcinogen, with the dietary fat content being varied (isocalorically) during, and frequently before or after, the carcinogen injections. Some of these studies found an enhancement when the dietary fat content of the diet was increased, but others saw no effect or even an inhibition of tumor development (Clinton et al., 1992; Glauert et al., 1981; Guillem et al., 1988; Hardman and Cameron, 1995; Rao et al., 2001; Rijnkels et al., 1997; Takeshita et al., 1997; Wargovich et al., 1990; Wijnands et al., 1999; Zhao et al., 1991). High-fat diets were found to influence the early stages of carcinogenesis more than the later stages (Bird et al., 1996). In several studies where fat was found to enhance colon carcinogenesis, fat was either added to a chow diet or was substituted for carbohydrate on a weight basis, so that the ratio of calories to essential nutrients was altered; therefore the effect could have been due to a lower consumption of essential nutrients rather than to an effect of fat (Glauert, 1993). In the Min model, high-fat diets were found to increase colon carcinogenesis in one study but not another (van Kranen et al., 1998; Wasan et al., 1997). Increasing the fat content of the diet has been found to increase the number

of ACF induced by colon carcinogens in several but not all studies (Baijal et al., 1998; Hambly et al., 1997; Ju et al., 2003; Koohestani et al., 1997; Kristiansen et al., 1995; Lafave et al., 1994; Liu et al., 2001; Morotomi et al., 1997; Parnaud et al., 1998; Rao et al., 2001; Wan et al., 2000). The type of fat (unsaturated vs. saturated) in the diet also produced conflicting results (Parnaud et al., 1998; Sakaguchi et al., 1984; Takeshita et al., 1997). ω-3 fatty acids can also influence colon carcinogenesis: feeding fish or flaxseed oil in place of corn oil, or eicosapentaenoic acid in place of linoleic acid, decreases the development of DMH- or AOM-induced colon tumors, but adding menhaden oil to a low-fat diet does not affect colon carcinogenesis (Bartoli et al., 2000; Dommels et al., 2003; Dwivedi et al., 2005; Latham et al., 1999; Minoura et al., 1988; Nelson et al., 1988; Rao et al., 2001; Reddy and Maruyama, 1986; Reddy and Sugie, 1988). Olive oil, high in ω-9 fatty acids, was also found to inhibit colon carcinogenesis when substituted for polyunsaturated fatty acids (Bartoli et al., 2000).

E. Pancreatic Carcinogenesis

Dietary fat has been studied extensively in animal models. A common model is induction of pancreatic tumors by azaserine; however, azaserine produces tumors in acinar cells (Grippo and Sandgren, 2005), whereas the primary site in humans is the ductal cell. Tumors can be produced in pancreatic ductal cells in hamsters, by the chemicals *N*-nitroso-*bis*-(2-oxypropyl)amine (BOP) and *N*-nitroso-*bis*-(2-hydroxypropyl)amine (BHP) (Grippo and Sandgren, 2005). A number of transgenic models have been developed (Grippo and Sandgren, 2005; Leach, 2004). A number of models used regulatory elements from the rat elastase gene, which targets acinar cells. These constructs produced acinar tumors or mixed acinar/ductal tumors (Grippo and Sandgren, 2005; Leach, 2004). Recently, a new model has been developed, which uses an oncogenic K-ras ($KRAS^{G12D}$) inserted into the endogenous K-ras locus (Hingorani et al., 2003). The gene has a Lox-STOP-Lox (LSL) construct inserted upstream. These mice are interbred with mice containing the Cre recombinase downstream from a pancreatic specific promoter, either PDX-1 or P48. The PDX-1-Cre;LSL-$KRAS^{G12D}$ mice develop pancreatic intraepithelial neoplasia (PanINs), which progress over time (Hingorani et al., 2003). In addition, when these mice are crossed to mice containing p53 mutations or Ink4a/Arf deficiency, the rapid development of pancreatic adenocarcinomas is observed (Aguirre et al., 2003; Hingorani et al., 2005).

Dietary fat has been found to influence tumorigenesis in both rats and hamsters. In rats, feeding high-fat diets after, or during and after, the injection of azaserine enhances the development of pancreatic tumors and putative preneoplastic lesions (O'Connor et al., 1985a; Roebuck, 1986; Roebuck et al., 1981a,b, 1985, 1987; Woutersen and van Garderen-Hoetmer, 1988; Woutersen et al., 1989a,b). Pancreatic carcinogenesis induced by *N*-nitroso(2-hydroxypropyl)(2-oxopropyl)amine in rats is also enhanced by feeding high-fat diets (Longnecker et al., 1985). In several of these studies, the effect cannot be attributed unequivocally to dietary fat because fat was substituted for carbohydrate on a weight basis. In hamsters, BOP-induced pancreatic carcinogenesis is also increased by feeding high-fat diets (Birt and Pour, 1983; Birt et al., 1981, 1989a, 1990; Herrington et al., 1997; Woutersen and van Garderen-Hoetmer, 1988; Woutersen et al., 1989a,b). Roebuck and colleagues (Roebuck, 1986; Roebuck et al., 1981a, 1985) found that polyunsaturated fat, but not saturated fat, enhanced pancreatic carcinogenesis, and that a certain level of essential fatty acids are required for the enhancement of pancreatic carcinogenesis. Increased linoleic acid was also found to increase metastases to the liver in hamsters (Wenger et al., 1999). Appel et al. (1994), however, found that increasing the linoleic acid content of the diet did not increase pancreatic carcinogenesis in either rats or hamsters. Birt et al. (1990) found that feeding a saturated fat (beef tallow) enhanced pancreatic carcinogenesis in hamsters greater than a polyunsaturated fat (corn oil). Studies using fish oil have produced differing results, depending on the experimental protocol. Substituting fish oil for oils high in polyunsaturated fats decreases (O'Connor et al., 1985b, 1989) or does not affect (Appel and Woutersen, 1994) the development of azaserine-induced preneoplastic lesions in rats. Adding fish oil to a diet-containing adequate polyunsaturated fatty acids enhances azaserine-induced carcinogenesis in rats and BOP-induced carcinogenesis in hamsters (Appel and Woutersen, 1995, 1996, 2003).

Finally, it has been observed in 2-year carcinogenesis studies in which corn oil gavage has been used as the vehicle for the carcinogen that a higher incidence of pancreatic acinar cell adenomas is present in corn oil gavage-treated male Fischer-344 control rats than in untreated controls (Eustis and Boorman, 1985; Haseman et al., 1985). This association was not observed in female rats or in male or female $B6C3F_1$ mice.

F. Mammary Carcinogenesis

The effect of dietary fat on mammary carcinogenesis in experimental animals has been examined extensively: over 100 experiments have been conducted (Fay et al., 1997; Freedman et al., 1990; Welsch, 1995). The primary model used is a rat model (usually the Sprague-Dawley strain) in which mammary tumors are induced by DMBA or MNU. Genetically engineered models have also been developed, in which the Erbb2 or simian virus 40 (SV40) T/t-antigens are overexpressed in mammary epithelial cells (Green and Hudson, 2005). The use of these models is advantageous because tumor latency, tumor size, and tumor progression can easily be quantified by palpation of mammary tumors as they appear. Increasing the fat content of the diet clearly enhances the development of mammary tumors (Fay et al., 1997; Freedman et al., 1990; Welsch, 1995). In the rat model, a high-fat diet increases tumorigenesis both when it is fed during and after carcinogen administration, and when it is fed only after carcinogen injection. Feeding a diet high in fish oil instead of a diet high in polyunsaturated fat decreases the incidence of DMBA-induced tumors in rats (Fay et al., 1997; Freedman et al., 1990; Welsch, 1995). A meta-analysis of experimental animal studies found that n-6 fatty acids strongly enhanced carcinogenesis, saturated fatty acids were weaker at enhancing carcinogenesis, monounsaturated fatty acids had no effect, and n-3 fatty acids weakly (but nonsignificantly) inhibited carcinogenesis (Fay et al., 1997).

G. Other Sites

Dietary fat has also been studied for its effect on experimental carcinogenesis in other organs. In the lung, dietary fat enhanced benzo[a]pyrene- (BP) or BOP-induced carcinogenesis in hamsters (Beems and van Beek, 1984; Birt and Pour, 1983), whereas in mice a high-fat diet did not affect spontaneous carcinogenesis in one study but the feeding of egg extracts enhanced it in another (Szepsenwol, 1964; Tannenbaum, 1942). In the prostate, several studies have found that high-fat diets enhance the growth of transplantable prostate tumors, but that inconsistent effects are seen in chemically induced prostate carcinogenesis models (Leung et al., 2002; Mori et al., 2001; Rose, 1997; Zhou and Blackburn, 1997).

IV. MECHANISMS BY WHICH DIETARY FAT MAY INFLUENCE CARCINOGENESIS

A. Membrane Fluidity

An important function of dietary fatty acids is their presence in membrane lipids. Altering the fatty acid content of the diet alters the composition of membrane lipids, particularly in certain tissues; feeding diets high in n-6 or n-3 fatty acids increases the concentrations of these fatty acids in membrane lipids (Murphy, 1990). The activities of membrane-bound enzymes are increased in membranes that are more fluid, that is, that have a higher content of polyunsaturated fatty acids (Murphy, 1990). The alteration by dietary fatty acids of the catalytic abilities of membrane-bound enzymes, such as cytochrome P-450, may play an important role in carcinogenesis.

B. Toxicity

One possible mechanism by which dietary fat may enhance carcinogenesis is by the toxicity of fatty acids or of metabolites that increase after the feeding of high-fat diets. Such toxicity would bring

about a proliferative response in the tissue to replace lost cells. Cellular genes involved in cell proliferation, including cellular oncogenes, would likely be increased.

In the colon, toxicity may play a role in the enhancement of carcinogenesis by dietary fat. One hypothesis for the effect of dietary fat is that dietary fat increases the concentration of metabolites with carcinogenic or promoting activity in the fecal stream. Bile acids, particularly secondary bile acids, have promoting activity in the colon (Narisawa et al., 1974; Reddy et al., 1976, 1977b); their concentration in the feces has been found to be increased by dietary fat in some but not all studies (Gallaher and Franz, 1990; Glauert and Bennink, 1983; Reddy et al., 1974, 1977a, 1980). Bile acids function as detergents; therefore, high concentrations may be toxic to epithelial cells in the colon. Several studies have shown bile acids to induce apoptosis (Bernstein et al., 2005). This may result in a compensatory increase in cell proliferation; most studies have found that increasing the concentration of bile acids *in vivo* or *in vitro* increases colon epithelial cell proliferation (Bartram et al., 1997; Cheng and Raufman, 2005; Cohen et al., 1980; Deschner and Raicht, 1979; Deschner et al., 1981; Milovic et al., 2002; Ochsenkuhn et al., 1999, 2003; Peiffer et al., 1997; Skraastad and Reichelt, 1988; Wargovich et al., 1983). In addition, several studies found that bile acids induced DNA damage (Bernstein et al., 2005).

C. Eicosanoid Metabolism

Another mechanism by which dietary fat may influence carcinogenesis is by altering the synthesis of eicosanoids. Fatty acids that are consumed in the diet can be metabolized to a variety of other compounds, including longer and more unsaturated fatty acids, prostaglandins, leukotrienes, thromboxanes, hydroperoxyeicosatetraenoic acids, and hydroxyeicosatetraenoic acids (Rosenthal, 1987). Altering the type of fatty acid in the diet has been found to change the amounts and composition of the eicosanoids that are produced by the body (McEntee and Whelan, 2002; Whelan and McEntee, 2004). n-3 Fatty acids antagonize the metabolism of arachidonic acid to eicosanoids, which may be a mechanism in their inhibition of carcinogenesis (McEntee and Whelan, 2002; Whelan and McEntee, 2004). Specific eicosanoids bind to receptors and cause specific alterations in gene expression and cellular function (Hanasaki and Arita, 2002; Kobayashi and Narumiya, 2002; Toda et al., 2002; Tsuboi et al., 2002), some of which may be related to carcinogenesis. It has been found that inhibition of eicosanoid synthesis inhibits tumor promotion in several tissues (Fischer et al., 1987; Mao et al., 2005; Richter et al., 2001; Steele et al., 2000).

D. Caloric Effect

The issue of whether the enhancing effect of fat in carcinogenesis is due to higher consumption of calories or more efficient utilization of energy has been examined in several tissues. The earliest study was conducted by Boutwell et al. (1949) using the mouse skin carcinogenesis system; they attributed most of the enhancing effect of dietary fat to an increased consumption of calories. Birt et al. (1989c), however, found that the promotion of skin carcinogenesis was enhanced even though the high-fat diets were pair fed. The greater caloric density of fat has also been proposed to play a role in colon tumorigenesis. Caloric restriction inhibits chemically induced colon carcinogenesis, even if the percentage of dietary fat in the diet is greatly increased (Klurfeld et al., 1987; Reddy et al., 1987). In the pancreas, the enhancement by dietary fat appears to be an effect of dietary fat rather than of an increased consumption of calories, as pair feeding does not inhibit the enhancing effect of dietary fat in hamsters (Birt et al., 1989a). Several studies have suggested that the enhancement of mammary carcinogenesis by dietary fat may be caused, at least in part, by an alteration in the efficiency of energy utilization (Boissonneault et al., 1986; Thompson et al., 1985; Welsch et al., 1990). Using a combined statistical analysis of over 100 animal experiments, Fay et al. (1997) found that energy intake was not responsible for the enhancing effect of polyunsaturated fats on mammary carcinogenesis, although there was a slight (but not significant) effect. Finally, caloric restriction has been found to inhibit tumorigenesis in many tissues in experimental animals (Boissonneault, 1991).

E. Effect on Initiation

Dietary fat may also affect the initiation stage of carcinogenesis. Since initiation involves the mutation of DNA, its alteration (by dietary fat or other agents) would mainly affect the structure of genes rather than their expression. In rat liver, dietary fat appears to enhance carcinogenesis primarily by an effect on initiation. In other tissues, many protocols have varied the levels of dietary fat during the time of carcinogen injections; therefore dietary fat may be affecting some aspect of initiation in these studies. Higher levels of dietary fat may enhance initiation of carcinogenesis by several mechanisms, including alterations in absorption of the carcinogen from the gut, transport to the target organ, uptake by the target organ, metabolism by cytochrome P-450 or other drug-metabolizing enzymes to a form which can react with DNA, and DNA repair. Several of these processes occur in membranes, whose lipid composition can be altered by changing the amount or type of dietary fat (Baldwin and Parker, 1985; Neelands and Clandinin, 1983). Increasing the fat content of the diet increases cytochrome P-450 and related activities (Cassanol et al., 1987; Hammer and Wills, 1980; Rutten and Flake, 1987; Wade et al., 1978). Western analysis has indicated that higher amounts of enzyme protein are present after feeding diets high in polyunsaturated fat (Kim et al., 1990). Therefore dietary fat may be affecting both gene expression and the surrounding matrix necessary for optimum enzyme activity. The metabolism of several chemicals, including hexobarbital, aniline, ethylmorphine, BP, and dimethylnitrosamine, is also enhanced by feeding diets high in polyunsaturated fatty acids (Hammer and Wills, 1980; Lam and Wade, 1980, 1981; Wade et al., 1978, 1982).

F. Lipid Peroxidation

Another way in which dietary fat could affect carcinogenesis is through lipid peroxidation. Polyunsaturated fatty acids are susceptible to lipid peroxidation; therefore diets high in polyunsaturated fat could result in increased consumption of oxidized lipids present in the diet or increased lipid peroxidation in the body. Lipid peroxidation could affect carcinogenesis in a number of ways. Several products of lipid peroxidation are very toxic (Chow, 1999) and could influence the carcinogenic process through toxicity as described earlier. Lipid peroxidation products have the potential to exert genotoxicity and therefore could bring about tumor initiation. One of the products of lipid peroxidation is malondialdehyde, which forms DNA adducts and is mutagenic (Marnett, 2002). Another potential product is the hydroxyl radical, which can form DNA adducts such as 8-hydroxyguanosine (Poulsen, 2005). Finally, oxidation products could act on signal transduction pathways leading to altered cell proliferation or apoptosis (West and Marnett, 2006).

G. Induction of Specific Gene Expression

Altering the level of dietary fat changes the expression of many genes. Most of the genes studied, however, are related to carbohydrate or lipid metabolism and are not likely to play a role in carcinogenesis (Hillgartner et al., 1995). Dietary fat may alter signal transduction pathways that lead to altered cell proliferation or apoptosis. Fatty acids have been found to bind to several transcription factors, including the peroxisome proliferator-activated receptors (PPARs), PPARα, PPARβ, and PPARγ; the liver X receptors (LXRs), LXRα and LXRβ; and hepatocyte nuclear factor-4 (HNF-4) (Jump, 2004). PPARα activators induce hepatic tumors, but only in specific rodent species (O'Brien et al., 2005); PPARγ, however, has antineoplastic properties (Wang et al., 2006). Several studies have examined effects on protein kinase C (PKC), which consists of at least 12 subtypes (Jump, 2004). Increasing the fat content of the diet has been found to increase PKC activity in the colon, skin, and mammary gland (Birt, 1995; Hilakivi-Clarke and Clarke, 1998; Lafave et al., 1994; Pajari et al., 1997; Rao et al., 2000; Reddy et al., 1996).

Several papers have been published on the effect of dietary fat on the expression of oncogenes and tumor suppressor genes. In the colon, Guillem et al. (1988) found that dietary fat did not affect the expression of either c-*myc* or c-H-*ras* oncogenes in normal or tumor tissue, whereas Singh et al. (1997)

found that dietary corn oil increased AOM-induced expression of *ras*. In contrast, fish oil was found to inhibit *ras* expression. In the mammary gland, DeWille et al. (1993) found that high corn oil diets increased *ras* mRNA levels in mammary tumors from MMTV/v-Ha-*ras* transgenic mice. However, high-fat diets decreased the frequency of *ras* mutations in rat mammary gland tumors induced by the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (Roberts-Thomson and Snyderwine, 1997). Substituting n-3 fatty acid-rich oils for corn oil decreased the expression of the H-*ras* oncogene in the mammary gland in one study but had no effect in the other (Karmali et al., 1989; Ronai et al., 1991).

V. SUMMARY AND CONCLUSIONS

Clearly there is much variability in studies of dietary fat and cancer, both in epidemiological and experimental studies. In epidemiological studies, a relationship between dietary fat and breast cancer has been found in correlational and case–control studies, but prospective studies do not support a role for dietary fat. Prospective epidemiological studies examining the role of dietary fat in the development of colon, pancreatic, and prostate cancers have produced conflicting results. The Women's Health Initiative intervention studies did not show any significant effects for dietary fat in the development of either colon or breast cancer in women. In experimental studies, dietary fat generally enhances chemically induced skin, liver, pancreatic, and mammary carcinogenesis, whereas conflicting results have been seen in colon carcinogenesis. Dietary fat appears to act primarily during the promotional stage of carcinogenesis in all of these models except the liver, where the effect of dietary fat is primarily on initiation. Because of the variability seen in studies of dietary fat and cancer (particularly prospective epidemiological studies), it cannot be stated unequivocally at this time that human cancer can be prevented by decreasing the fat content of the diet.

The mechanisms by which high-fat diets enhance experimental carcinogenesis are unclear, but probably involve several mechanisms, some of which are organ specific. Nearly all of the mechanisms by which dietary fat may influence carcinogenesis involve alterations in genetic expression. The determination of genes which are turned on or off by dietary fat or its metabolic or oxidative products will likely provide answers as to the role of dietary fat in carcinogenesis.

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46 Fatty Acids and Renal Disease

Stuart K. Ware

CONTENTS

I. INTRODUCTION

It has long been known that diet affects kidney function. However, whether lipids and their metabolic products affect the development of kidney disease is still under investigation. Various polyunsaturated fatty acids (PUFAs) serve as precursors for the production of eicosanoids (ECs), which are known to affect blood flow and blood pressure in the kidney, platelet aggregation, and inflammation. EC metabolism appears altered in a variety of renal pathophysiological states. Enhanced synthesis of certain ECs has been reported in various renal diseases and may delay further deterioration of kidney function, and the administration of such ECs often retards the development of or improves the disease. However, other ECs may contribute to renal disease as suggested by the improvement in renal function that occurs when inhibitors of these particular compounds are administered. Thus, the dietary PUFA intake may modulate some renal diseases, because the types of ECs produced are dependent on the quantity and composition of the dietary fatty acids. Indeed, a reduction in the progression of renal injury has been demonstrated in experimental animals given fatty acid precursors of ECs. Because certain ECs seem to promote renal injury, whereas others appear to protect the diseased kidney from further damage, the dietary significance of these observations are not yet clear. Other, noneicosanoid-mediated effects of fatty acids that may be beneficial or harmful to renal function include changes in blood rheological properties, membrane composition and function, and serum lipid concentrations.

II. BASIC RENAL FUNCTION

The kidneys are the guardians of the internal environment. That is, maintenance of the composition and volume of the extracellular fluid (and indirectly the composition of the intracellular fluid) is the primary function of the kidneys. Body balances of water and of many of the electrolytes of the extracellular fluid such as sodium, potassium, chloride, and inorganic acids are regulated primarily by the kidneys (i.e., the intake and/or metabolic production of such substances must equal their excretion and/or metabolic consumption). The kidneys are also important in excreting waste products such as urea, uric acid, and creatinine and in the elimination from the body of many foreign chemicals such as drugs, pesticides, food additives, and their metabolites. Other significant functions of the kidneys include the regulation of arterial blood pressure through their role in regulating sodium balance and blood volume, their participation in the renin–angiotensin system, and their production of various vasoactive substances such as ECs; the regulation of erythrocyte production by the bone marrow via the renal hormone erythropoietin; the hydroxylation of vitamin D to its active form; and gluconeogenesis during starvation.

Each kidney is composed of roughly 800,000 microscopic units called nephrons, which are considered to be the basic functional units of these organs (Figure 46.1). Since each nephron is capable of forming urine by itself, kidney function can be described by explaining the function of a single nephron. Each nephron is composed of a filtering component called the renal corpuscle (consisting of glomerular capillaries and Bowman's capsule), a renal tubule extending from it, and a peritubular capillary bed. The glomerular capillaries extend into the hollow Bowman's capsule, much like a fist pushed into an inflated balloon. The space inside the capsule (balloon) is Bowman's space, and it is into this cavity that fluid filters from the glomerular capillaries by crossing the capillary and capsular membranes. The vessel delivering blood to the glomerular capillaries is called the afferent arteriole (it descends from the renal artery), and blood leaving the glomerular capillaries enters into an efferent arteriole. Bowman's space is continuous with the first portion of the tubule, and the glomerular filtrate entering Bowman's space simply continues on into the tubule. The tubule itself, composed of a single layer of epithelial cells continuous with that of Bowman's capsule,

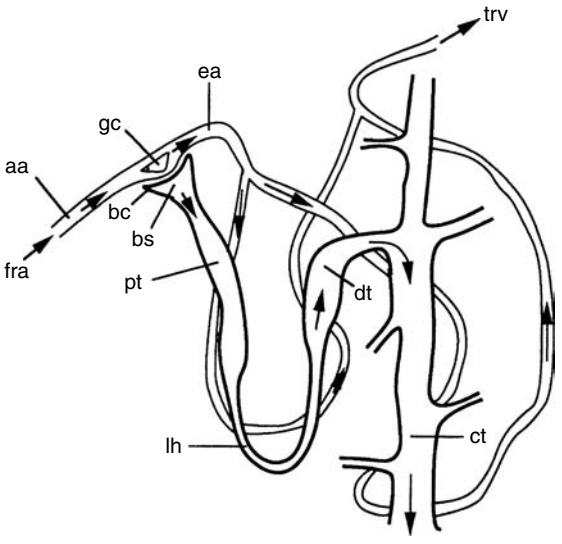

FIGURE 46.1 Nephron structure. aa, afferent arteriole; bc, Bowman's capsule; bs, Bowman's space; ct, collecting tubule; dt, distal tubule; ea, efferent arteriole; fra, blood from renal artery; gc, glomerular capillaries; lh, loop of Henle; pt, proximal tubule; trv, blood returning to renal vein. Arrows show direction of blood and filtrate flow. The blood vessels branching from ea are the peritubular capillaries.

is divided into segments corresponding to differences in morphology and function. The proximal tubule drains Bowman's capsule and extends into the deeper portion (medulla) of the kidney. The tubule then forms a hairpin loop (the loop of Henle) and returns toward the kidney surface (cortex) as the distal tubule. The distal tubule eventually joins the collecting tubule, which again runs deep into the kidney. The collecting tubule conveys fluid from several nephrons into the renal pelvis. The fluid is not altered in composition after it leaves the collecting tubule; thus, fluid in the renal pelvis is identical to urine, and urinary structures distal to this point are used simply to transport or temporarily store the urine.

The renal corpuscle acts as a high-pressure filter. Normally, about 20% of the blood plasma entering the glomerular capillaries is filtered into Bowman's space. The remainder moves on into the efferent arteriole. This arteriole soon divides into a set of capillaries, the peritubular capillaries, which are distributed to all portions of the renal tubule. This arrangement allows for the transport (not filtration) of water and solutes between the tubular lumen and the peritubular capillaries (Figure 46.2). The peritubular capillaries eventually drain into the renal vein, which returns blood to the general systemic circulation. Although not shown in Figure 46.1, the peritubular capillaries of a given nephron usually associate with the tubules of several nephrons, and each tubule is supplied with peritubular blood from several other nephrons.

There are three basic processes by which urine is formed: filtration, reabsorption, and secretion (Figure 46.2). Owing to the pressure of the blood in the glomerular capillaries, an essentially protein-free plasma is filtered into Bowman's space. Fluid that becomes glomerular filtrate must pass through several membranes making up the renal corpuscle (capillary endothelium, basement membranes, and Bowman's capsule epithelium). These membranes do not hinder the passage of small molecules (such as water, electrolytes, and nutrients) but large protein molecules cannot cross. As this filtrate passes through the renal tubule, its composition and volume are altered by the processes of reabsorption and secretion. Each substance in plasma is precisely regulated by a particular combination of filtration, reabsorption, and/or secretion, and these three basic processes are controlled by complex homeostatic mechanisms occurring both inside and outside the kidney. For most substances, the rate of reabsorption is the primary mechanism used to control their concentrations in the body fluids (notable exceptions are potassium and hydrogen ions that are controlled

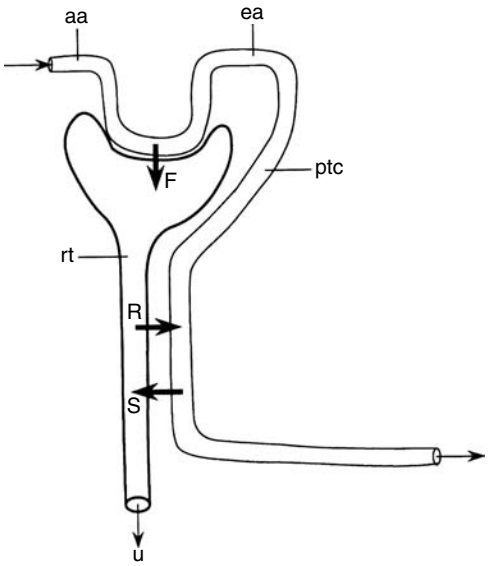

FIGURE 46.2 Basic nephron function. aa, afferent arteriole; ea, efferent arteriole; F, glomerular filtration; ptc, peritubular capillary; R, reabsorption; rt, renal tubule; S, secretion; u, urine flow (excretion).

primarily by secretion). That is, most substances in plasma are filtered into Bowman's space, and then as the filtrate flows through the renal tubule, unwanted substances fail to be reabsorbed and are thus excreted in urine, whereas needed substances are reabsorbed back into the blood. The kidneys filter large quantities of water and solutes from the plasma into the renal tubules each day (the glomerular filtration rate [GFR] is normally about 125 mL/min; that is, each minute about 125 mL of protein-free plasma is filtered into Bowman's space). Again, the rate at which such filtered substances are reabsorbed is the key to their regulation. Reabsorption of many solutes occurs by active transport. Water moves passively by osmosis once an osmotic gradient is established by active solute (primarily sodium chloride) reabsorption. Between 70% and 90% of the glomerular filtrate is reabsorbed from the proximal tubule, including nutritionally important solutes such as glucose, vitamins, and amino acids.

To summarize, the glomerular filtrate (plasma minus proteins) derived from the renal blood traverses the renal tubule and enters the pelvis of the kidney as urine. Along the way, substances are selectively reabsorbed (water, electrolytes, and nutrients) or secreted (potassium and hydrogen ions) by the tubular epithelium. Reabsorption plays a much greater role than does secretion in the formation of urine. About 99% of the water in the glomerular filtrate is eventually reabsorbed. Constituents of the filtrate not reabsorbed to the same extent as water will therefore be concentrated in the urine, and their concentrations in the extracellular fluids will decrease. In this way, the nephron separates substances that are to be conserved from those that are to be eliminated. For additional information on basic renal function, the reader should consult textbooks on renal physiology such as those of Rennke and Denker (2006) and Brenner et al. (1987a).

III. RENAL EICOSANOIDS AND LEUKOTRIENES

The ECs and leukotrienes (LTs) are groups of bioactive lipids synthesized from 20-carbon polyunsaturated essential fatty acids, and enzymes involved in their metabolism are present in the tissues of the kidneys, especially the renal medulla (Figure 46.3). The numbering of the compounds

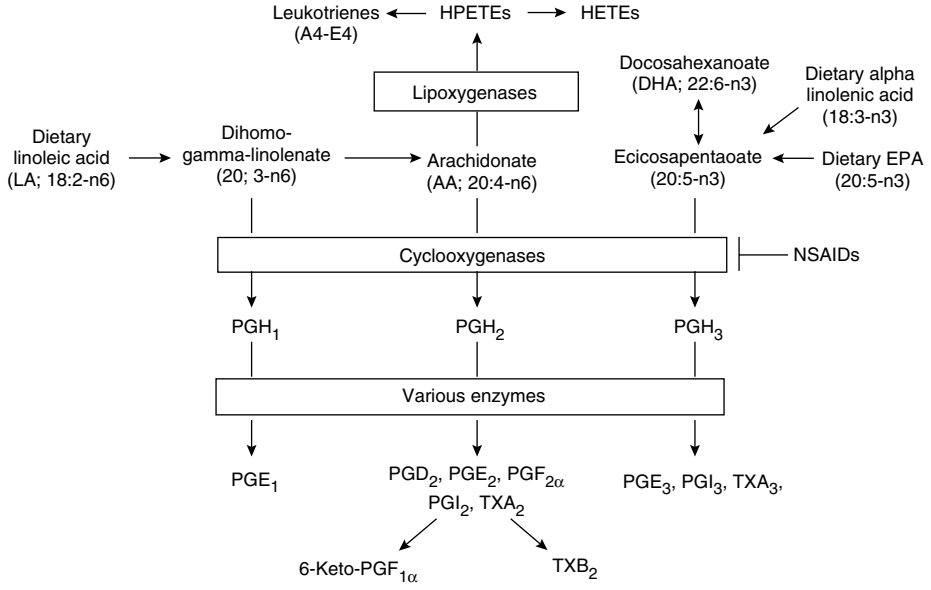

FIGURE 46.3 Renal production of eicosanoids and leukotrienes. EPA is also a source of leukotrienes of the five series.

denotes the number of double bonds (those derived from arachidonic acid (AA) have two, the eicosapentaenoic acid (EPA)-derived ECs have three, and the LTs have four). The ECs and LTs mostly function as autocrine and paracrine mediators. There are specific receptors for all ECs and LTs, allowing for pharmacological blockade of specific compounds. In general, ECs mediate local symptoms of inflammation, control of blood flow through vasoconstriction or vasodilation, blood coagulation, fever, and pain. The cyclooxygenase (COX) II isoform is responsible for inflammation and pain. The COX I isoform is responsible for platelet (coagulation) functions. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the COX II isoform. LTs also play an important role in inflammation, and blocking their receptors may be useful in the management of some inflammatory diseases.

The most plentiful fatty acid precursor is arachidonic acid (AA; 20:4n-6), which is synthesized from the essential dietary fatty acid linoleic acid (LA; 18:2n-6). Thus, the membrane concentrations of AA are influenced by dietary levels of LA (the first step in EC synthesis is the release of the 20-carbon fatty acid from membrane phospholipids by phospholipase A_2 or from diacylglycerol by phospholipase C). LA is desaturated and elongated to form AA, and therefore both the intake and enzymatic regulation of this dietary lipid can alter the amount of AA available for EC biosynthesis. ECs are not stored, and the rate-limiting step in their biosynthesis appears to be the release of AA from membrane phospholipids into the intracellular environment in response to various stimuli that activate phospholipase A_2 and other acylhydrolases. Such stimuli include renal ischemia, mechanical trauma, stimulation of renal nerves, angiotensin II, and catecholamines. COX acts on AA to form the endoperoxide prostaglandin H_2 (PGH_2), which is then converted to the stable metabolites PGE_2 (a potent vasodilator), $PGF_{2\alpha}$ (which may be a weak vasoconstrictor), PGD_2 (a vasodilator), and the unstable metabolites thromboxane A_2 (TXA_2; a potent vasoconstrictor and stimulator of platelet aggregation) and PGI_2 (prostacyclin; a potent vasodilator and platelet inhibitor). The major ECs produced by the kidneys appear to be PGE_2 and PGI_2, with smaller amounts of $PGF_{2\alpha}$, TXA_2, and PGD_2, and the production of these ECs may be significantly increased in some renal disorders. Indeed, an upregulation of AA metabolism generally accompanies renal pathophysiological conditions (Lefkowith and Klahr, 1996). Different segments of the nephron, renal vasculature, and renal interstitium may synthesize different amounts and types of ECs (Figure 46.4). For example, in rats, PGI_2 is the primary product of the renal arteries and afferent arterioles, where it has a major influence on renal vascular tone, whereas PGE_2 is found in high concentration in the glomerulus correlated with its action on renin release (Terragno et al., 1978). The renal pattern of EC production can also be species specific. Human glomeruli produce mostly PGI_2 (Sraer et al., 1983), compared to rat glomeruli which synthesize primarily PGE_2. The ECs have diverse actions in the kidneys, including the modulation of renal blood flow (RBF) and its regional distribution (Herbaczynska-Cedro and Vane, 1973), GFR (Dworkin et al., 1983), water (Dunn, 1984), and solute (Kaojaren et al., 1983) flux across renal tubular membranes, and renin secretion (Larsson et al., 1974). However, under normal conditions, RBF and GFR are probably not EC dependent. The ECs are eventually metabolized in the kidney to compounds with minimal biological activity.

AA is converted by lipoxygenase enzymes into hydroperoxyeicosatetraenoic acids (HPETEs), which undergo dehydration to monohydroxy forms (HETEs). At least three such enzymes exist. The 12- and 15-lipoxygenases generate 12- and 15-HETE, whereas the 5-lipoxygenase pathway forms LTA_4 and 5-HETE. LTA_4 can then be converted to LTB_4 and LTC_4, and these products released from the cell. LTs D_4 and E_4 can then be produced from LTC_4 in plasma. It appears that the kidney can produce 5-lipoxygenase products, but whether glomerular cells can do so is uncertain (Pirotzky et al., 1984). Cells in the kidney also contain 12- and 15-lipoxygenase enzymes. LTs appear to have vasoconstrictive properties in the kidneys, probably by direct actions on vascular smooth muscle cells, at least in the rat (Rosenthal and Pace-Asciak, 1983). The renal vasculature in the dog may be insensitive to the LTs (Chapnick, 1984), and human studies are lacking. The LTs also play a role in immunological and inflammatory reactions by stimulating leukocyte chemotaxis and their degranulation (Goetzl et al., 1977; Ford-Hutchinson et al., 1980). Even if all of the known lipoxygenase

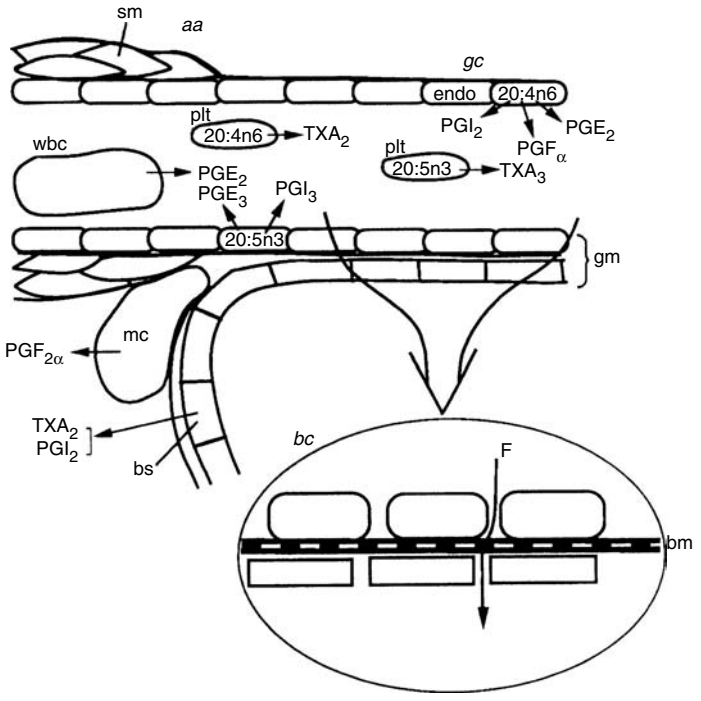

FIGURE 46.4 Sources of eicosanoids in the kidney. aa, afferent arteriole; bc, Bowman's capsule; bm, basement membrane; bs, Bowman's space; endo, endothelium; F, filtration; gc, glomerular capillary; gm, glomerular (renal corpuscle) membrane; mc, mesangial cell; plt, platelet; sm, smooth muscle; wbc, white blood cell.

enzymes are not present in renal cells, infiltrating neutrophils and other inflammatory cells may contain them. Thus, the lipoxygenase pathway may be especially important in inflammatory renal diseases. LT receptors are found in renal cells, and both LTC_4 and LTD_4 stimulate mesangial cell (glomerular) contraction, which may modulate glomerular hemodynamics and filtration. LTC_4 is known to decrease both the RBF and GFR and to alter the permeability of the glomerular membrane (Badr et al., 1984). The LTs may also modulate renal function through the activation of phospholipases and the subsequent production of COX metabolites (Cramer et al., 1983). The physiological actions of the lipoxygenase products on the kidney are not as well understood as those of the COX products. The term *eicosanoid*, as used in this chapter, refers primarily to the prostaglandins and thromboxanes (collectively called the prostanoids) rather than to the lipoxygenase metabolites (the LTs). A renal cytochrome P-450-dependent monooxygenase has been found that converts AA into various products (Schwartzman et al., 1986), but the renal significance of this pathway is largely unknown. Rats treated with fish oil showed increased hepatic concentrations of cytochrome P-450, but renal concentrations were not changed (Valdes et al., 1995).

When eicosapentaenoic acid (EPA; 20:5n-3) is the substrate for COX, the products are PGE_3 and PGI_3 (Knapp et al., 1986), which are vasodilators, and TXA_3. Unlike TXA_2, TXA_3 is not a potent vasoconstrictor and does not stimulate platelet aggregation (Needleman et al., 1979). Whereas n-6 PUFAs increase AA and the 2-series ECs, the n-3 PUFAs competitively inhibit the conversion of n-6 precursor fatty acids to AA; suppress production of the 2-series ECs, and the PGI_3 produced decreases platelet aggregability. Thus, enriching the diet with EPA at the expense of AA may be of benefit in diseases characterized by platelet activation and/or vascular smooth muscle contraction (Leaf and Weber, 1988). Very high does of EPA (about 10 g/day) are required to reduce the platelet production of TXA_2 by 85% in humans (Knapp et al., 1986), but little information is available regarding the dose-response relationship between fish oil and renal TXA_2 production in humans.

It is apparent that there is upregulation of AA metabolism with several renal diseases (Lianos et al., 1991; Yanagisawa et al., 1993) resulting in the production of he beneficial ECs PGE_2 and PGI_2 that suppress immune function and enhance renal hemodynamics, but also produced are TXA_2 and LTs that oppose these actions (Badr, 1992; Remuzzi et al., 1992). We now know that glomerular infiltration by platelets and neutrophils is obligatory for the upregulation of AA metabolism, probably by bringing in the necessary enzymes (such as COX and thromboxane synthetase), and also for the accompanying renal dysfunction leading to proteinuria (Couser, 1993; Wu et al., 1993). It is possible that the upregulation of AA results from activation of the inducable COX isozyme (COX 2) (Obanion et al., 1992) in leukocytes and resident mesangial cells by cytokines (Rzymkiewicz et al., 1994) or nitric oxide (Tetsuka et al., 1995) derived from leukocytes, macrophages, and mesangial cells. In this context, protection from inflammatory renal damage may occur by the use of inhibitors of COX 2 (Masferrer et al., 1994) or nitric oxide synthesis (Narita et al., 1995). Glomerular influx of monocytes and macrophages occurs at a later stage in renal disease and, by interaction with resident glomerular cells, causes glomerulosclerosis (GS) (Couser, 1993; Nikolic-Paterson et al., 1994). Since both n-3 fatty acid supplementation and essential fatty acid deprivation exert an anti-inflammatory effect by decreasing neutrophil chemotaxis (Lee et al., 1985), reducing LTB_4 synthesis (Lefkowith et al., 1990) and cytokine production (Endres et al., 1989), and decreasing nitric oxide production (Boutard et al., 1994) and the secretion of platelet-activating factor (PAF) (Sperling et al., 1987), such dietary changes may be of benefit in the treatment of inflammatory renal diseases such as glomerulonephritis (GN) and in preventing sclerosis.

The functions of ECs on the kidneys have been determined with the use of both COX inhibitors and exogenous ECs. NSAIDs are potent inhibitors of COX, and conventional doses of NSAIDs have been shown to reduce urinary ECs by 50%–80% (Gafni et al., 1978). The enzyme inhibition is probably greater in the renal cortex (where most of the glomeruli are located) than in the medulla (composed mostly of renal tubules) (Attallah, 1979). NSAIDs may, under certain circumstances, have adverse effects on renal function (Kimberly and Plotz, 1977; Ciabattoni et al., 1984). When NSAIDs are given to healthy individuals, no obvious changes occur in the RBF or GFR (Gullner et al., 1980); thus, under these conditions, renal functions do not seem to be EC dependent. However, individuals subjected to circulatory compromise (such as with heart failure, shock, or volume depletion) and those with preexisting renal dysfunction may react to NSAIDs with a reduction in kidney function that leads to acute renal failure (ARF) (Walshe and Venuto, 1979). This is thought to occur because the vasoconstrictive actions of catecholamines and angiotensin II (found in high concentrations with circulatory failure), or the already low RBF, are being counteracted by the intrarenal vasodilatory ECs (the most important may be PGI_2, since it is produced in renal vessels). The full ischemic action of the vasoconstrictors becomes evident when EC production is impaired by the NSAIDs. The more profound the circulatory compromise or renal failure the ECs are counteracting, the more deleterious will be the effects of COX inhibition. The harmful effects of NSAIDs on renal function are usually reversible once the drugs are discontinued, but not always (Kleinknecht et al., 1980). Thus, whenever blood pressure and cardiac output are threatened, the levels of circulating vasocontrictors increase and sympathetic activity is enhanced. To protect the function of the kidneys, renal vasodilator ECs are produced in response to such vasoconstrictive forces. Patients at high risk for developing complications with NSAIDs include those with low circulating blood volume, chronic renal failure (CRF), and the elderly (Henrich and Diamond, 1989). In some renal disorders, NSAIDs may improve renal functions; a reduction in proteinuria in patients with nephrosis has been noted (Arisz et al., 1976).

Drugs that inhibit the formation of the vasoconstrictor TXA_2 without causing a reduction in vasodilator ECs could be of advantage where the maintenance of renal function is dependent upon such vasodilators (Ciabattoni et al., 1984; Patrignani et al., 1984). Thromboxane receptor antagonists could also e therapeutically important, since they may selectively inhibit TXA_2 action without affecting the action of other ECs (Harris et al., 1984). It should be pointed out that by inhibiting certain metabolic pathways, enzyme-inhibiting drugs may shunt substrates to alternative pathways.

For example, inhibition of thromboxane synthetase may shunt endoperoxides toward an enhanced synthesis of the vasodilators PGE_2 and PGI_2, and indomethacin (an NSAID) could divert AA toward the vasoconstrictive and chemotaxic lipoxygenase pathway. Thus, an enzyme inhibitor may have actions that decrease the synthesis of undesired metabolites and increase the synthesis of beneficial ECs, or vice versa.

Some studies indicate that additional dilatory ECs are synthesized by the kidneys with renal failure or disease (Blum et al., 1981; Lianos et al., 1983; Schambelan et al., 1985), most likely as a compensatory reaction to the impaired renal function; however, exceptions have been noted (Ciabattoni et al., 1984). It appears that with certain renal diseases, vasodilatory ECs are important in sustaining the remaining functions of the kidney. The source of the additional vasodilatory ECs in some renal diseases may be inflammatory cells and fibroblasts (Nagle et al., 1976). In animal models of ARF, tubular necrosis due to impaired RBF may be prevented if vasodilator ECs are given before or at the time of the renal ischemia (Werb et al., 1978). However, deciphering the normal effects of renal ECs on renal function based on studies utilizing exogenous ECs may be difficult, because intravenous and intrarenal infusions may not distribute the ECs to the sites within the kidney where they are normally produced and have their actions.

Despite the evidence for enhanced EC production in experimental and clinical renal disease, the actual sources of the ECs remain equivocal, because both circulating and renal ECs appear in urine. Possible sources include infiltrating blood cells such as leukocytes (Goldstein et al., 1978) and platelets (Needleman et al., 1976) as well as the renal cells themselves (Ardaillou et al., 1983). Human studies have suggested that the major urinary metabolite of TXB_2 (2,3-dinor-TXB_2) is a sensitive marker of *in vivo* platelet activation, whereas urinary TXB_2 reflects the renal synthesis of the parent compound TXA_2 (FitzGerald et al., 1983; Catella et al., 1986). However, increased urinary TXB_2, together with 2,3-dinor-TXB_2, has been documented in patients with extrarenal platelet activation and normal renal function (Reilly et al., 1986). Under some circumstances, urinary EC excretion parallels the urine flow rate (Walker et al., 1981), and it is unclear if this actually represents an increased synthesis of ECs. Nevertheless, most investigators assume that urinary excretion rates of TXB_2 and 2,3-dinor-TXB_2 represent the synthesis rates of TXA_2 by renal and extrarenal sources, respectively. Urinary 6-keto-$PGF_{1\alpha}$ is thought to reflect intrarenal PGI_2 synthesis (Pugliese and Ciabattoni, 1983). Since infused PGE_2 and $PGF_{2\alpha}$ are not recovered as such in the urine, urinary PGE_2 and $PGF_{2\alpha}$ are usually considered of renal origin (Frolich et al., 1975).

To summarize the basic actions of the ECs (Table 46.1), renal vasoconstriction is caused by TXA_2, $PGF_{2\alpha}$, and possibly by the LTs, and these actions are countered by PGE_2 and PGI_2, which are potent vasodilators. Renal vasodilation is also elicited by PGE_1, PGD_2, PGE_3, and PGI_3. However, since PGI_2 and PGE_2 stimulate renin release and the subsequent production of the vasoconstrictor angiotensin II, under certain circumstances their indirect actions may result in vasoconstriction. Platelet aggregation is enhanced by TXA_2 and inhibited by PGI_2 and PGI_3. Additionally, from an inflammatory and immunological perspective, LTs are chemotactic, and PGE_2, PGI_2, and the 3-series prostaglandins may suppress neutrophil and T-cell function and reduce antibody production. The major renal ECs are PGE_2 and PGI_2, which are derived from dietary n-6 PUFAs. For additional information on renal ECs and LTs, see the reviews by Morrison (1986) and Ballermann et al. (1986).

IV. RENAL DISEASE

There are a large number of renal and extrarenal disorders that alter the function of the kidneys. As discussed earlier, the kidneys have many important functions, all of which are liable to suffer when the kidneys are affected by disease processes. This is especially true when the renal disease is chronic.

Many diseases that affect the kidneys cause a reduction in the GFR. However, compensatory mechanisms occur to help preserve homeostasis of the body fluids (Deen et al., 1974; Brenner, 1985). First, there is hypertrophy of the remaining functional nephrons, and their performance is

TABLE 46.1
Principal Actions of Renal Eicosanoids and Leukotrienes

Compound	Renal Actions
Leukotrienes	Vasoconstriction, proinflammatory
PGE_1	Vasodilation, suppresses immune functions
PGE_2	Vasodilation, suppresses immune functions, stimulates renin release
PGI_2	Vasodilation, inhibits platelets, suppresses immune functions, stimulates renin release
$PGF_{2\alpha}$	Vasoconstriction
PGD_2	Vasodilation
TXA_2	Inhibits platelets
	Vasoconstriction, stimulates platelets, proinflammatory
PGE_3	Vasodilation, suppresses immune functions
PGI_3	Vasodilation, suppresses immune functions, inhibits platelets
TXA_3	Not as vasoconstrictive nor pro-platelet as TXA_2

The 1 and 2-series ECs and the leukotrienes are derived from dietary n-6 PUFAs; the 3-series ECs originate from dietary n-3 PUFAs. The major renal ECs are PGE_2 and PGI_2.

enhanced such that the reduction in the total GFR is proportionately less than the reduction in the functional renal mass. That is, single-nephron GFR (SNGFR) increases, although the overall GFR may decrease. Second, as the plasma concentration of solutes increases, more of the solutes will be filtered into Bowman's space at a given GFR, because solute filtration is proportional to the plasma concentration of the solute. Thus, the content of solutes in the extracellular fluid will not increase indefinitely provided some glomerular filtration occurs. Third, reabsorption rates may decrease and secretion rates increase. Thus, more solutes are excreted by functional changes in these two mechanisms, and this serves further to reduce the accumulation of compounds as the kidneys fail.

A reduction in the GFR may occur because of various circulatory problems, such as hypotension, heart failure and circulating volume depletion resulting from edema or blood loss. It can also result from damage to nephrons caused by immune disorders, infections, nephrotoxins, renal vascular diseases, hypertension, and chronic urinary tract obstruction. When the overall GFR is reduced, circulating volume overload may occur, resulting in more hypertension and further nephron injury and loss. With nephron injury comes proteinuria (protein in the urine) and thus edema resulting from a reduction in blood oncotic pressure. Thus, despite a compensatory increase in the GFR in some nephrons, the above described positive-feedback cycle between nephron damage and a reduced GFR can ultimately lead to renal failure. The physiological disturbances and clinical symptoms which then occur are referred to as uremia. With chronic nephron loss, tubule damage, and reduced GFR, one would anticipate bone disease due to impaired vitamin D production, anemia caused by an inadequate erythropoietin secretion, an inability adequately to excrete water and various solutes, proteinuria, hematuria (blood in the urine), metabolic acidosis due to decreased acid excretion, edema, elevated blood urea and creatinine (azotemia), and other complications of CRF. Without dialysis or transplantation, death may ensue.

Acute renal failure (ARF) refers to a rapid decline of renal function in a patient whose renal function was previously normal. The GFR may drop precipitously within a few hours. Azotemia develops rapidly and is the clinical hallmark of ARF. ARF can occur with a variety of renal and extrarenal disorders, such as diminished perfusion of the kidneys (owing to heart failure blood loss, or shock) resulting in a reduced GFR and/or tubular necrosis, from inflammatory or immunological processes, from metabolic derangements, or from obstructions to urine flow. Toxic substances (such as lead, mercury, or some antibiotics) can alter renal function and may cause tubular necrosis. The most common cause of ARF is as a complication of catastrophic illness, and the pathological

process is usually assumed to be acute tubular necrosis. Treatment is directed toward correcting the primary problem so that the renal pathological process can heal itself. Dialysis may be appropriate in some cases. In ARF, it is often possible to reverse the deterioration in renal function if prompt treatment is initiated.

Chronic renal failure (CRF) is a syndrome in which there is a progressive and usually irreversible decrease in the GFR even when the disease that caused the renal damage has disappeared or abated. CRF (like ARF) occurs in association with a variety of primary renal diseases (such as inflammation and infection of the kidneys), systemic diseases (diabetes mellitus, hypertension, atherosclerosis, and lupus erythematosus), and nephrotoxins, with the most common histological manifestations of CRF being glomerular damage. Some investigators believe that immunological mechanisms account for the initial damage in many chronic renal diseases; however, the rate of progression may be influenced by a variety of nonimmunological factors. At the end of their clinical course, patients are said to have end-stage renal disease, and dialysis or transplantation may be necessary. More than 40,000 patients enter treatment for end-stage renal disease each year in the United States, most often due to diabetes and hypertension (Brazy, 1993).

By way of summary, all causes of acute and CRF can be classified into three basic groups. *Prerenal causes* of kidney failure include renal diseases resulting from a disordered perfusion (inadequate blood flow) of an otherwise normal kidney due to changes in the function of structures such as the heart or renal arteries, or drug effects on renal perfusion. Most cases of ACF are due to prerenal causes. *Renal causes* of failure include diseases of the small arteries feeding the glomeruli and/or the glomeruli themselves, disorders of other parts of the nephron and the interstitial tissue surrounding it, and intratubular obstructions. These disorders cause direct damage to the nephrons. *Postrenal causes* of kidney failure usually entail obstruction of the urinary system distal to the kidney. Obstructions of the urinary tract occur due to things such as tumors, prostatic hypertrophy, spasm, or drug effects, and these are among the most reversible causes of renal failure if caught early. ARF occurs abruptly and may be treated effectively if intervention is started early (some cases of ARF resolve spontaneously). CRF is insidious, slow to develop, and progressive and may be lethal without transplantation.

Some of the more common renal diseases, which may present as either acute or CRF, will be briefly discussed. Nephritis is an inflammation of the renal glomeruli. Azotemia, hematuria, and proteinuria are common, as is hypertension. There are many forms of nephritis recognized clinically, and several of these are dealt with later in this chapter with regard to their modulation by renal ECs and dietary fatty acids. Nephritis is thought to be immunologically mediated. Its causes are not well understood, but it often occurs following an infection or may develop as part of another disease (such as lupus erythematosus). Inflammation of the glomerulus increases the permeability of the glomerular membrane and causes proteinuria and hematuria. Progressive glomerular injury frequently results in GS (a thickening and hardening of the glomerular vasculature leading to obliteration of the glomerular capillary bed) and thus further disruption of kidney function, often leading to end-stage renal disease.

Nephrosis refers to renal disease where glomerular changes cause edema and massive proteinuria; hypertension is usually absent. Hyperlipidemia is common with nephrosis, but the pathogenesis of this disease is not completely understood (attenuation of mevolonate degradation by the kidneys is thought to be a contributory mechanism). The glomerular lesion in nephrosis is not inflammatory. Causes of nephrosis include infectious agents, poisons, and various metabolic disturbances.

Nephrotoxic renal disease occurs when a toxin or drug capable of destroying renal cells (especially tubules) is introduced into the body. Some nephrotoxins, such as those that cause nephrotoxic serum nephritis (NSN), damage the glomeruli, usually by causing an immune reaction there. Renal injury may also result from deposition of biologically active compounds such as immunoglobulins and lipoproteins or their lipid constituents in kidney tissues. Other factors that may initiate and sustain renal injury include renal blood coagulation and various factors released from renal cells and infiltrating leukocytes and platelets. For additional information on renal dysfunction, the reader

should consult textbooks on nephrology such as those of Brenner et al. (1987b), Johnson (2003), and Brenner (2004).

A. Obesity and Renal Disease

High body mass index (BMI) has been reported to be a strong risk factor for the development of end-stage renal disease in humans, independent of blood pressure and diabetes mellitus (Hsu et al., 2006). Obesity (as an increased abdominal waist circumference) is one of the defining criteria of the metabolic syndrome (along with hypertriglyceridemia, low serum LDL level, raised blood pressure, and an elevated fasting blood glucose level), and this syndrome has been shown to be a strong independent risk factor for chronic kidney disease (Chen et al., 2004; Reisin and Alpert, 2005). Kurella and coworkers (2005) showed that as the number of traits of the metabolic syndrome increased in nondiabetic adults, so did the odds of developing chronic kidney disease. The mechanisms linking metabolic syndrome to renal disease may include impaired pressure natriuresis, insulin resistance, excess excretory load, endothelial cell dysfunction, chronic inflammation, hyperfiltration, and a prothrombotic status (Zhang et al., 2005). Obesity in dogs caused by a high-fat diet is associated with glomerular hyperfiltration, thickening of the glomerular membrane, proliferation of mesangial cells, activation of the rennin–angiotensin system, and expansion of Bowman's capsule (Henegar et al., 2001) and obesity is associated with the eventual development of GS (Praga, 2002). Diet-induced obesity in mice has been shown to cause lipid accumulation in the kidneys, GS, and proteinuria, and the effects were mediated by a sterol regulatory element-binding protein (SREBP) pathway (high fat feeding stimulates this pathway) that activates genes involved in fatty acid and cholesterol synthesis (Horton et al., 2002; Jiang et al., 2005). It has also been reported that inhibition of cholesterol synthesis by HMG-CoA reductase inhibitors may protect against diabetic and nondiabetic renal disease (Bianchi et al., 2003; Guan, 2004). Studies have linked obesity with the presence of low-grade systemic inflammation (Ziccardi et al., 2002; Pedersen et al., 2003) and adipose tissue may contribute to this by secreting inflammatory cytokines such as interleukin-6 (Axelsson et al., 2004) and activation of inflammation-associated signaling pathways (Perreault and Marette, 2001; Hirosumi et al., 2002). The principal source of obesity-related cytokines may come from macrophages that infiltrate adipose tissue rather than adipocytes themselves (Weisberg et al., 2003), and many of these cytokines are postulated to play a role in renal pathophysiology (Wolf et al., 2002) supporting the suggestion that obesity and lipid-lowering therapies have the potential to improve renal diseases associated with the metabolic syndrome (Schelling and Sedor, 2004). Leptin is a hormone secreted by adipocytes that acts as a regulator of food intake and energy expenditure (it decreases food intake and increases energy expenditure). Blood leptin levels are increased with chronic kidney disease and correlate with C-reactive protein levels, implying that inflammation may be associated with the hyperleptinemia in patients with chronic kidney disease (Mak et al., 2006).

V. FATTY ACIDS, EICOSANOIDS, AND RENAL DISEASE

A. Nonimmunological Renal Disease

a. Subtotal Renal Ablation

The rat with subtotal renal ablation (SRA; partial infarction of one kidney and contralateral nephrectomy) has been used extensively to study the mechanisms underlying the progression of nonimmunological renal disease (Brenner, 1985). Such rats develop proteinuria, hypertension, GS, and chronic progressive renal failure in the viable portion of the remnant kidney (Chanutin and Ferris, 1932; Shimamura and Morrison, 1975); however, the pathogenesis of these abnormalities has not been completely elucidated. Compensatory hypertrophy of the residual renal mass occurs and is accompanied by a marked increase in plasma flow per nephron and in the SNGFR despite a decrease in the total GFR (Deen et al., 1974). It has been suggested that an increase in glomerular capillary pressure

secondary to increased transmission of the systemic blood pressure into the glomerular capillary bed (possibly mediated by vasodilator ECs) may be the adaptive response resulting in the increased SNGFR. However, the enhanced pressure and filtration may ultimately be responsible for the sclerosis and progression of the renal disease (Hostetter et al., 1981; Brenner et al., 1982; Klahr et al., 1988), but this hypothesis has been challenged (Purkerson et al., 1985; Jones et al., 1987; Yoshida et al., 1988). Observations by Berstrom et al. (1986) show that a reduction of blood pressure has a beneficial effect on intraglomerular hemodynamics and retards GS in humans with chronic renal disease regardless of its etiology. Dietary supplementation with either n-6 or n-3 fatty acids has been shown by some investigators to lower systemic blood pressure (Lacono et al., 1975; Norris et al., 1986) and thus may help to reduce such glomerular damage (if it occurs). However, not all workers have found a blood pressure-lowering effect with dietary PUFAs (Puska et al., 1985; Bruckner et al., 1987; Ware et al., 1990, 1992).

It has been shown that renal TXA_2 in rats with SRA is increased (Stahl et al., 1986). Long-term administration of an inhibitor of TXA_2 synthesis in rats with a remnant kidney decreased both proteinuria and urinary TXB_2 excretion, improved renal histology, and enhanced both the single-nephron plasma flow and the SNGFR (Purkerson et al., 1985). It thus appears that in animals with reduced renal mass, TXA_2 has deleterious physiological effects. Stahl et al. (1986) also reported that the glomerular production of PGE_2 is greater in rats with SRA compared to sham-operated controls, and that indomethacin significantly reduces the GFR in these animals. The reduced rate of filtration has been verified for rats (Nath et al., 1987), rabbits (Kirschenbaum and Serros, 1981), and dogs (Altscheler et al., 1978) with reduced renal mass and subjected to COX inhibition. Thus, enhancement of vasodilatory EC production may contribute to the augmented renal hemodynamic response to partial renal ablation. Ikeda et al. (1989) found that rats with SRA showed enhanced TXA_2 and PGI_2 production by the aorta. The former may be a pathological response to the hypertension induced by reduced renal mass, whereas the enhanced prostacyclin synthesis appears to be adaptive.

Similar renal functional changes (hyperfiltration, elevated RBF, glomerular hypertension) are observed in patients with diabetes mellitus during the early phase of diabetic nephropathy (Christiansen et al., 1981). The glomerular production of PGE_2 and $PGF_{2\alpha}$ is enhanced in diabetic rats, but the TXA_2 production is normal (Schambelan et al., 1985). Thus, increased synthesis of vasodilatory ECs may be a compensatory mechanism in diabetic nephropathy as it appears to be in animals with reduced renal mass. It is generally believed that n-3 fatty acids (e.g., EPA) are effective against many renal diseases, including diabetic nephropathy. This was most recently shown by Hagiwara et al. (2006) where they demonstrated that EPA improves the diabetic nephropathy of type 2 diabetic mice, and that this was accompanied by decreased serum triglycerides, improved glucose tolerance, reduced tubulointerstitial fibrosis, and down-regulation of monocyte chemoattractant protein-1. The imposition of a restricted calorie intake may be sufficient to prevent the proteinuria and histologic damage to the kidneys in a rat model of diabetic nephropathy, possibly by normalizing the oxidative and carbonyl stress imposed by a higher dietary intake (Nangaku et al., 2005). If this can be extended to humans, it suggests that the glomerular and tubulointerstitial damage characteristic of human diabetic nephropathy may be due in part to local oxidative stress associated with fuel metabolism.

Partial nephrectomy in rats suggests that LA plays a role in preventing the structural and functional deterioration that may occur as a consequence of the compensatory hypertrophy in the remaining nephrons (Barcelli et al., 1982). The LA-treated rats showed a decrease in proteinuria, no sclerosis of the enlarged glomeruli, less tubule and interstitial tissue damage, stabilization of renal function, and no evident deterioration of health. The renal production of PGE_2 was increased, and although TXA_2 production may also have been increased, PGE_2 production was quantitatively greater. The improved renal function was not due to changes in blood pressure or blood lipids, because no significant alterations of these parameters were noted. Others have observed that an LA-enriched diet is associated with an improvement or maintenance of renal function in rats with

SRA and may prevent some of the pathological changes leading to the development of GS (Barcelli et al., 1985; Heifets et al., 1985, 1987a; Izumi et al., 1986; Ingram et al., 1996). These effects of LA were often abolished by the administration of indomethacin (a COX inhibitor) suggesting that the improved renal function may be EC dependent. It has been shown that LA feeding significantly changes the lipid composition of kidney tissues and results in increased concentrations of renal LA and AA (Heifets et al., 1987a). Thus, altering LA in the diet enhances the membrane phospholipid content of n-6 fatty acids and may alter EC production. However, renal function is improved by feeding LA to SRA rats without apparent changes in urinary EC excretion (Heifets et al., 1987a). Another possible mechanism of action could be a reduction in blood pressure with LA feeding. It has been shown that LA-enriched diets attenuate the hypertension that usually occurs in rats with SRA (Heifets et al., 1985; Izumi et al., 1986), but this is not a consistent finding (Barcelli et al., 1982). Furthermore, membrane fluidity changes could occur with changes in membrane PUFA content (Beitz and Forster, 1980) and this might change membrane permeability characterists and/or alter hormone receptor activity in the target tissues.

It is known that dietary protein restriction is beneficial in CRF (Barsotti et al., 1983; Brazy, 1993). Ito et al. (1987) demonstrated that a low-protein high-linoleate diet had more benefit in partially nephrectomized rats than either diet alone, and it was postulated that the additive effects may have been mediated by an increased PGE_2 production from the dietary LA and its subsequent effect on renal hemodynamics. Since elevated blood lipids may contribute to the development of GS and renal injury (Kasiske et al., 1985; Keane and Guijarro, 1996), the LA-enriched diet might be of benefit because of its hypocholesterolemic effect. Muhlfeld and co-workers (2004) have shown that hyperlipidemia worsens the renal pathology and function (in part through increased renal expression of cytokines) of B6.ROP Os/+ mice, a model of reduced renal mass. Daily supplementation with n-3 PUFAs to patients with impaired renal function consuming a high-protein diet increased RBF and GFR (Schapp et al., 1987). Total renal resistance was reduced by 20%. Thus, n-3 fatty acids appear to improve renal function primarily by eliciting renal vasodilation.

A 13%–20% LA diet improved renal function in humans, as evidenced by an increased excretion of creatinine (Adam and Wolfram, 1984). Increasing the amount of fat in the diet usually results in a decreased carbohydrate intake and elevated plasma glucagon. Glucagon is known to increase RBF and creatinine excretion, and this might explain some of the dietary fat effects on renal function. Even an intake of 6%–8% LA increased creatinine excretion compared to a 0%–4% LA diet (Adam and Wolfram, 1984). Hirschberg et al. (1984) reported that a diet deficient in essential fatty acids was not detrimental to rats with SRA. However, glomerular lesions were found to be more severe in rats with SRA fed a low-LA diet (Heifets et al., 1987a).

As platelet aggregation in the glomerular capillaries and renal vasculature may play a role in the progression of renal disease in the SRA rat model, especially if there is endothelial damage by factors such as hypertension, it is significant that dietary LA may alter platelet aggregation. McGregor et al. (1980) demonstrated that 0%–6% dietary LA was correlated with a decreased susceptibility of platelets to aggregate (thrombin induced) and a prolongation of the clotting time of platelet-rich plasma. Similar results on platelet function have been reported when diets were enriched with n-3 PUFAs (Leaf and Weber, 1988). Increased fibrinolytic activity has been reported in rats fed increased levels of PUFAs (Rondeau et al., 1986), and this may also favorably alter the progression of renal disease by reducing intraglomerular thrombosis.

Barcelli et al. (1986) demonstrated that there was improvement in renal function in rats with SRA when they were fed diets rich in various PUFAs. Rats fed evening primrose oil (high in LA and gamma-linolenic acid [GLA]) showed enhanced production of PGI_2, and it has been shown by others that GLA increases PGE_1 and PGE_2 production (Kernoff et al., 1977) and improves renal function (Ingram et al., 1996). Rats fed safflower oil (high in LA) showed greater synthesis of PGE_2 and PGI_2. Rats fed salmon oil (n-3 rich) demonstrated an increase in PGE_3 production and a decrease in urinary excretion of TXB_2.These changes in ECs were associated with improved renal function. All three oil diets reduced serum triglycerides, and since a lowering of serum lipids has been associated

with improved renal function (Keane et al., 1988), this may help to explain their beneficial effect on the kidneys. There is, however, some question as to whether triglycerides are important in this regard (Hirschberg et al., 1984). In studies using animal models of SRA, diets supplemented with n-3 fatty acids improved renal function and ameliorated structural damage (Barcelli et al., 1985, 1986; Scharschmidt et al., 1987), but this is not a universal finding because some studies have found EPA to worsen the renal disease in rats with partial nephrectomy (Hirschberg et al., 1984; Scharschmidt et al., 1985). Logan and coworkers (1990) found fish oil to enhance the GFR and RBF in uninephrectomized rats, and the changes were associated with a decrease in the dienoic ECs and a rise in the 3 series. These authors caution that because hyperperfusion and hyperfiltration of nephrons is thought to lead to progressive renal disease in rats with SRA, dietary modifications that elicit increases in GFR and RBF may eventually lead to GS and renal failure. Since PUFAs are the precursors of ECs, it is presumed that by substitution of EPA for AA, the COX metabolites produced will decrease platelet aggregation and reduce renal vasoconstriction (Table 46.2). Various studies have demonstrated that rats fed n-3 PUFAs show a lower renal synthesis of dienoic ECs (Schoene et al., 1981; Croft et al., 1984; Ito et al., 1988). Prostaglandin E_3 was found in renal tissue after 22 weeks on n-3 fatty acid supplementation (Ferretti et al., 1981). Thus, the synthesis of diene ECs by the kidney decreases with increased n-3 consumption and PGE_3 production increases. However, Kasiske et al. (1989) found that fish oils decreased renal prostacyclin (PGI_2) production but not the production of TXA_2. It is not clear why these discrepancies occur, but they may be related to the oxidative status of the oil, dosage, duration of treatment, and other factors. Fish oils also improve glomerular function in diabetic rats (Sinha et al., 1990) that have renal impairments similar to those in rats with SRA. Thus, fatty acid supplements have direct effects on renal ECs and subsequent renal function, but the mechanisms are not clearly understood. It has been reported that n-3 fatty acid supplements reduce blood viscosity and increase red blood cell deformability in humans both with (Clark et al., 1989) and without (Terano et al., 1983; Woodcock et al., 1984) renal disease, but the reduced viscosity does not apply to plasma alone (Ware et al., 1992). Since uremic patients have been found to have impaired red cell deformability (Kikuchi et al., 1982), n-3 PUFAs, by improving deformability,

TABLE 46.2
Renal Diseases and Associated Fatty Acids/Eicosanoids

		Compounds	
Disease	**Characteristics**	**Harmful[a]**	**Beneficial[b]**
Nonimmunological			
Partial Nephrectomy (SRA)	Eventual renal failure in remaining viable tissue	TXA_2	PGE_2, PGI_2, PGE_3, LA, EPA
Hypertension	Kidneys regulate blood pressure by controlling blood volume	TXA_2	PGE_2, PGE_3, LA, EPA
Immunological			
Nephritis	Renal inflammation and injury; many forms recognized	TXA_2, LTs	PGE_1, PGE_2, PGI_2, PGE_3, PGI_3, EPA
Nephrosis	Noninflammatory renal injury	TXA_2	EPA
Nephrotoxicity	Nephrotoxin-induced renal injury	TXA_2	EPA

Saturated fatty acids appear harmful in most renal diseases. LA may be helpful in some immunological renal diseases, but without effect or harmful in others. Supplementing the diet with LA increases the 2-series ECs and reduces the 3-series ECs; EPA does the opposite.

[a] May be responsible for some of the disease etiology.
[b] Eicosanoids may be elevated as a compensatory response.

may improve these rheological disturbances. Several studies suggest a role for lipid abnormalities in glomerular injury, and a strong correlation between plasma lipids and progression of renal disease in humans has been shown (Moorhead et al., 1982; Keane and Guijarro, 1996). A greater intake of cholesterol in the diet accelerates the development of GS in animals (Keane et al., 1988; Klahr and Harris, 1989; Kasiske et al., 1990), and lowering serum lipids ameliorates the sclerosis in rats with SRA (Keane et al., 1988; Keane and Guijarro, 1996). Daily exercise decreases serum cholesterol and triglycerides in rats with SRA and improves renal function compared to that of sedentary rats with SRA (Heifets et al., 1987b). Moreover, some dietary saturated fatty acids may contribute to renal impairement by causing hypercholesterolemia, especially in individuals in whom some degree of renal dysfunction already exists. Indeed, an increased intake of saturated fats has been shown to worsen the renal disease in mice with glomerular inflammation (Kelley and Izui, 1983). In this context, dietary monounsaturated and polyunsaturated fatty acids have been shown to lower blood lipids and thus may improve renal function. Deposition of lipid in glomerular cells occurs in a variety of renal diseases (Grond et al., 1986a), and it has been postulated that lipids may be nephrotoxic (Moorhead et al., 1982). Hypertriglyceridemia has been implicated in renal dysfunction in uremic rats. However, Hirschberg et al. (1984) showed that triglyceride-rich diets were not harmful to rats made uremic by SRA or to mice with immune-mediated glomerular injury with regard to survival, renal histology, or development of azotemia. These results suggest that a higher intake of dietary lipids may not necessarily contribute to hypertriglyceridemia, and therefore the nephrotoxicity of dietary lipids is still not clearly understood. Diets rich in PUFAs could have various roles in the glomerular scarring caused by GS: They could alter the early compensatory hemodynamics by changing the renal EC levels; they could inhibit platelet aggregation and intraglomerular thrombosis; and they may contribute to membrane functional changes.

b. Hypertension

The kidneys play a primary role in both the development and prevention of hypertension. Certain forms of hypertension may result from excess renin-angiotensin activity, sympathetic nervous stimuli, or excess renal vasopressor ECs. A common view suggests that a deficiency in renal EC vasodilators is fundamental to essential hypertension (hypertension without apparent cause). Infusion of prostacyclin into patients with essential hypertension reduces blood pressure, but whether this means that PGI_2 is involved in the etiology of the disease is not known. It is possible that PGI_2 released into renal venous blood could escape inactivation in the lungs and thus modulate resistance in systemic vascular beds. Renal ECs are important regulators of sodium excretion and blood volume through their effects on renal vascular resistance, GFR, and renal sodium reabsorption. By modulating renin release, ECs regulate the levels of circulating vasoconstrictors (such as angiotensin II) and aldosterone (which acts on the kidneys to increase sodium reabsorption in the distal and collecting tubules). Increased renal vascular tone may be present in many cases of essential hypertension, possibly due to increased activity of the sympathetic nervous system. Studies indicate that essential hypertension is rare in persons with a normal renal circulation free of excessive vascular tone.

Spontaneously hypertensive (SH) rats have been used as a model of essential hypertension in humans. Although the exact pathogenesis of the genetic hypertension in these rats has not been determined, several renal abnormalities have been established. Included are an enhanced renal vascular resistance and sensitivity to vasopressor agents (Fink and Brody, 1979), increased renal production of vasodilatory PGE_2 and PGI_2 (Limas and Limas, 1977; Konieczkowski et al., 1983), and enhanced sodium reabsorption (Farman and Bonvalet, 1975). Martineau and colleagues (1984) found reduced renal PGE_2 synthesis in SH rats and suggested that it may be contributory to the hypertension. Watanabe et al. (1989) found renal phospholipids in SH rats to contain a relatively higher level of AA and relatively lower levels of EPA and dihomo-gamma-linolenic acid compared to normotensive controls. Thus, more substrate availability for the 2-series ECs might somehow contribute to the hypertension in these animals, possibly by augmenting the production of TXA_2.

Indomethacin administration to SH rats causes a rise in systolic blood pressure (Levy, 1977), suggesting that at least one AA metabolite is involved in attenuating the hypertension. Renal TXA_2 synthesis is enhanced in SH rats (Shibouta et al., 1981), and inhibition of thromboxane synthetase appears to delay the development of the hypertension (Uderman et al., 1982; Purkerson et al., 1986). Therefore, an increased synthesis of TXA_2 may play a role in the development of hypertension, possibly by causing an enhanced renal vascular resistance (Grone et al., 1986b; Stegmeier et al., 1987). Little is known regarding TXA_2 levels in human hypertension, but dietary saturated fats are known to be associated with salt retention and hypertension (Preuss, 1977). In contrast to results with SH rats, EC excretion rates in humans with essential hypertension are reduced (Tan et al., 1978; Grose et al., 1980). Despite this, COX inhibition is associated with a further rise in blood pressure (Donker et al., 1976) and an enhanced response to the vasoconstrictor angiotensin II (Negus et al., 1976). Thus, the use of NSAIDs in hypertensive patients may worsen the disease.

Depletion of dietary LA, which is the precursor of the dienoic ECs, results in both biochemical and physiological essential fatty acid deficiency (EFAD) symptoms. When rats are placed on such a diet, PGE_2 excretion in urine falls significantly (Cox et al., 1982). Since PGE_2 is a natriuretic compound, the animals fed a high-salt diet show enhanced sodium reabsorption and develop hypertension. The EFAD rats also demonstrate a defect in water excretion possibly due to the natural antagonism of PGE_2 for antidiuretic hormone, which is now somewhat lacking. These effects are reversible when the rats are fed LA and probably result from the action at the tubular level rather than by an alteration of RBF or GFR. An EFAD diet in rabbits and rats causes elevated blood pressure, increased sensitivity to the vasopressor angiotensin II (O'Brien and Pipkin, 1979), and increased renal sodium reabsorption (Rosenthal et al., 1974). Goldblatt's hypertension resembles clinical renal hypertension and can be produced in experimental animals by decreasing the kidney blood flow by placing a clip on the renal artery. Occasionally, the contralateral kidney is removed. The addition of LA to the diet of rats attenuates the development of two-kidney, one-clip Goldblatt's hypertension (Reddy et al., 1989) and reduces the rise in blood pressure following subtotal nephrectomy (Izumi et al., 1986; Heifets et al., 1987a). Codde et al. (1985) found no difference in blood pressure in rats with the one-kidney, one-clip model of hypertension fed either cod liver oil/linseed oil (n-3 rich) or sunflower oil (n-6 rich), and Watanabe et al. (1989) could not improve the hypertension in SH rats by feeding n-6 fatty acids. However, the antioxidant status of the diets was not clearly defined and may contribute to the observed results. A LA-enriched diet significantly delays the onset and magnitude of NaCl-induced hypertension in the Dahl S strain of rats, a strain that is highly susceptible to salt-induced hypertension. It appears that this effect may be due to a 2.5-fold rise in the renal PGE_2 production in these rats (Tobian, 1983). LA-supplemented diets in SH rats (Schoene et al., 1980), salt-loaded rats (MacDonald et al., 1981), and essential hypertensive patients (Comberg et al., 1978) reduce blood pressure. LA in the rat increases PGE_2 production (Schoene et al., 1980); thus, LA appears to reduce blood pressure by an increased synthesis of vasodilatory ECs and by increasing sodium and water excretion. Therefore, endogenously produced ECs may, in some cases, protect against the development of high blood pressure, but this is certainly not a universal finding (Izumi et al., 1986). Although PGE_2 and PGI_2 cause the release of renin from the kidney (Jackson et al., 1982a), LA is reported to inhibit the activity of renin *in vivo* (Reddy et al., 1987). Reddy et al. (1989) found that LA reduced blood pressure in two-kidney, one-clip hypertensive rats, whereas indomethacin administration did not affect this response; thus, the hypotensive action of LA is most likely due to inhibition of the activity of renin in peripheral tissues rather than an alteration in EC synthesis. Thromboxane A_2, a renal vasoconstrictor, may inhibit renin release (Hackenthal et al., 1990). The secretion of renin from the kidney eventually results in the production of angiotension II and to reduced renal excretion of sodium, which may lead to volume expansion. Thus, the effects of the renal ECs on blood pressure are complex. Furthermore, one must distinguish between alterations in EC levels causing the pathological changes and alterations in EC levels that are compensatory responses to changes in blood pressure, salt balance, or circulating hormones.

Eicosopentaenoic acid given to rats has been reported to have protective effects against epinephrine (adrenaline)-induced renal functional impairment (Sadjak et al., 1987), which may be due to

inhibition of the renal vasocontriction that epinephrine causes. This n-3 PUFA also antagonizes the vascular effects of angiotensin II, and the effects may be independent of trienoic EC production. The administration of n-3 fatty acids to humans and experimental animals, both with and without hypertension, has often resulted in a lowering of blood pressure (Sanders et al., 1981; Singer et al., 1983). Fish oil feeding (10% of calories) was shown to lower blood pressure in the borderline hypertensive rat receiving 1% NaCl (Mills et al., 1989) through mechanisms other than salt and water metabolism. This strain of rats is produced by backcrossing the SH rat with its normotensive parent strain, and such animals become hypertensive with high-salt intake. Bond et al. (1989) found dietary fish oil to attenuate the development of hypertension in the deoxycorticosterone acetate (DOCA)-alt hypertensive rat unrelated to alterations of net sodium balance. It also lowered total, LDL, and HDL cholesterol levels. However, Watanabe et al. (1989) could not decrease hypertension in SH rats by feeding n-3 fatty acids. Stroke-prone SH rats (a well-established animal model of malignant hypertension) fed a high-salt diet develop renal damage, and this renal impairment can be prevented or minimized with dietary fish oil but not with canola or safflower oils (Hobbs et al., 1996). It was suggested that the prevention of hypertensive renal damage may occur as a result of an enhanced incorporation of long-chain n-3 fatty acids in the kidney (such as EPA and docosahexanoic acid [DHA]); n-6 fatty acids exacerbated the development of renal failure in these salt-loaded rats. Fish oil feeding is also usually accompanied by a reduction in AA in such tissues (Otten et al., 1993), but this is not a consistent finding (Cao et al., 1995). Miyazaki and coworkers (2000) examined the effects of dietary oils and docosahexaenoic acid (DHA; 22:6-n3) on renal injury in the stroke-prone SH rat. Compared to soybean oil, both rapeseed and safflower oil-supplemented diets had adverse effects on renal function, whereas the DHA-supplemented diet inhibited the development of proteinuria and suppressed the hypertension in these rats.

LA-enriched diets appear to attenuate hypertension and lessen the renal disease caused by SRA in rats. The mechanism(s) could include an enhanced production of vasodilator ECs (PGE_2 and PGI_2), an inhibition of renin activity, a decrease in TXA_2 synthesis and platelet aggregation, less intraglomerular coagulation, and a decrease in plasma lipids. Thromboxane A_2 production is usually elevated in renal disease and its inhibition improves the pathological condition. Thus, in rats with SRA, excess renal TXA_2 production could be one of the primary mechanisms that eventually lead to end-stage renal disease. Prostaglandins E_2 and I_2 are usually elevated with these renal disorders, possibly as a compensatory mechanism. Fish oil (rich in n-3 fatty acids) also attenuates renal disease and hypertension, possibly by reducing TXA_2 synthesis and by increasing the production of PGE_3 and PGI_3. Changes in cell membrane fatty acid composition reflect the PUFA components of the diets, and these compositional changes could lead to changes in membrane function (ion channels, receptors, and membrane flexibility) that may favorably alter renal physiology. Studies on how PUFAs modify membrane functions and thus impact renal disease and hypertension deserve further study. GLA lowers blood pressure in SH rats, and Yu et al. (2006) showed that this pressure lowering effect involves increases in the vasodilatory epoxyeicosatrienoic acids (EETs) and decreases in the vasoconstrictive hydrosyeicosatetraenoic acid (20-HETE). The EETs and 20-HETE are cytochrome P450-derived ECs from AA, and a GLA-enriched diet apparently alters this P450-catalyzed AA metabolism.

c. Polycystic Kidney Disease

Polycystic kidney disease (PKD) is the most common life threatening genetic disease, affecting about 13 million people worldwide. PKD is a genetic disorder characterized by the progressive development of multiple cysts in both kidneys. The disease can also damage other organs. There are two major forms of PKD that are distinguished by their patterns of inheritance. Autosomal dominant PKD is generally a late onset disorder characterized by progressive renal cyst development bilaterally. Manifestations include renal function abnormalities, hypertension, and renal insufficiency. Autosomal dominant PKD affects about 1 in 500 people, and approximately 50% of patients with this disorder develop end-stage renal disease by 60 years of age, requiring some form of renal

replacement therapy (e.g., dialysis). Autosomal recessive PKD is much rarer than the autosomal dominant form (it is estimated to occur in 1 in 20,000–40,000 people) and is often lethal. The signs and symptoms of the condition are usually apparent at birth or in early infancy.

Several studies have investigated the effects of dietary lipids on the development of PKD. A flaxseed- or flax oil-supplemented diet (resulting in the replacement of LA with alpha-linolenic acid) modifies renal PUFA metabolism such that the ratio of omega-3 to omega-6 fatty acids increases (Ogborn et al., 1999, 2002), and this is associated with the production of less inflammatory ECs (Gibson et al., 1992). This diet reduces renal injury in a rat model of PKD (the Han:SPRD-cy rat) (Ogborn et al., 1999; Sankaran et al., 2004). One of the simplest ways to reduce AA and other omega-6 PUFAs is by feeding alpha-linolenic acid, and fish oil supplements appear beneficial in several renal conditions, such as IgA nephropathy and diabetic nephropathy (McCarty, 1998) and also in PKD (Ogborn et al., 2002; Lu et al., 2003). A high-fat diet increases the pathological injury in PKD (Jayapalan et al., 2000; Sankaran et al., 2004). Ogborn et al. (2003) demonstrated that conjugated linoleic acid (CLA) reduced the renal inflammation and fibrosis in the Han:SPRD-cy rat model of PKD by reducing the renal production of PGE_2, and it is known that diets high in n-3 fatty acids also reduce the production of PGE_2.

B. Immunological Renal Disease

Unlike the nonimmunological renal diseases, immunologically mediated renal diseases are not characterized by an increased SNGFR. In fact, most nephrons show a normal or reduced filtration rate. What mechanisms lead to progression of renal disease in these disorders is not clear. It is apparent that invasion of mononuclear cells and lymphocytes play a greater role in these diseases than in renal diseases where hemodynamic changes are more clearly involved.

a. Nephritis

Nephritis is an inflammation of the kidney, usually caused by infections or autoimmune processes. It usually affects the membrane of the renal corpuscle such that the membrane becomes highly permeable to the filtration process (e.g., proteins enter into the glomerular filtrate and are excreted). There are various subtypes of nephritis, including GN (inflammation of the glomeruli), interstitial nephritis and tubulo-interstitial nephritis (inflammation of the interstitial tissues between renal tubules), pyelonephritis (caused by a urinary tract infection that reaches the kidneys), and lupus nephritis (caused by systemic lupus erythematosus).

Kelley et al. (1986) demonstrated that renal TXA_2 synthesis is positively correlated to the severity of lupus nephritis in both MRL/lpr and NZB/NZW F_1 hybrid mice. However, neither renal PGE_2 nor PGI_2 was consistently increased as the disease became more severe. Thus, as the GN becomes more extreme, renal TXA_2 production is enhanced, and this vasoconstrictor may contribute to the renal disease. Prostaglandin E_1 (a vasodilatory COX product of dihomo-gamma-linolenic acid derived from LA) markedly increases survival in mice with lupus nephritis (Zurier et al., 1977a, 1978) and improves the histological appearance of the glomeruli (Zurier et al., 1977b). Kelley and coworkers showed beneficial renal effects with exogenous PGE_1 and PGE_2 in both MRL/lpr and NZB/NZW mice (Kelley et al., 1981). These ECs may suppress T- and B-cell function (Kelley et al., 1979; Rogers et al., 1980), reduce the activity of natural killer cells (lymphocytes distinct from B and T cells) (Brunda et al., 1980), and depress neutrophil function (Fantone et al., 1983). Thromboxane B_2 excretion is elevated in mice and humans with lupus nephritis, and the source is thought to be renal (Patrono et al., 1985; Kelley et al., 1986). Renal prostaglandin E_2 secretion is also increased in humans with lupus (Kimberly et al., 1978a), and renal synthesis of prostacyclin (PGI_2) appears to play a role in maintaining the RBF and GFR in patients with chronic GN (Ciabattoni et al., 1984). Ibuprofen, a nonselective COX inhibitor altering both renal and platelet EC production (Kimberly et al., 1978b), caused a deterioration of renal function in patients with GN (Ciabattoni et al., 1984), as did aspirin (Kimberly et al., 1978a), but sulindac, a compound that selectively inhibits renal

and platelet TXA_2 formation (Ciabattoni et al., 1980), did not impair renal function nor alter urinary PGE_2 or 6-keto-$PGF_{1\alpha}$ (renal PGI_2) excretion, but production of TXA_2 decreased significantly (Ciabattoni et al., 1984). Ibuprofen decreased urinary 6-keto-$PGF_{1\alpha}$, PGE_2, and TXB_2 in patients with GN, which may be related to the observed decrease in plasma creatinine clearance (Patrono et al., 1985). It appears that a predominance of renal vasodilator ECs in GN is beneficial to the maintenance of kidney function. Clark and coworkers (1993) reported that fish oil did not improve renal function or reduce disease activity in humans with lupus nephritis after 1 year of dietary supplementation. Studies have demonstrated some improvement in systemic lupus erythematosus with high doses of EPA (20 g/day) (Walton et al., 1991), or little or no improvement with smaller doses (Moore et al., 1987; Westberg and Tarkowski, 1990), but the effects of dietary n-3 PUFAs on human lupus nephritis remain equivocal (Lefkowith and Klahr, 1996).

A diet deficient in LA has been shown to have (1) a beneficial effect on the GN of the NZB/NZW mouse (Hurd et al., 1981) and (2) no effect on murine GN (Hirschberg et al., 1984). Thus, it is possible that at least one of the LA metabolites plays a role in the pathogenesis of this disease, and that when LA is removed from the diet, the production of such dienoic EC(s) is reduced.

EPA has been shown to have significant beneficial effects on kidney function in murine models of lupus nephritis (Prickett et al, 1983; Robinson et al., 1985), possibly by impairing TXA_2-mediated changes in platelet and renal function. A diet enriched in EPA prolonged survival and reduced proteinuria in NZB/NZW F_1 mice compared to mice receiving beef tallow (Prickett et al., 1981). The group fed beef tallow showed severe glomerular injury on autopsy. Thus, a diet high in EPA prevents or delays the development of GN in NZB/NZW F_1 mice. However, this may be due to inadequate n-6 fatty acids, because n-6-deficient diets have also been noted to diminish GN (Hurd et al., 1981). Additionally, dietary n-3 fatty acids appear to have beneficial effects on the MRL/lpr murine model of lupus; progression of renal injury was suppressed and survival prolonged (Kelley et al., 1985). It was noted that renal PGE_2 and TXB_2 production was reduced compared to safflower oil-treated animals and that trienoic EC production increased. Immunological changes were also observed, such as decreased lymphoid hyperplasia and reduced circulating immune complexes. The reduced PGE_2 with n-3 supplementation initially appears difficult to reconcile with the beneficial effects usually attributed to the pharmacological use of that EC (Kelley et al., 1981). However, TXA_2 is diminished by n-3 PUFAs, and TXA_2 appears to accelerate renal diseases by stimulating lymphocyte production, platelet aggregation, and renal vasoconstriction. It is possible that the increased synthesis of renal PGE_3 helped to offset the reduction in PGE_2; overall, the n-3 PUFAs may ameliorate the deleterious effects of renal TXA_2. Clark et al. (1989) found that n-3 fatty acids given to humans with lupus nephritis improved blood rheology, reduced collagen-induced platelet aggregation, and, at high doses (18 g/day), improved the serum lipids by reducing both triglycerides and cholesterol. Platelet EPA was increased, whereas platelet AA was decreased, and the production of PGI_3 was enhanced while that of PGI_2 remained unchanged. Thus, the n-3 PUFAs may reduce the progression of renal atherosclerotic disease and GS. Furthermore, these authors observed a reduction in the neutrophil release of LTB_4, and elevated levels of this LT have been implicated in the pathogenesis of lupus nephritis (Spurney et al., 1991). PAF has been implicated in the glomerular injury observed with lupus, most likely because of its inflammatory and vasoconstrictive properties (Baldi et al., 1990). How dietary fatty acids may alter PAF synthesis or secretion by platelets in renal disease is not known. Bhattacharya and coworkers (2003) showed that fish oil plus antioxidants may prevent kidney disease in NZB/W mice, and that both n-3 and n-6 dietary lipids are susceptible to lipid peroxidation.

Yang and Cook (2003) have shown that feeding conjugated linoleic acid (CLA; isomers of LA found in dairy products and meat) to autoimmune-prone NZB/W F_1 mice after the onset of proteinuria had a beneficial effect on cachexia (mice fed regular rodent chow lost 25% more weight than mice fed CLA) and the CLA-fed mice survived an average of 1.7× longer. Other workers have shown CLA feeding to have a number of health benefits, including reducing atherosclerosis in hamsters (Nicolosi et al., 1997), modulating immune responses (O'Shea et al., 2004), improving

type II diabetes mellitus (Belury et al., 2003), reducing body fat while enhancing lean body mass in mice (DeLany et al., 1999; West et al., 2000), and anticancer properties (Wang et al., 2006). Park and coworkers (2005) found that CLA-feeding may improve survival in male Fischer 344 rats after developing renal disease due to a high protein diet.

NSN is a disease of the glomerulus that occurs because of antibody binding to glomerular structures and the ensuing host immune response to the bound antibody. Its progression is extremely rapid following nephrotoxin injection, and proteinuria and renal failure quickly ensue (Sakai et al., 1984). Glomerular TXB_2 production is significantly elevated within 2 h of the initiation of the disease (Lianos et al., 1983), and inhibitors of TXA_2 synthesis result in attenuation in the acute RBF and GFR changes. Glomerular PGE_2 and, to a lesser extent, TXA_2 are elevated at 1 and 14 days after nephrotoxin administration (Stork and Dunn, 1985); these changes are accompanied by increases in RBF and GFR. However, the inhibition of TXA_2 synthesis appears to have only a short-term beneficial effect despite a continued high glomerular TXA_2 production, whereas COX inhibition results in a significant deterioration in renal function. Since both PGE_2 and TXA_2 are increased with NSN, the major hemodynamic effects are most likely mediated by PGE_2 rather than by TXA_2. Thus, early renal vasoconstriction by TXA_2 production probably reduces renal function in rats with NSN, whereas later stages are characterized by an improvement in RBF and GFR by enhanced renal PGE_2 production. At day 14, PGE_2 production by glomeruli was about twice that of TXB_2 (Stork and Dunn, 1985). Pretreatment with aspirin, however, has been reported to prevent the proteinuria of rats with NSN without changing either RBF or GFR (Kurokawa and Sakamoto, 1982), possibly by altering some part of the glomerular pathological process. Rats with NSN that were treated with PGE_1 showed less proteinuria and fewer glomerular effects, but the deposition of antibody into the glomerulus was not altered (Kunkel et al., 1982). Lianos and coworkers (1985) found that rats with NSN showed enhanced lipoxygenation of AA to 12-hydroxyeicosatetraenoic acid, which is chemotactic and proinflammatory. Therefore, lipoxygenase products of AA metabolism may mediate some aspects of the glomerular injury. A diet deficient in LA did not alter the course of the disease in rats with NSN; in fact, a greater deposition of fibrin in their glomeruli was noted (Dubois et al., 1982), implying that an LA-deficient diet in rats with this disease may be harmful.

Immune complex-mediated glomerulonephritis (ICGN) can be induced in animals by the intraperitoneal injection of human plasma, apoferritin, or bovine serum albumin. Glomerular PGE_2 and TXB_2 production may be elevated with ICGN (Rahman et al., 1985). Since indomethacin therapy results in 30%–40% reductions in RBF and GFR, the decreased EC production (e.g., PGE_2) suggests a compensatory role for EC vasodilators in renal function related to this disease. Saito et al. (1984) reported that TXA_2 synthesis inhibition in rabbits resulted in less severe ICGN. Thromboxane synthetase inhibition resulted in increased PGI_2 in rats treated with mercuric chloride and was associated with a decline in TXB_2 excretion, less proteinuria, and 25% greater survival (Papanikolaou, 1987) Herring oil (rich in EPA) increased the survival rate of these animals to 100% and prevented the deposition of antibody in the glomeruli. Rats treated with mercuric chloride without dietary intervention had a survival rate of 60%. When mercuric chloride-treated rats were given evening primrose oil (which contains LA and GLA), survival was increased to 100%, proteinuria decreased, TXB_2 synthesis was reduced, and PGE_2 production was enhanced. In mice with ICGN, exogenous PGE_1 has been reported to reduce the proteinuria and lessen the pathological changes in the glomeruli (Kelley and Winkelstein, 1980; McLeish et al., 1980). Similar findings were reported with administration of AA (McLeish et al., 1982b). When high levels of dietary LA were supplemented to apoferritin-injected mice, the LA diet resulted in reduced proteinuria and serum creatinine and attenuated the pathological characteristics of ICGN (Barcelli et al., 1984; Kher et al., 1985), but it was not determined if the effects of LA were due to enhanced dienoic EC production. The reduction of ICGN with PGE_2 and PGE_1 may be due to a reduction in the synthesis of antiapoferritin antibody (McLeish et al., 1982a). Thus, a direct effect of ECs on the immune system may be related to the beneficial effects of these ECs on immunemediated renal disease.

Fish oil, rich in n-3 PUFAs, has been demonstrated to be beneficial in protecting against ICGN (Barcelli et al., 1984) and reduced the severity of GN in the mouse (Robinson et al., 1985) and rat (Thaiss and Stahl, 1987). The effects of the n-3 PUFAs may be related to changes in the dienoic and trienoic ECs by effectively reducing TXA_2 and substituting PGE_3 for PGE_2.

Mesangial nephropathy is a common form of GN in which antibody deposition occurs in the mesangial cells of the glomeruli. A significant number of patients with this disease will eventually progress to renal failure. Despite a preliminary report suggesting a beneficial effect of the n-3 fatty acids (Hamazaki et al., 1984), another study could not confirm these earlier findings (Bennett et al., 1989). However, Pestka et al. (2002) showed that fish oil impaired the development of immunopathology in mice with immunoglobulin A (IgA) nephropathy (IgAN). This is important given that human IgAN is the most common type of GN in the world even though the specific causes for overreactive IgA responses in IgAN are not known, although it is known that interleukin-6 deficient mice are resistant to IgA nephropathy (Pestka and Zhou, 2000). That n-3 fatty acids have these effects may not be surprising in that epidemiologic studies of fish oil consumption link it with very low incidences of autoimmune and inflammatory disorders.

There is good evidence that platelets are involved in GN. Platelet aggregation can be stimulated by immune complexes, and platelets may release factors that enhance the deposition of immune complexes in the cells of the glomerulus (Cochrane, 1971). Platelets may release substances that also enhance the inflammatory process (Baldi et al., 1990), attract white blood cells (Weksler and Coupal, 1973), and alter glomerular permeability leading to proteinuria (Camussi et al., 1984). Platelet-inhibitor drugs may be used in glomerular renal diseases where their use has delayed progression to end-stage renal disease. Of course, the beneficial effect of platelet inhibitors may also be due to both platelet and renal EC production changes (such as a reduction in TXA_2) and the consequent effects on renal hemodynamics. Dietary modification of platelet aggregation may be achieved by altering the intake of fatty acids. Both n-3 and n-6 PUFAs have been shown to reduce platelet aggregation to various agonists (McGregor et al., 1980; Leaf and Weber, 1988).

b. Nephrosis

Nephrosis refers to noninflammatory disease of the kidney. Some authors may use this term to include any damage or disease of the kidneys, including inflammatory changes (nephritis). Other authors may use the term nephropathy to indicate global renal diseases, reserving the terms nephrosis for noninflammatory kidney disorders and nephritis for inflammatory renal diseases. We will follow this latter terminology. Nephrosis may result from longstanding diabetes mellitus resulting in angiopathy of glomerular capillaries and nodular GS, or from hypertension that causes pathological changes in the small arteries and arterioles of the kidneys resulting in ischemic damage to various kidney structures.

Nephrosis (often characterized by increased glomerular permeability to proteins that may precede the development of renal failure and GS) can be induced in rats by the administration of adriamycin (Bertani et al., 1986), and this is a commonly used experimental model of human chronic kidney disease. These animals exhibit enhanced glomerular TXB_2 production concomitant with proteinuria (Remuzzi et al., 1985). The proteinuria associated with nephrosis is attenuated by TXA_2 synthetase inhibition, which also reduced urinary TXB_2 excretion to normal levels. Thus, TXA_2 may change permeability characteristics of the glomerular membrane. Thromboxane synthetase inhibitors used in humans with nephrosis also resulted in decreases in proteinuria and TXB_2 excretion. Indomethacin treatment in nephrotoxic humans reduced both proteinuria and GFR (Arisz et al., 1976). Thus, despite deterioration in the GFR, COX blockade improved the proteinuria attendant with this disease. The lessened proteinuria is probably not a result of the reduced GFR but rather due to an alteration in glomerular permeability (Tiggeler et al., 1979). It is known that adriamycin causes apoptosis of renal tubular cells, and Chen and co-workers (2006) have shown that prostacyclin (PGI_2) can protect the renal tubular cells from this programmed cell death.

Nephrosis appears to be associated with a thrombotic tendency due to platelet hyperactivity (Bang et al., 1973). Platelet-rich plasma from patients with nephrosis demonstrates enhanced aggregation to various agonists. The proteinuria with this disease causes hypoalbuminemia, and this is thought to contribute to an enhanced platelet production of the proaggregatory TXA_2 (Jackson et al., 1982b) and to defective platelet production of the antiaggregatory PGD_2. It has been shown that dietary fish oils protect against structural and functional deterioration in nephrotic rats, possibly by improving the plasma lipid profile (Ito et al., 1988).

c. Nephrotoxicity

The use of cyclosporine to prevent graft rejection is often associated with nephrotoxicity (Klintmalm et al., 1981), which can manifest as both acute and CRF. The mechanisms for the renal toxicity are not clear. Cyclosporine may increase urinary TXB_2 excretion (Kawaguchi et al., 1985), but renal PGE_2 and PGI_2 synthesis does not appear to change. The increased TXA_2 synthesis is thought to be at least partly responsible for the decline in GFR that accompanies the use of this drug. The mechanism may be due to the effect of TXA_2 on increasing glomerular vascular resistance and thus reducing RBF, or it may result from TXA_2 reducing the glomerular surface area by eliciting mesangial cell contraction (Schor et al., 1981; Scharschmidt et al., 1983).

When fish oil (rather than olive oil) is used as the vehicle to administer cyclosporine to rodents, some protection from renal tubular damage and reduced GFR has been reported. This is probably due to the effect of n-3 fatty acids to reduce the renal production of TXA_2. Fish oil also appears to be beneficial in experimental models of cyclosporine nephrotoxicity (Elzinga et al., 1987), and evening primrose oil (rich in LA and GLA) normalizes renal function in cyclosporine-treated rats, possibly by changing the renal phospholipid fatty acid profile (Mills et al., 1994). Liver transplant recipients receiving cyclosporine plus fish oil showed increased GFR and RBF and reduced renal vascular resistance compared to such patients receiving cyclosporine plus corn oil (Badalamenti et al., 1995), and there are various reports of improved renal function in renal allograft patients receiving n-3 fatty acids (Berthoux et al., 1992; Homan van der Heide et al., 1992). Holm and coworkers (2001) demonstrated that n-3 fatty acids can preserve renal function in hypertensive heart transplant patients taking cyclosporine to prevent rejection. The n-3 fatty acids prevented increases in blood pressures and vascular resistances, and also prevented a rise in plasma creatinine and a decrease in the GFR (in contrast to controls). However, Kooijmans-Coutinho and coworkers (1996) found no differences in renal function or rejection episodes between renal transplant patients receiving cyclosporine plus coconut oil (rich in short-chain saturated fatty acids) and those receiving cyclosporine plus fish oil, and there is other research indicating that fish oil supplementation does not alter renal function in kidney transplant patients, both with and without cyclosporine treatment, and in normal volunteers (Hansen et al., 1995). The nephrotoxic effects induced in rats by intramuscular injection of gentamicin was attenuated by fish oil supplementation, but not by sunflower oil (n-6 rich) (Abdel-Gayoum et al., 1995). In a review of the literature, Lefkowith and Klahr (1996) concluded that fish oil supplements may be of more consequence to patients with new transplants than to those with established allografts.

Whether ECs modulate renal events in ARF (as caused by nephrotoxins or ischemia) is unclear. Vasoconstriction usually occurs in the early phases of ARF, and a deficiency of vasodilator ECs has been proposed as the mechanism (Fine, 1970). However, since it has been found that PGE_2 and PGI_2 production and excretion are usually enhanced during the development of ARF (McGiff et al., 1970; Torres et al., 1979), this hypothesis is probably not valid. The enhanced EC production may be the result of phospholipase activation by tissue injury and ischemia attendant with ARF. Indomethacin administration causes further declines in the RBF and GFR in rabbits with ARF (Torres et al., 1975). It has been shown that PGE_1 has protective actions on the kidneys in ARF (Mauk et al., 1977). Furthermore, PGE_2 and PGI_2 infusions provide some degree of protection against renal failure induced experimentally (Casey et al., 1980). It is possible that vasodilatory ECs are produced in

the first few days of ARF to enhance kidney function. Acute tubular necrosis is associated with enhanced PGE_2 during the recovery phase of the disease, but cause and effect have not been clearly established (Funaki et al., 1982).

To summarize, it appears that immunological renal diseases are usually associated with increased rates of renal TXA_2 production. Inhibition of the action or synthesis of this EC often results in an amelioration of the disease. Thromboxane may alter glomerular filtration independent of changes in hemodynamics, possibly by altering glomerular permeability or surface area. The role of vasodilatory ECs is less clear, because they are increased in some immunological renal diseases but not others. However, the administration of exogenous vasodilatory ECs (usually PGE_2 and PGI_2) appears to be of benefit, possibly by having a negative modulatory effect on the proliferation and/or function of B and T cells, antibody production, and the action of neutrophils, in addition to their vasodilatory properties, which augment the RBF and GFR. LA-enriched diets may be helpful in some diseases, but they have either no effect in others or may possibly be harmful. In lupus nephritis, an LA-deficient diet was of benefit. Fish oils almost always proved beneficial in immunological renal diseases; however, it is not clear whether the attenuation of the disease process is due to increased n-3 fatty acids or to concomitant decreases in n-6 fatty acids. The postulated mechanisms of action include a reduction in plasma lipids (especially triglycerides), anti-inflammatory properties (Lee et al., 1985), reduced TXA_2 synthesis, and enhanced trienoic EC production. Both EPA and DHA are known to be anti-inflammatory n-3 fatty acids, and they may have synergistic properties with each other (Robinson et al., 1993).

C. Diet and Renal Patients

Nutritional management is a critical aspect of the treatment of CRF. An excellent review is presented by Grundy (1990).

Since hyperlipidemia is a common finding in patients with renal disease, dietary recommendations to improve the serum lipid profile may serve to prevent associated disorders. A number of investigators have demonstrated an increased incidence of myocardial infarction and coronary artery disease in patients with chronic renal disease, but some dispute any causal connection between lipid abnormalities seen in renal failure and the atherosclerotic lesions that frequently develop in such patients. However, the GS occurring in some renal disorders is thought to be partly due to elevated lipoproteins. In CRF, plasma triglycerides are elevated, HDL cholesterol is usually low, and LDL cholesterol is often elevated. A deficiency of lipoprotein and hepatic lipase has been well documented, and this causes a reduction in triglygeride-rich lipoprotein clearance from the blood. Both lipoprotein and apoprotein metabolism appears to be abnormal in renal patients.

Diets traditionally prescribed for patients with CRF substitute carbohydrate and fat for protein. Low-protein diets have been used to alleviate the symptoms of uremia in patients with renal dysfunction, and there is evidence that protein restriction may slow the renal pathogenesis (Klahr and Purkerson, 1988). However, the high-carbohydrate intake may exacerbate the hypertriglyceridemia. Since recent advances in our understanding of atherogenesis implicate lipids as a major contributing factor, the importance of treating the hyperlipidemia associated with renal disease is warranted. A low-carbohydrate diet with a high polyunsaturated/saturated fat ratio (P/S) can help to normalize triglyceride and cholesterol levels in CRF. Fats should provide about 40% of the total caloric requirement, and the P/S ratio should be high (Tsukamoto et al., 1982). Thus, a PUFA-rich diet comprising both n-3 and n-6 fatty acids and low in carbohydrates may reduce-triglycerides, increase high-density lipoprotein (HDL) cholesterol, and decrease the development of atherosclerosis in CRF patients. Fat intake may be increased further (at the expense of carbohydrates) if serum triglycerides remain high. Regular exercise may also be effective in lowering the triglycerides and improving the plasma lipid profile (Goldberg, 1984). In fact, exercise improves glucose tolerance, reduces anemia, and decreases the requirement for antihypertensive medications as well. Occasionally, lipid-lowering drugs may be required. In addition to their pathogenic role in atherosclerosis, lipids may themselves

contribute to glomerular and tubular injury. The possible nephrotoxic actions of lipids may be ameliorated with the correction of lipid abnormalities accompanying chronic renal disease.

VI. CONCLUSIONS

The beneficial effects of altering the dietary supply of n-3 and n-6 fatty acids in various renal pathologies may be due in part to changes in the mono-, di-, and trienoic ECs thus produced. Acting as local hormones, these ECs have profound effects on vascular musculature and platelet aggregability and may modulate the immunological and inflammatory events associated with some renal diseases. Thus, alterations in the balance of the various ECs may affect kidney function and thus change the clinical and pathological courses of some renal diseases. It has been demonstrated that renal ECs are important factors in the maintenance of kidney function in patients with preexisting renal or circulatory disease, because COX inhibition precipitates deleterious effects on these patients. The increased vasodilator EC production in many chronic renal diseases and in animal models of acute and CRF suggest a compensatory mechanism to the compromised renal function. Renal TXA_2 synthesis is usually found to be elevated in animals and humans with diminished renal function. Thus, pharmacological doses of PGE_1 or PGE_2 or thromboxane inhibition have been shown to have beneficial effects in a variety of renal diseases. Often both n-6 and n-3 dietary supplements will improve a given renal disorder. Since the effects of these two fatty acids on the formation and metabolism of AA are totally different, changes in EC synthesis may not be the only mechanism responsible for the improved renal function. Since changes in PUFA intake can change the phospholipid composition of cell membranes, changes in membrane function may also be involved. Membrane changes could alter fluidity and permeability, solute transport, enzyme activities, receptor functions, and immunological and inflammatory reactions, and these changes could be independent of alterations in EC production. Omega-3 PUFA-supplemented diets may alter the production of tumor necrosis factor and interleukin-1 in macrophages and thus attenuate the inflammatory response (Endres et al., 1989). PUFAs may also improve kidney function through their actions on serum lipids. Changes in blood fibrinolytic activity and blood viscosity may improve the rheological disturbances that could contribute to renal insufficiency. At the present time, we do not fully understand the patho-physiological role of ECs in renal disease, and additional research is essential. Further studies are warranted to define the cellular origin of the ECs, to determine the extent to which renal diseases can be ameliorated by modulating EC synthesis or action via the use of specific enzymatic inhibitors or receptor blockers, and to determine how changes in fatty acid membrane composition affect renal processes. The possible relationship between hyperlipidemia and the progression of renal disease has received much attention, but further work is needed to elucidate fully the relationship and the extent to which renal disease can be changed by altering the serum lipids. It may turn out that modifications in dietary fats could be a relatively easy and practical way to attenuate or prevent some renal diseases. Indeed, dietary PUFA modulation appears to be a useful strategy that is recently finding increased clinical application.

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47 Fatty Acid Metabolism in Diabetes

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CONTENTS

I. INTRODUCTION

The two major abnormalities in both insulin-dependent diabetes mellitus (IDDM, type 1) and noninsulin-dependent diabetes mellitus (NIDDM, type 2) are hyperglycemia and dyslipidemia.

In normal as well as many pathological conditions, the metabolism of carbohydrates, especially glucose, is closely linked to the metabolism of lipids. Insulin is intimately involved in the control of carbohydrate and lipid metabolism. There is either an absolute (in IDDM) or relative (in NIDDM) deficiency of insulin in diabetes or peripheral resistance to insulin, particularly in obese NIDDM subjects. Thus, in untreated as well as poorly controlled diabetic subjects, hyperlipidemia is often associated with hyperglycemia. Close relationship has also been reported for fasting plasma glucose and fasting and meal-stimulated free fatty acid (FFA) levels (Coates et al., 1994). Newgard and McGarry (1995) recently advanced the concept that important signal for insulin secretion may reside at the linkage between glucose and lipid metabolism, namely the generation of malonyl coenzyme A (CoA) that promotes fatty acid esterification and inhibits oxidation. The treatment of hyperglycemia by both diet and exercise, oral agents (sulfonylureas, biguanides, thiazolidinediones), or insulin often also results in the partial reduction of hyperlipidemia.

A major proportion of fatty acids are present in the esterified form as a component of phospholipids, triglycerides, or cholesterol esters. In contrast, only a small fraction of total fatty acids in the body (plasma and tissues) is in the free form (nonesterified or FFAs). The metabolism of fatty acids is intimately linked to the metabolism of lipids and lipoproteins. The differences in the composition of lipids and lipoproteins between control and diabetic subjects are both quantitative and qualitative. In diabetes the most common abnormalities in lipid metabolism, and therefore of fatty acids, are elevated triglycerides (TGs) and very-low-density lipoproteins (VLDL), altered lipogenesis, and accelerated lipolysis (Randle, 1963; Tarrant et al., 1964; Fredrickson et al., 1967; Saudek and Eder, 1979; Brown et al., 1982; Dunn, 1988, 1990; Garg and Grundy, 1990; Fagot-campagna et al., 1997). An increase in small dense low-density lipoprotein (LDL), an integral part of insulin resistance syndrome, has been recognized as an independent risk factor for coronary heart disease (CHD) and may be a predictor of NIDDM (Austin et al., 1995, 1996). The increased VLDL levels are due to increased production as well as decreased clearance. As a consequence, there is an increase in FFA in the plasma of diabetic subjects. Increased levels of plasma TG, total and LDL-cholesterol, apolipoprotein B (apo B), apolipoprotein A-IV (apo A-IV), and phospholipids are also observed in poorly controlled IDDM subjects (Attia et al., 1997). The increased level of lipoproteins containing apoprotein B appears to be due to increased secretion rather than intracellular degradation due to increased FFA flux from liver (Ginsberg, 1996). In poorly controlled diabetic patients there is also an increased nonenzymatic glycosylation of LDL apoprotein B (Steinbrecher and Witztum, 1984; Taskinen, 1987), increased oxidation of LDL lipoproteins (Dimitriadis et al., 1996) and apo A-IV levels (Verges et al., 1994) and decreased high-density lipoprotein (HDL) and HDL_2-cholesterol (Verges et al., 1994). The low levels of HDL are due to a decreased production (Golay et al., 1987) and an increased catabolism due to the increased hepatic TG lipase activity (Kasim et al., 1987). Autoantibodies to LDL are also increased in NIDDM subjects with and without atherosclerosis and are accompanied by high levels of polyunsaturated fatty acids (PUFAs) in LDL (Griffin et al., 1997). Abnormalities in lipid metabolism have been reported in animal models of diabetes, namely, streptozotocin-treated rat, d*b*/d*b* mouse, and in newer models of NIDDM, the obese SHR/N-cp rat and obese WKY/N-cp rat (Hennig and Dupont, 1983; Cunnane et al., 1985; Michaelis et al., 1986; Bhathena et al., 1989a). The abnormalities of lipid metabolism also accompany various complications of diabetes, namely retinopathy, nephropathy, peripheral neuropathy, coronary arterial disease, microangiopathy, lipodystrophy, and ketoacidosis (Vannini et al., 1984; Appel et al., 1985; Winocour et al., 1987; Stacpoole et al., 1988; Vandongen et al., 1988). The vascular complications of diabetes may be in part due to increased lipoperoxidation of LDL in erythrocytes and other cell membranes (Rabini et al., 1994).

This chapter covers only the alterations in plasma and tissue fatty acid composition, the role of dietary fatty acids in different types of diabetes and its complications, the metabolism and hormonal regulation of fatty acids in diabetes, and how the control of diabetes by diet and exercise, oral hypoglycemic and hypolipidemic agents, and insulin affects fatty acid metabolism. Though earlier studies will be referred to, this chapter concentrates more on the studies carried out in the past 10 years. Only a few references are cited; several more could have been easily added.

II. FATTY ACID COMPOSITION OF TISSUE LIPIDS

Dietary fatty acids have been shown to influence the fatty acid composition of lipids in plasma and tissues in normal as well as in pathological conditions such as diabetes (Horrobin, 1989; Popp-Snijders and Blonk, 1995; Berry, 1997). The trend in past 40–50 years indicate increased consumption of saturated fats, *trans* fatty acids (TFA), vegetable oils rich in linoleic acid and decreased consumption of long-chain PUFA (arachidonic acid, AA; eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA) leading to increased incidence of NIDDM and related disorders (Simopoulos, 1994).

The fatty acid composition of cholesterol esters appear to be related to the risk of development diabetes. The risk is increased when cholesterol esters are rich in saturated fatty acid, palmitoleic acid, γ-linolenic acid (GLA), and dihomo-γ-linolenic acid (DHLA), and low in linoleic acid (Vessby et al., 1994). The prevalence of diabetes may also be correlated with the dietary ratio of ω6–ω3 fatty acids (Berry, 1997). Besides diet, genetic factors may also play a role in altering fatty acid composition of cholesterol esters (Vessby et al., 1994). Feeding very long-chain fatty acids (C24–C34) increases these fatty acids in phosphatidyl choline but not in other phospholipids of some organs such as rod of outer segments in normal as well as diabetic rats (Suh et al., 1994).

In untreated and poorly controlled diabetic subjects, desaturases involved in the metabolism of fatty acids are decreased (see below) and 18-carbon fatty acids with a low number of double bonds predominate both in plasma and in tissues. Thus, even though the composition of fatty acids in plasma reflects that in the diet, metabolites may be different. Mikhailidis et al. (1986) fed DHLA to controls and IDDM subjects. In controls there was an increase in DHLA as well as its metabolite AA in erythrocyte lipids, but in IDDM subjects there was no proportionate increase in AA in erythrocyte lipids, indicating a possible defect in Δ5 desaturase. Similarly, conversion of DHLA to prostaglandin E_1 (PGE_1) was also inhibited in IDDM subjects but not in controls (Mikhailidis et al., 1986). Decreased AA in plasma and 6-keto-$PGF_{1\alpha}$ in urine have been reported in IDDM (Pottathil et al., 1985). Bassi et al. (1996) reported decrease in plasma phospholipid ω6 fatty acids in IDDM subjects with mild ketosis, which was primarily due to decrease in AA. There was also an increase in 20:4 to 20:3 ratio indicating decreased Δ5 desaturase activity during mild ketosis. Fatty acids in platelets from control nondiabetic subjects showed a significant inverse correlation between linoleic acid and AA. No such relationship was observed in IDDM and NIDDM subjects, indicating that both Δ5 and Δ6 desaturases are impaired in the diabetic state (Jones et al., 1986). Diabetic children in good control showed a direct correlation between apo A-I and PUFAs and the ratio of PUFA/saturated fatty acids in TGs, while APO A-II correlated with the ratio of DHLA and AA (Ewald et al., 1982).

Some differences have been observed in the fatty acid compositions of plasma lipids between IDDM and NIDDM subjects (Seigneur et al., 1994). A decrease in EPA was observed in the livers of diabetic subjects (Singer et al., 1980) but, in another study, EPA in liver TGs was high (greater than 30%) in diabetic subjects without hyperlipoproteinemia (Singer et al., 1984). In another study, no significant differences were observed in plasma concentrations of AA, EPA, DHA or the saturated fatty acids (palmitic and stearic) between normal and poorly controlled diabetic Melanesian subjects (Rao and Erasmus, 1996). In red blood cells of IDDM (van Doormaal et al., 1984) as well as NIDDM (Prisco et al., 1989a) subjects, phospholipids have significantly higher saturated fatty acids and decreased PUFA. A similar fatty acid profile was observed in plasma of IDDM subjects (van Doormaal et al., 1984; Juhan-Vague et al., 1986), which was normalized when diabetes was improved by insulin infusion (van Doormaal et al., 1984). However, in another study insulin treatment had no significant effect on the fatty acid composition of erythrocyte membrane phospholipids (Persson et al., 1996). In NIDDM subjects with hyperlipidemia, there is an increase in saturated fatty acid and monounsaturated fatty acid (MUFA) in plasma lipids and a decrease in ω6 PUFA metabolites and C_{20}–C_{22} PUFA (Bohov et al., 1997). Diabetic subjects with macroangiopathy had significantly lower EPA and DHA acid but higher AA in platelet phospholipids than diabetic subjects without macroangiopathy and control subjects (Morita et al., 1983; Prisco et al., 1989b). Increased AA has also been observed in LDL and in erythrocyte membrane phospholipids of diabetic subjects

compared to controls (Rabini et al., 1994). Similarly, in patients with diabetic nephropathy, levels of DHLA, α-linolenic acid (ALA) and DHA are low in plasma phospholipids compared to control subjects (Das, 1995). Alteration in fatty acid composition is also observed in milk of diabetic mothers, especially lower levels of long-chain PUFA (Jackson et al., 1994), indicating altered metabolism in the mammary gland (Bitman et al., 1989).

Since hyperlipidemia and other abnormalities of lipid and lipoprotein metabolism associated with coronary artery disease are usually present in diabetic subjects, especially obese NIDDM, they are prudently recommended to consume diets low in total fat and saturated fatty acids and high in PUFA (Mann et al., 1976; American Heart Association, 1986; American Diabetes Association, 1987; Kissebah and Schectman, 1988; Margolis and Dobs, 1989; Clandinin et al., 1993). It is therefore not surprising to find higher levels of linoleic acid, GLA, and AA as well as EPA and DHA and lower levels of saturated fatty acids in plasma and tissues of well-controlled diabetic subjects (Ewald et al., 1982; O'Dea and Sinclair, 1985; Mann, 1986). Increased levels of EPA have been observed in plasma (Glauber et al., 1988; Landgraf-Leurs et al., 1990), red blood cells (Glauber et al., 1988), erythrocyte membranes (Kamada et al., 1986), platelets (Tilvis et al., 1987; Landgraf-Leurs et al., 1990), and pancreatic B cells (Lardinois, 1987) in diabetic subjects given a daily supplement of fish oils. Since fatty acids from the ω-3 family compete with those from the ω-6 family (Holman, 1986), feeding fish oil decreases linoleic acid and AA from tissue lipids, especially phospholipids (Dang et al., 1989; Landgraf-Leurs et al., 1990).

As stated earlier, atherosclerosis is a common abnormality of diabetes. Thickness of carotid intima-media is one of the predictor of atherosclerosis. In a population-based study, Ma et al. (1997) observed positive correlation between saturated fatty acid and MUFA composition of plasma phospholipids and cholesterol esters and carotid artery intima-media thickness. There was a negative correlation for PUFA and carotid artery intima-media thickness. This study further indicates the benefit of reducing dietary intake of saturated fatty acids to reduce the incidence of cardiovascular complications in diabetic subjects. The importance of MUFA in addition to ω3 fatty acids in reducing cardiovascular risk in Asian Indian diabetic subjects has been stressed (Peterson et al., 1994).

In animal models of diabetes, a somewhat different picture is observed. Most of the data on the fatty acid composition of various tissues in the diabetic state have come from animal studies. Significant changes in fatty acid composition of lipids, especially phospholipids, have been observed in many but not all tissues. In streptozotocin diabetic rats, the incorporation of palmitate, oleate, and linolenate into erythrocyte membrane phospholipids is reduced but arachidonate incorporation is not affected (Arduini et al., 1995). This is partially reversed by the treatment with propionyl-L-carnitine treatment (Arduini et al., 1995). In alloxan diabetic sheep (Henderson et al., 1982) and streptozotocin diabetic rat (Takahashi et al., 1987), there is a decrease in saturated fatty acids and an increase in PUFA in liver TGs, which are the major lipids. TG levels are also elevated in the hearts of diabetic animals (Denton and Randle, 1967; Murthy and Shipp, 1977; Paulson and Crass, 1982; Murthy et al., 1983), and the level of linoleic acid is increased (Gudbjarnason et al., 1987). Increased levels of TGs in the hearts of diabetic animals are due to both stimulation of esterification (synthesis) (Murthy and Shipp, 1977; Paulson and Crass, 1982) and an inhibition of lipolysis (Paulson and Crass, 1982; Murthy et al., 1983). In phospholipids, however, there is a significant decrease in PUFA in diabetic animals (Henderson et al., 1982; Takahashi et al., 1987), indicating a shift in PUFA from phospholipids to TGs. Similar decreases in AA and other PUFAs have been observed in whole liver (Holman et al., 1983) and in mitochondrial and microsomal lipids from diabetic rats (Labonia and Stoppani, 1988; Burke and Fenton, 1989; Dang et al., 1989). There was however, an increase in DHA in mitochondrial and microsomal membranes (Labonia and Stoppani, 1988; Burke and Fenton, 1989). Modulation of adipose tissue fatty acid by dietary fatty acids has been reviewed by Clandinin et al. (1993). Lipid changes in the heart in diabetes have been reviewed by Dhalla et al. (1992). Decreases in PUFA, especially AA, are also observed in adipocytes and from streptozotocin diabetic rat (Field et al., 1988, 1989, 1990; Clandinin et al., 1993) and in heart muscle of alloxan (Gudbjarnason et al., 1987) as well as streptozotocin (Holman et al., 1983) diabetic rat compared

to controls. The levels were normalized when the diabetic rats were fed diets with high PUFA/saturated fatty acid ratios (Field et al., 1988, 1990).

Dietary fatty acids produced no significant change in brush border membrane phospholipids (Keelan et al., 1987) or cholesterol esters in controls as well as the streptozotocin diabetic rats. Lack of alteration in lipid composition by dietary fatty acids in intestinal brush border membrane as opposed to a significant effect in liver and adipose tissue may be due to the fact that both the liver and adipose tissue are actively involved in lipid metabolism as opposed to the intestine. Also, unlike liver and adipose tissue, the intestine is not insulin sensitive (Shiau and Holtzapple, 1980) and therefore is less likely to show metabolic responses to dietary fatty acids. Abnormalities of PUFA metabolism were also observed in T cells (Singh et al., 1988), glomeruli (Kanzaki et al., 1987), testes (Wilder and Coniglio, 1984), and diaphragm and renal cortex (Chorvathova and Ondreicka, 1983) of streptozotocin diabetic rats. In the streptozotocin diabetic rat, dietary fatty acids did not alter AA content in plasma phospholipids, but in aortic phospholipids there was a significant decrease in AA, which was increased by feeding diets high in ω-6 fatty acids (Takahashi et al., 1988). In genetically diabetic d*b*/d*b* mice, decreases in AA in phospholipids in the pancreas and in TGs in the liver have been observed and were normalized by dietary essential fatty acids (EFAs) (Cunnane et al., 1985).

Thus, most tissues show a decreased level of AA in phospholipids in streptozotocin-treated animals, even when they are fed diets high in linoleic acid and GLA such as soybean oil and evening primrose oil, clearly indicating a defect in Δ5 and Δ6 desaturases. Streptozotocin produces hypoinsulinemia by destroying pancreatic B cells resembling IDDM in humans with absolute insulin deficiency, and insulin is required for normal functioning of desaturases.

III. METABOLIC EFFECTS OF DIETARY FATTY ACIDS IN DIABETES

A. Unsaturated Fatty Acids

There are at least four families of unsaturated fatty acids. However, fatty acids from only two families, ω-6 and ω-3, are considered essential. The two most common EFAs are linoleic acid (18:2ω6) and ALA (18:3ω3). They are required for normal growth and other biological processes and cannot be synthesized in humans owing to the lack of specific desaturases required to insert double bonds at the correct positions. Both acids, linoleic and ALA, as well as oleic acid are involved in cholesterol transport and oxidation. Most of the other biological effects of both of these fatty acids appear to be through their conversion to longer chain, more unsaturated fatty acids, such as GLA and AA from linoleic acid and EPA from ALA. GLA, in fact, is 170 times as active as linoleic acid in lowering cholesterol levels (Horrobin and Manku, 1983). High cholesterol-lowering activity has also been reported for AA (Kingsbury et al., 1961). These fatty acids are precursors for leukotrienes (LTs) and prostanoids that are biologically active. In addition, AA is a major component of cellular membrane structure, and the nature and unsaturation of the membranes determine the biological function (see below). The importance of EFA in human and animal nutrition has been reviewed by Sinclair (1984). It has been shown that dietary PUFA also regulate transcription of genes thereby influencing the metabolic directions of fuels (Clarke et al., 1997). Though precise mechanism of how PUFA regulate gene transcription is not known, it may be via modulation of transcription factor peroxisome proliferator-activated receptor (PPAR) action.

In diabetes, in general there is increased concentration of saturated fatty acid and MUFA and decreases in PUFA (see above). EFAs of both families have been shown to have several beneficial effects in normal and diabetic subjects. Complications of diabetes, namely, retinopathy, nephropathy, and peripheral neuropathy, may occur in chronic EFA deficiency (Sinclair, 1984). However, some deleterious effects of EFA have also been observed. Dietary fish oils contain proportionately higher concentrations of EPA and DHA. Their role in diabetes will be discussed separately.

B. ω-6 Fatty Acids

Most studies in human diabetic subjects used vegetable oils rich in linoleic acid. Few studies have used dietary AA and evening primrose oil that contain appreciable amounts of GLA as well as linoleic acid. In IDDM and NIDDM the beneficial effects include decreased cholesterol and TG levels especially with high doses of GLA (Chaintreuil et al., 1984); reversal of abnormalities in lipid metabolism caused by very low-fat diet (Piper et al., 1986); improved platelet function (Monnier et al., 1983), possibly by increased prostanoid and LT synthesis and secretion; enhanced erythropoiesis and decreased glycosylated hemoglobin levels (van Doormaal et al., 1988); and increased bleeding time (O'Dea and Sinclair, 1985), thereby reducing the risk of coronary artery disease. In addition, linoleic acid has been shown to have a protective effect in diabetic retinopathy (Houtsmuller et al., 1980; Howard-Williams et al., 1985), and GLA has beneficial effect in the prevention and treatment of diabetic polyneuropathy (Jamal and Carmichael, 1990; Keen et al., 1993). The beneficial effect of GLA on neurophysiological parameters in diabetic subjects has been reviewed by Horrobin (1997). Similarly, increased linoleic acid in LDL- and HDL-cholesterol in NIDDM subjects appear to protect against the oxidation of LDL (Dimitriadis et al., 1996). An epidemiological study in Australian aborigines also reported a beneficial effect of diets low in fat but high in PUFA in preventing the development of diabetes and cardiovascular diseases (Naughton et al., 1986). Diabetic subjects also showed a greater insulin response to PUFA compared to saturated fat.

Yam et al. (1996) reported increased incidence of NIDDM, cardiovascular diseases, hypertension, and obesity with hyperinsulinemia and insulin resistance as the underlying causes in Israeli population, which has the highest consumption of PUFA to saturated fatty acid ratio in the world. Thus, instead of being beneficial, high consumption of PUFA may have serious long-term effects. In animal studies, high PUFA intake has been shown to increase incidence of variety of tumors. Higher concentrations of long-chain PUFAs, especially C_{20}–C_{22} in muscle membrane phospholipids are shown to reduce insulin resistance, while linoleic acid appears to increase insulin resistance (Simopoulos, 1994).

Most of the studies in animals have been carried out in streptozotocin diabetic rats. Beneficial effects of PUFA have been observed after both short- and long-term feeding (Keelan et al., 1989). Diabetic rats fed PUFA had lower plasma and urinary glucose (Rajotte et al., 1988), lower glycosylated hemoglobin (Rajotte et al., 1988; Keelan et al., 1989), lower relative decline in glucose after intravenous glucose load, lower cholesterol and TG levels, and near-normal microsomal glucose-6-phosphatase activity (Keelan et al., 1989). However, in another study no improvement was seen during oral and intravenous glucose tolerance tests (Rajotte et al., 1988). In diabetes there is an increase in passive transport of both glucose and lipids. Saturated fatty acids further increase passive transport of lipids (Thomson et al., 1988). PUFAs decrease the enhanced glucose (Thomson et al., 1987a,b) and galactose (Thomson et al., 1987a) transport, possibly by increasing intestinal brush border membrane alkaline phosphatase activity (Thomson et al., 1987b), and also by enhancing lipid transport (Thomson et al., 1988). Others have not observed the beneficial effect of PUFAs on intestinal glucose uptake (Keelan et al., 1989).

As seen in human diabetic subjects, EFAS, especially GLA, improves nerve conductance in streptozotocin diabetic rats and improves symptoms of diabetic polyneuropathy (Julu, 1988; Tomlinson et al., 1989; Cameron and Cotter, 1994; Dines et al., 1995). Ascorbyl GLA appears to be more effective in improving neurovascular defect in diabetic rats than GLA, ascorbate or the combination of the two (Cameron and Cotter, 1996a). Similar synergistic effect was observed when GLA was given in combination with antioxidant (Cameron and Cotter, 1996b) or the aldose reductase inhibitors (Cameron et al., 1996; Cameron and Cotter, 1997). The beneficial effect of GLA may be in part due to increased prostacyclin production. A beneficial effect of AA on diabetes-related embryopathy has also been reported (Pinter et al., 1988). Reece et al. (1996) reported *in vivo* decreased incidence of neural tube defect in diabetic rats fed safflower oil. The effect was attributed to the conversion of linoleic acid to AA. In human skin fibroblast cultured in the presence of lipoprotein-deficient

serum, linoleic acid and AA increased carbohydrate oxidation as measured by increased pyruvate dehydrogenase activity (Loriette et al., 1987).

Dietary PUFA has been reported to increase glucose utilization (Awad, 1981; Field et al., 1990) along with increased insulin binding (Field et al., 1988) and improved insulin sensitivity (Field et al., 1990). Others, however, have reported increased insulin resistance in liver and muscle in diabetic rats fed linoleic acid and saturated fatty acids (Storlien et al., 1987). In obese as well as insulin-treated diabetic rats, PUFA from corn oil appears to suppress lipogenic enzyme gene expression stimulated by insulin (Iritani et al., 1995; Iritani and Fukuda, 1995). PUFAs compared to saturated fat, decreases insulin binding, autophosphorylation of the receptor and the kinase activity in liver, which are partially reversed by pioglitazone (Iritani and Fukuda, 1995). In streptozotocin diabetes, T-cell-dependent immune function is decreased. Although dietary linoleic acid did not improve the T-cell function, diets low in linoleic acid further decreased the immune function (Singh et al., 1988). In diabetic mice, dietary linoleic acid increases the incorporation of AA in phospholipids of the pancreas and liver (Cunnane et al., 1985).

Several studies in animal models of diabetes have reported detrimental effects of EFA and beneficial effects of feeding EFA-deficient diets. Thus, in diabetes prone BB rats, feeding an EFA-deficient diet, which lowers the concentrations of AA, decreased the incidence of spontaneous diabetes (Lefkowith et al., 1990). It is possible that AA or its eicosanoid metabolites may be responsible for the inflammatory injury in this model of autoimmune diabetes (Lefkowith et al., 1990), which resembles human IDDM. In this regard, it is interesting to note that injection of arachidonate in BB rats increased the concentrations of thromboxane B_2 (TXB_2) and trioxilin A_3 and decreased 6-keto-$PGF_{1\alpha}$ (Pace-Asciak et al., 1988). This increased ratio of TXB_2 to 6-keto-$PGF_{1\alpha}$ is in line with the prothrombotic nature of platelets associated with diabetes (Pace-Asciak et al., 1988). Similarly, in nonobese diabetic mice, EFA deficiency decreases the incidence of diabetes and has the protective effect on autoimmunity (Benhamou et al., 1995). There is an increase in splenocyte interleukin-4 production and a reduction in interferon-γ production, while in macrophages there is an increase in tumor necrosis factor-α (TNF-α) and interleukin-1 and a reduction of PGE_2 indicating altered eicosanoid metabolism (Benhamou et al., 1995). The beneficial effect of EFA deficiency was also observed in low-dose streptozotocin-treated diabetic mice, possibly by decreasing the lipid mediators of autoimmunity such as prostaglandin and LTs (Wright et al., 1988). The destruction of pancreatic B cells was also low in EFA-deficient mice (Wright et al., 1988; Fraser et al., 1997). EFA deficiency also prevents diabetes in low-dose streptozotocin mice treated with cyclosporin A (Wright et al., 1995), the later is known to increase the severity of diabetes in streptozotocin-treated mice. Also streptozotocin- and alloxan-treated diabetic rats were less EFA deficient than control rats when fed diets deficient in EFA (Riisom et al., 1981). The levels of AA in liver and heart phospholipids were higher in diabetic rats than in controls fed an EFA-deficient diet (Riisom et al., 1981). The reason for this is not clear. It could be that the concentration of AA in phospholipids is well preserved in diabetes or that a specific phospholipase (phospholipase A_2) may be less active. It is also possible that processes of desaturation and elongation of fatty acids are less affected in diabetes. The latter possibility is less attractive because desaturases have been shown to be decreased in diabetes.

C. ω-3 Fatty Acids and Fish Oils

In diabetic subjects, dietary supplementation of long-chain PUFAs present in fish oils has been studied more extensively than that of other fatty acids. The two predominant PUFAs in fish oils are EPA and DHA. In nondiabetic but hyperlipidemic subjects, fish oil feeding has been shown to have more beneficial effects, especially in reducing the risk of heart disease, and few deleterious effects, as reviewed by Mueller and Talbert (1988), Gibson (1988), Margolis and Dobs (1989), Harris (1989), Nestel (1990), Flaten et al. (1990), and Kinsella et al. (1990). In a prospective study with Physicians, moderate consumption of fish had no effect on lowering the risk of cardiovascular disease (Morris et al., 1995). In diabetic subjects, however, supplementation with fish oils has not produced beneficial

TABLE 47.1
General Effects of Dietary Fish Oils in Normal and Diabetic Subjects

Reduce risk of heart disease
Lower TGs and lipoproteins
Increase HDL-cholesterol (HDL_2 and HDL_3)
Reduce thrombogenicity of platelets in microcirculation
Reduce platelet aggregation
Decrease incidence of NIDDM
Increase insulin secretion and insulin sensitivity
Prevent insulin resistance caused by high fat and saturated fat diets
Improve fluidity of cell membranes in IDDM but not NIDDM subjects
Decrease blood pressure in hypertensive individuals
Decrease as well as increase plasma or blood viscosity
Increase total and LDL-cholesterol
Increase fasting and postprandial blood glucose
Increase hepatic glucose output
Impair insulin secretion (in diabetic patients)
Increase nonenzymatic glycosylation
Altered eicosanoid production
Increase bleeding time
Impair red cell deformity
Decrease vitamin E absorption
Increase caloric intake and hence weight gain
Cause stomach and intestinal irritation

effects to the extent seen in nondiabetic subjects. The role of dietary fish oils in diabetic subjects has been reviewed by Axelrod (1989), Sorisky and Robbins (1989), and Vessby (1989). Fish oil concentrate K-85, containing 92% of total fatty acids as ω3 fatty acids has been shown to lower serum TG and VLDL in nondiabetic hypertriglyceridemic subjects (Mackness et al., 1994). Its effect in diabetic subjects however, needs to be explored. ω3 Fatty acids also have antihypertensive effects (Knapp, 1996). The known metabolic and biological effects of fish oils in nondiabetic and diabetic subjects are summarized in Table 47.1.

In diabetic subjects, the most consistent beneficial effect of dietary fish oils is the lowering of plasma TG levels (Popp-Snijders et al., 1986, 1987; Schectman et al., 1988, 1989; Borkman et al., 1989; Mori et al., 1989; Rillaerts et al., 1989; Schmidt et al., 1989; Stacpoole et al., 1989; Bagdade et al., 1990; Landgraf-Leurs et al., 1990; Rivellese et al., 1996; Sirtori et al., 1997), though in one study no significant lowering of TG was observed in NIDDM (Kasim et al., 1988). Singer et al. (1984) observed a negative correlation between TG and EPA in the liver of diabetic subjects. In most studies (Haines et al., 1986; Schectman et al., 1988; Vandongen et al., 1988; Mori et al., 1989; Stacpoole et al., 1989) but not all (Borkman et al., 1989; Rillaerts et al., 1989), total cholesterol and LDL-cholesterol were increased. However, this deleterious effect was negated by increases in HDL-cholesterol (especially in HDL_2 and HDL_3 subfractions) (Vandongen et al., 1988; Mori et al., 1989; Rillaerts et al., 1989; Schectman et al., 1989; Schmidt et al., 1989; Bagdade et al., 1990). There was also a lowering of the cholesterol/phospholipid ratio (Kamada et al., 1986) and the cholesterol/HDL-cholesterol ratio (Rillaerts et al., 1989; Schmidt et al., 1989), which is a measure of atherogenic index. Decrease in plasma VLDL TG and FFA and increase in long-chain ω3 fatty acids in erythrocyte phospholipids have been observed after 6 months of feeding fish oil to NIDDM subjects (Rivellese et al., 1996) but had no significant effect on blood glucose control in these subjects. Fish oil increases lipoprotein lipase (LPL) activity in NIDDM (Kasim et al., 1988) but has no effect in IDDM (Bagdade et al., 1990). In a study comparing ω3 fatty acids from fish oil (EPA + DHA) with that from linseed oil (linolenic acid), fish oil decreased plasma TGs in NIDDM subjects but

linseed oil was without effect (Goh et al., 1997) indicating that preformed long-chain ω3 fatty acids are more effective in lowering lipid levels in diabetic subjects than linolenic acid. ω3 Fatty acids are also more readily incorporated in brain and other tissues as compared to those from vegetable oils. Recognizing the need for preformed long-chain PUFA for brain development and function, infant milk formulas are now being supplemented with fish oil (Clandinin et al., 1992; Carlson, 1994). This has led to improved brain and visual function in infants (Makrides et al., 1994; Uauy et al., 1994). The beneficial effects of fish oil on lipid metabolism in NIDDM subjects are potentiated by high-fiber intake (Sheehan et al., 1997).

Dietary fish oils have several deleterious effects on carbohydrate metabolism in diabetic subjects. There is an increase in fasting and postprandial glucose (Glauber et al., 1988; Kasim et al., 1988; Schectman et al., 1988; Borkman et al., 1989; Friday et al., 1989; Vessby, 1989), increased levels of glycosylated hemoglobin (Schectman et al., 1988), increased hepatic glucose output (Glauber et al., 1988), but unaltered glucose disposal (Glauber et al., 1988; Friday et al., 1989) and thus worsened glycemic control. However, in a multicenter study, ethyl ester of ω3 fatty acid did not worsen the glycemic control (Sirtori et al., 1997). In another study, fish oil improved glucose homeostasis in some but not all subjects (Zak et al., 1996). Short-term feeding of fish oil was shown to increase gluconeogenesis from glycerol but not the overall glucose production or the glycemic control (Puhakainen et al., 1995). Owing to the increase in gluconeogenesis from glycerol, long-term feeding of fish oil is anticipated to deteriorate glucose control. Insulin secretion is also impaired by fish oil feeding (Glauber et al., 1988; Lardinois et al., 1988), but plasma insulin levels are generally not altered (Borkman et al., 1989; Friday et al., 1989). In some diabetic subjects, fish oil feeding also increases the daily insulin requirement for metabolic control (Stacpoole et al., 1989). Dietary fish oil, however, prevents insulin resistance caused by a high-fat diet or by saturated fat (Storlien et al., 1987; Borkman et al., 1989) and improves insulin sensitivity (Popp-Snijders et al., 1987; Vessby, 1989). Beneficial effects of dietary ω-3 fatty acids have been observed in a diabetic subject with acanthosis nigricans and lipodystrophy (Sherertz, 1988); however, this needs to be confirmed. ω3 Fatty acids also increased fluidity of erythrocyte membranes in IDDM (Kamada et al., 1986; Tilvis et al., 1987), but no change was seen in NIDDM subjects (Popp-Snijders et al., 1987; Rabini et al., 1993). Dietary fish oil affects several hormones besides insulin. Thus, fish oil feeding decreased glucagon and somatomedin-C (Bhathena et al., 1991) and β-endorphin (Bhathena et al., 1993).

In diabetic subjects there is a decrease in thromboxane but normal prostacyclin production (Tilvis et al., 1987), and increased platelet aggregation (Landgraf-Leurs et al., 1990). ω-3 Fatty acids further lower thromboxane production *in vitro* in platelets stimulated with collagen or adenosine diphosphate (Haines et al., 1986; Tilvis et al., 1987; Landgraf-Leurs et al., 1990), but thromboxane production from exogenous AA is increased (Tilvis et al., 1987). Fish oil feeding either decreased platelet aggregation (Landgraf-Leurs et al., 1990) or prolonged the lag phase before aggregation (Haines et al., 1986). Harris et al. (1997) reported that antiatherogenic effect of fish oil may also be in part due to enhance arterial nitric oxide (NO) production, which has vasodilatory effects. Other beneficial effects of dietary ω-3 fatty acids in diabetic subjects include decreased blood viscosity (Rillaerts et al., 1989), lowering of blood pressure (Kasim et al., 1988), and increased neutrophil but not monocyte chemotaxis (Schmidt et al., 1989). The formation of eicosanoids and their effects in diabetes are discussed later in this chapter.

ω-3 Fatty acids in fish oils are highly unsaturated and are easily oxidized. Vitamin E decreases this peroxidation, and hence in some diabetic subjects, especially children, high intake of fish oil causes vitamin E deficiency, which may lead to neurological disturbance when severe malabsorption problems exist (Margolis and Dobs, 1989). The apparent differences in the observed fish oil effects in different studies are partly due to different doses of fish oil concentrate as well as the length of feeding.

Very few studies on the effects of dietary fish oils have been carried out with animal models of diabetes. In diabetes-prone BHE rats fed fish oil, thyroxine increased fatty acid synthesis in liver and adipose tissue (Pan and Berdanier, 1990). In low-dose streptozotocin-treated mice, fish oil decreased elevated blood glucose and improved immune function (Linn et al., 1989) but increased the development of retinopathy (Hammes et al., 1996). In streptozotocin-treated rats, ω-3 fatty acids had no

effect on glucose, TG, or cholesterol levels, but the development of cardiomyopathy was partially blocked, possibly by improving Ca^{2+} transport activity in cardiac sarcoplasmic reticulum (Black et al., 1989). However, in diet induced, insulin resistant mildly diabetic rats, fish oil did reduce plasma TG and cholesterol and corrected hyperinsulinemia (Luo et al., 1996). Infusion of perilla oil rich in ALA compared to soybean oil in streptozotocin-treated diabetic rats increased the proportion of EPA and decrease AA in serum and liver phospholipids accompanied by a decrease in TXA_2 production (Ikeda et al., 1995). These changes were independent of plasma insulin levels. In hepatic microsomes, ω-3 fatty acids decreased the incorporation of AA acid in phospholipids and also decreased Δ5 desaturase activity. There was a corresponding increase in DHA in hepatic microsomes (Dang et al., 1989), indicating a competition between ω-3 and ω-6 fatty acids. ω3 Fatty acids also increase cholesterol to phospholipid ratio in liver microsomal membranes of normal as well as experimental diabetic rats (Igal and de Gomez Dumm, 1997). In β-TC3 insulinoma cell line, EPA-potentiated glucose-stimulated insulin secretion without affecting glucose metabolism (Konard et al., 1996).

From human studies, it is thus clear that in diabetic subjects ω-3 fatty acids appear to have some beneficial effects on lipid metabolism and may decrease the severity of cardiac disorder and lower the incidence of coronary artery disease. However, ω-3 fatty acids have detrimental effects on carbohydrate metabolism and worsen glycemic control even though insulin sensitivity is improved. Also, the beneficial effects on lipid metabolism cannot be sustained by prolonged fish oil treatment and are reversed when fish oil feeding is stopped. Thus, on the basis of the present knowledge, it is not prudent to supplement high doses of ω-3 fatty acids for a prolonged time to diabetic subjects. The beneficial effect of lowering hypertriglyceridemia in diabetic subjects can be achieved by substituting starch for simple sugars in the diet. The consumption of fish is more beneficial than fish oil for diabetic individuals. The reasons for this are beyond the scope of this chapter.

D. Saturated Fatty Acids

Several studies have shown that saturated fat intake is a risk factor for hyperinsulinemia. Folsom et al. (1996) observed a positive correction between saturated fatty acid percentage in plasma phospholipids and insulin levels. Rasmussen et al. (1996) compared the effect of butter and olive oil in NIDDM subjects and showed that saturated fat increase insulin response more than MUFAs.

Saturated fatty acid (palmitate) also increases glucose-induced insulin release but not basal release, which is reversed by epinephrine. Palmitate appears to act via increasing calcium flux and the mobilization of intracellular calcium (Warnotte et al., 1994). Though saturated fatty acids stimulate glucose transport in isolated rat adipocytes acutely, prolonged treatment induces insulin resistance via postreceptor defect (Hunnicutt et al., 1994). The stimulation of glucose transport by saturated fatty acids in adipocytes is via stimulation of insulin receptor autophosphorylation (Hardy et al., 1991).

E. *Trans* Fatty Acids

The average estimated consumption of TFA in the United States is 6%–8% of total daily intake (Senti, 1988). Most of it comes from margarines and other hydrogenated oils used for frying (Enig et al., 1990), from milk (Parodi, 1976), and in small quantities from some green vegetables. In humans and animals, dietary TFAs are readily and reversibly incorporated into plasma and tissue lipids, especially TGs, and to a small extent in phospholipids (Moore et al., 1980; Vidgren et al., 1998). TFAs have also been found in human milk after feeding diets high in TFA (Aitchison et al., 1977). In phospholipids TFAs are normally incorporated in position 1, where saturated fatty acids are also preferentially incorporated. Mitochondrial enzymes can metabolize TFAs via 2-*trans*-enoyl CoA or 3-*trans*-enoyl CoA depending on the position of the double bond (Schettler, 1986).

The biological effects of TFAs are not fully understood. Compared to oleic acid, TFAs have been shown to elevate serum TG (Anderson et al., 1961; Mensink and Katan, 1990), total cholesterol (Vergroesen, 1972; French et al., 2002), and LDL-cholesterol (Laine et al., 1982; Mensink

and Katan, 1990), and to lower HDL-cholesterol (Mensink and Katan, 1990). Mattson et al. (1975), however, did not observe the elevation in cholesterol or TG. The TFAs also elevate apo B and lower apo A-I compared to oleic acid (Mensink and Katan, 1990). Thus, the effects of TFAs on lipid and lipoprotein metabolism are worse than those of saturated fatty acids (Mensink and Katan, 1990). The TFAs of the ω-9 family (18:1ω9) inhibit Δ6 desaturase involved in the conversion of linoleic acid to GLA in the liver and heart but have no effect on Δ5 desaturase in the ω-6 family. They also decrease Δ9 desaturase (Hill et al., 1982). In monkeys, Barnard et al. (1990) reported that, unlike *cis* isomers, TFAs had no effect on erythrocyte membrane fluidity but affected insulin receptors by decreasing the number and increasing the affinity, an effect similar to that observed with saturated fatty acids (Ginsberg et al., 1979; Gould et al., 1982; Berlin et al., 1989). In brain TFAs are less potent in inhibiting binding of opiates to their receptors and in decreasing membrane viscosity compared to corresponding *cis* fatty acids (Remmers et al., 1990). They also alter the concentration of dopaminergic neurotransmitters in brain (Acar et al., 2003).

It is important to note that these studies were carried out in normal humans and animals. No studies on the effects of TFA in diabetic subjects have been reported. Since in most studies, TFAs have been shown to be as atherogenic as saturated fatty acids (and certainly more than unsaturated fatty acids) and also to lower insulin receptor number, it is possible that, compared to unsaturated fatty acids, TFAs will have deleterious effects in diabetic subjects. It is therefore advisable for diabetic individuals to restrict their intake of fats containing TFAs. Studies on the effects of TFAs on lipid profile, glucose homeostasis, and insulin and other hormones in diabetic subjects are long overdue.

F. Free Fatty Acids

One of the complications of lipid metabolism in diabetes is the increased lipolysis, which results in the increased plasma concentration of FFAs. Thus, increases in plasma FFAs have been observed in both IDDM and NIDDM subjects with or without ketoacidosis (Liewendahl and Helenius, 1976; Yue et al., 1981; Linfoot et al., 2005). Intra abdominal fat appears to be the precursor for increased lipolysis (Kissebah, 1996). In control subjects as well as in NIDDM subjects elevated levels of FFA inhibits insulin-stimulated glucose uptake in skeletal muscle by suppressing glycolysis and increasing insulin-stimulated glycogen synthesis (Kim et al., 1996) and hence may play a significant role in the pathogenesis of insulin resistance in NIDDM (Reaven and Chen, 1988; Randle et al., 1994; Boden, 1996; Charles et al., 1997). In NIDDM subjects, cigarette smoking further aggravates insulin resistance by increasing plasma FFA and decreasing lipid peroxidation (Targher et al., 1997). Elevated levels of FFA are also observed in the myocytes of diabetic animals (Kenno and Severson, 1985). FFAs can be both oxidized and re-esterified within tissues without oxidation (Felber et al., 1987). Increased availability of FFA increases fat oxidation (Lillioja et al., 1985; Felber et al., 1987). Elks (1990) suggested that increased availability of FFA for oxidation by muscle and other tissues may lead to the impairment of carbohydrate oxidation, thereby leading to the glucose intolerance seen in obese diabetic subjects. However, rate of lipid oxidation by muscle is reduced in NIDDM subjects (Kelley and Simoneau, 1994). Glucose-fatty acid cycle appears to act via inhibition of PDH complex by acetyl CoA and NADH (Wieland, 1983). In diabetes PDH level falls significantly and glucose utilization by inhibiting fatty acid oxidation is much less in diabetic monocytes than that of normal cells (Abdel-aleem et al., 1995). Increased activity of carnitine palmitoyltransferase-I (CPT-I) and carnitine acetyl transferase and decreased activity of PDH complex is also observed in diabetic d*b*/d*b* mice leading to increased oxidation of fatty acid and decreased oxidation of glucose (Makar et al., 1995). Glucose-fatty acid cycle may also operate in patients with insulin resistance and hyperlipidemia (Kumar et al., 1994). FFAs appear to stimulate insulin release (Crespin et al., 1973) and inhibit glucagon release (Madison et al., 1968) from pancreatic islets. However, long-term exposure of islets to high levels of FFA results in beta cell dysfunction and diminishes glucose-induced insulin secretion (Zhou and Grill, 1994; Girard, 1995; Newgard and McGarry, 1995; Hirose et al., 1996).

In pubertal IDDM subjects insulin has no significant effect on plasma FFA (Caprio et al., 1994). Higher concentrations of FFA in blood are associated with morphological changes in the arteries of diabetic rats (Reinila, 1981), which may be due to their high-detergent activity (Shaw, 1985). Elevated FFA in plasma in diabetes and other disorders result in hypercorticoidism and initiates a positive feedback loop between adipocytes and hypothalamic-pituitary-adrenal axis (Widmaier et al., 1995). In the fasting state and in diabetes, high levels of FFA increase platelet aggregation (Gjesdal et al., 1976; Mikhailidis et al., 1981), possibly by inhibiting vascular adenosine diphosphate activity and thereby decreasing the concentration of adenosine, an inhibitor of aggregation and a vasodilator as compared to adenosine diphosphate, which stimulates platelet aggregation (Barradas et al., 1987). Another mechanism may involve inhibition of prostacyclin (PGI_2) synthesis and acceleration of its degradation. Significant decreases in PGI_2 have been observed in the aorta and bladder of streptozotocin diabetic rats (Colwell et al., 1983; Jeremy et al., 1986, 1987). Recently NO has been suggested to play a role in FFA-induced insulin secretion since, islets from prediabetic Zucker fatty diabetic rats, FFA-induced significant rise in NO and reduced insulin secretion (Shimabukuro et al., 1997b).

IV. METABOLISM OF FATTY ACIDS IN DIABETES

The metabolism of fatty acids includes synthesis, desaturation and elongation, oxidation, and formation of eicosanoids. Desaturation and elongation of EFA is necessary for the synthesis of AA, which is generally not obtained from the diet and is required for eicosanoid formation (see below). AA is also a vital component of phospholipids of cellular membranes. Alteration in AA would lead to abnormal membrane formation and result in altered unsaturation and fluidity of the membranes.

A. Desaturation

There are four specific microsomal desaturases that are involved in the desaturation of fatty acids (Sinclair, 1984; Holman, 1986; Axelrod, 1989). Δ9 desaturase is involved only in the ω-9 and ω-7 series and converts palmitic acid to palmitoleic acid and stearic acid to oleic acid. Δ6, Δ5, and Δ4 desaturases are involved in the desaturation of fatty acids of all four families. Though not clearly demonstrated, it is presumed that the same desaturase is involved in the insertion of double bonds in specific positions in fatty acids of different families, that is, that there is only one each of Δ9, Δ6, Δ5, and Δ4 desaturases. Thus, there is interaction and competition between fatty acids of different families for the desaturase. This interaction has been reviewed by Holman (1986). Usually fatty acids with greater unsaturation are preferentially desaturated. Thus, fatty acids of the ω-3 family would be preferentially desaturated compared to other families, and fatty acids of the ω-7 and ω-9 families will be desaturated more only when the availability of EFAs is diminished.

In human diabetic subjects and experimental diabetic animals, the activity of all four desaturases is decreased. Though desaturase activity is present in liver microsomes, the alteration in fatty acid composition in diabetes, especially low levels of AA, in several tissues such as platelets, kidney, heart, testes, and plasma indicate that desaturation possibly occurs in these tissues as well. It is important to note that Δ5 and Δ6 desaturases are not present in human skin (Chapkin et al., 1986). The decrease in all four desaturases explains higher levels of fatty acids with 16- and 18-carbon atoms and lower levels of fatty acids with 20- and 22-carbon atoms in diabetic subjects and experimental diabetic animals (see above) except those fed fish oil, which supplies preformed EPA and DHA.

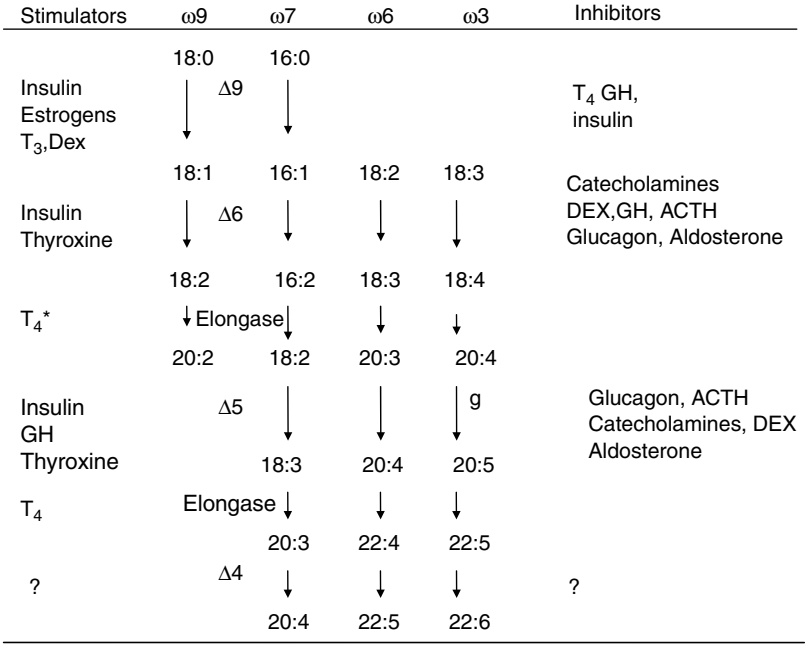
A decrease in Δ9 desaturase has been reported in diabetic rats (Friedmann et al., 1966; Gellhorn and Benjamin, 1966; Mercuri et al., 1974; Eck et al., 1979; Faas and Cater, 1980; Garg et al., 1986; Dang et al., 1988). Since it is not involved in the metabolism of EFA, Δ9 desaturase is of less importance. On the basis of direct *in vitro* studies in liver microsomes as well as on altered fatty acid composition, decreased Δ9 desaturase activity has been observed in liver, platelets, aorta, and plasma (Faas and Carter, 1980; Garg et al., 1986; Dang et al., 1988). Dietary carbohydrates

also modulate the desaturases in the normal as well as the diabetic state (Worcester et al., 1979). Different sugars have quantitatively different effects on Δ9 desaturase in liver and adipose tissue. In liver, dietary fructose stimulates Δ9 more than glucose in normal as well as diabetic rats (Mercuri et al., 1974; Worcester et al., 1979). Prasad and Joshi (1977) reported 20-fold stimulation of hepatic Δ9 desaturase by fructose in diabetic rats. In adipose tissue, fructose produced less stimulation of Δ9 desaturase than glucose (Worcester et al., 1979). Of all the four desaturases, Δ9 desaturase is most affected in diabetes (Gellhorn and Benjamin, 1964; Friedmann et al., 1966; Eck et al., 1979). Studies on the activity of Δ9 desaturase in human diabetic subjects are lacking. It is, however, possible that the decrease in Δ9 desaturase may also occur in diabetic humans especially in poorly controlled IDDM subjects, since insulin plays an important role in desaturation (see below).

A decrease in Δ6 desaturase has been suggested on the basis of fatty acid composition as well as direct measurement of the enzyme in diabetic humans (Tilvis and Miettinen, 1985; Jones et al., 1986; Tilvis et al., 1986; Keen et al., 1993). The enzyme is also decreased in experimental diabetic animals (Friedmann et al., 1966; Eck et al., 1979; Faas and Carter, 1980; Dang et al., 1988; Shin et al., 1995) and is increased after insulin treatment (Shin et al., 1995). It is a rate-limiting enzyme in the conversion of linoleic acid to AA (Marcel et al., 1968). Decreased Δ6 desaturase activity or its fatty acid metabolic products have been shown in several tissues including liver, kidney, aorta, platelets, testes, and plasma (Faas and Carter, 1980; Dang et al., 1988). Erythrocytes appear to be devoid of this enzyme (Shin et al., 1995). Riisom et al. (1981) reported increased Δ6 desaturase activity in liver microsomes of alloxan- and streptozotocin diabetic rats fed EFA-deficient diets compared to control rats fed the same diet. They also observed higher levels of AA in liver and heart phospholipids in diabetic rats than in control rats. It is important to note that in this model, EFA deficiency actually reduced the severity of diabetes and that diabetic rats were less EFA deficient than control rats. Therefore, increased Δ6 desaturase activity is not unexpected. The fatty acid composition in liver and heart, however, did not parallel the activity of desaturase in liver microsomes. It is possible that AA is better preserved in phospholipids or that lipolysis is decreased when diabetes is accompanied by EFA deficiency.

Decreases in Δ5 desaturases in several tissues have also been shown or suggested in diabetic humans (Stone et al., 1979; Tilvis and Miettinen, 1985; Jones et al., 1986; Tilvis et al., 1986; El Boustani et al., 1989; Bassi et al., 1996) and experimental animals (Holman et al., 1983; Wilder and Coniglio, 1984; Dang et al., 1988). Studies on the activity of Δ4 desaturase in humans and animals are lacking. Studies reporting changes in desaturases on the basis of fatty acid composition should be viewed with caution. Besides desaturases, fatty acid concentration in tissue and plasma can be changed because of utilization, oxidation, and membrane lipid degradation and synthesis.

The activity of desaturases, especially Δ6 and Δ5, is modulated by several factors. Two important factors are diet and hormones. Cholesterol feeding has been shown to decrease Δ5 and Δ6 desaturase activity in rats (Huang et al., 1985, 1990; Garg et al., 1986). However, dietary cholesterol stimulates Δ9 desaturase (Garg et al., 1986). This would tend to increase concentration of unsaturated 18-carbon fatty acids including oleic acid. No direct study of dietary cholesterol on desaturases has been carried out in humans, but increased levels of linoleic acid and decreased levels of AA observed in some hypercholesterolemic subjects may indicate a decrease in desaturase activity. Similarly, studies in diabetic animals with cholesterol feeding have not been done. Primrose oil, which is relatively high in GLA, may be beneficial in diabetic subjects because it bypasses the critical step in the formation of AA. TFAs have been reported to decrease desaturase activity (Brenner, 1981; Hill et al., 1982; Holman, 1986). High-fat diets also decrease the activity of Δ9, Δ6, and Δ5 desaturases (Garg et al., 1986). PUFAs increase Δ6 and Δ5 desaturases (Pugh and Kates, 1984; Garg et al., 1986). Both increases (Pugh and Kates, 1984) and decreases (Garg et al., 1986) have been observed in Δ9 desaturase by dietary PUFAs compared to saturated fats. Theoretically, fish oils, which are rich in EPA and DHA, are clearly beneficial for diabetic subjects because they need not be desaturated or elongated. However, high doses of fish oil compete with AA for the formation of eicosanoids. Eicosanoids formed from EPA have effects on platelet function diametrically opposite to those formed from AA (see below) and hence may not be of advantage for diabetic subjects.



* In microsomes but not in mitochondria

FIGURE 47.1 Hormonal control of fatty acid desaturation and elongation. The stimulatory and inhibitory effects of various hormones on different desaturases and elongase are shown. Note that the hormonal control of Δ4 desaturase is not yet defined. *Abbreviations:* T_3, triiodothyronine; T_4, thyroid hormone; Dex, dexamethasone; GH, growth hormone; ACTH, adrenocorticotropic hormone. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

Like lipases, desaturases are also controlled by hormones. There is indirect, as well as, direct evidence that hormones control desaturation of fatty acids. Indirect evidence comes from the alteration of fatty acid composition in diabetic, thyroidectomized, hypophysectomized, and adrenalectomized animals where one or more hormone is lacking. Figure 47.1 summarizes the stimulatory and inhibitory effects of various hormones on desturation and elongation. Insulin plays an important role in fatty acid desaturation. Insulin treatment in humans (Tilvis et al., 1986; El Boustani et al., 1989) as well as experimental diabetic animals (Gellhorn and Benjamin, 1964; Prasad and Joshi, 1977; Eck et al., 1979; Faas and Carter, 1980) stimulates desaturases. Faas and Carter (1980) reported that insulin increased Δ6 desaturase levels, yet AA decreased in the liver, which they suggested could be due to increased utilization in excess of increased synthesis by desaturase. Glucagon and catecholamines, which antagonize the effect of insulin, decrease desaturase activity (de Gomez Dumm et al., 1975, 1976a,b; Brenner, 1981). Thus, decreased insulin and elevated glucagon in diabetic subjects would account for decreased desaturase activity in diabetes. Desaturase activity is also influenced by lipid-lowering agents. Using a ratio of 20:4 to 20:3 as an index of Δ5 desaturase activity, Matsui et al. (1997) showed that bezafibrate increases Δ5 desaturase activity and thereby improving insulin sensitivity.

Insulin has been shown to stimulate as well as inhibit Δ9 desaturase. Estrogens, triiodothyronine and synthetic glucocorticoid, dexamethasone, stimulate Δ9 desaturase (Marra and de Alainz, 1995; Stanton et al., 2001), while thyroid and growth hormones inhibit it (Gueraud and Paris, 1997). In obese women insulin stimulates Δ5 desaturase (Medeiros et al., 1995). Insulin and thyroxine also stimulate Δ5 and Δ6 desaturases while catecholamines, glucagon, aldosterone, adrenocorticotropic hormone (ACTH) and dexamethasone inhibit both desaturases. Thus, decreased insulin and increased glucagon in diabetic subjects would account for decreased desaturase activity in diabetic state. However, Liu et al. (2000) reported increased Δ6 desaturase activity in diabetes.

Testosterone, aldosterone, and corticosterone inhibit Δ6 desaturase in HTC cells (Marra et al., 1988). The hormonal control of Δ4 desaturase is not well defined. Δ4 Desaturase is also involved in the conversion of EPA to DHA in brain (Anderson et al., 1990). However, it is not clear whether any hormone or neuropeptide play a role in its modulation in brain. The TFAs of the ω9 family (18:1ω9) inhibit Δ6 desaturase involved in the conversion of linoleic acid to GLA in the liver and heart but have no effect on Δ5 desaturase in the ω6 family. Others have reported that TFAs also inhibit Δ5 desaturase and can also inhibit cyclooxygenase and lipooxygenase and thereby decrease eicosanoid production. They also decrease Δ9 desaturase (Hill et al., 1982). The effects of TFAs on desaturases in diabetes have not been elucidated.

B. Elongation

Elongation of fatty acids is carried out in the endoplasmic reticulum and in microsomes. Studies on the conversion of 18-carbon fatty acids to 20- and 22-carbon fatty acids have concentrated more on desaturases. Chain elongation is catalyzed by discrete enzymes using CoA derivatives as substrates (Sprecher, 1982). The elongases act much faster than desaturases (Sinclair, 1984). As observed for desaturation, there is also competition for elongation between the families of fatty acids. Thus, GLA is elongated to a greater extent than ALA. Unsaturated fatty acids of the ω-6 family increase elongation while saturated fatty acids decrease elongation of fatty acids of both families (Christiansen et al., 1968). No specific defect has been reported to occur in elongation of fatty acids in diabetes. Thyroxine has been shown to stimulate elongase in liver microsomes but not in mitochondria. The effects of other hormones on elongases have not been reported in normal or diabetic state.

C. Oxidation of Fatty Acids in Diabetes

Fatty acids are normally oxidized in liver mitochondria via β oxidation to acetyl CoA and then to CO_2 via the citric acid cycle. Short- and medium-chain fatty acids enter the mitochondria where they are esterified to CoA before oxidation. Long-chain fatty acids, however, are first esterified in the cytosol and then transported across mitochondrial membrane by CPT-I and CPT-II (McGarry et al., 1989) where they undergo β oxidation via fatty acyl-CoA dehydrogenase. The oxidation of short, medium-, and long-chain fatty acids is catalyzed by three isoenzymes. In general, unsaturated fatty acids are more readily oxidized than saturated fatty acids and hence saturated fatty acids are stored in tissue lipids more than unsaturated fatty acids. In human muscle, oxidation of long-chain fatty acids is controlled by glucose plus insulin via regulation of entry into mitochondria. However, mitochondrial uptake and oxidation of medium-chain fatty acids is not dependent on glucose and/or insulin (Sidossis et al., 1996). In diabetes, owing to the lack of insulin, oxidation of glucose is reduced and is compensated by increased oxidation of fatty acids. The ratio of fatty acid oxidation to esterification is higher in streptozotocin diabetic rats than in control animals and is not significantly affected by insulin treatment (Moir and Zammit, 1994). β oxidation of fatty acids in heart can be down regulated by intra mitochondrial acetyl CoA derived from carbohydrate oxidation (Lopaschuk and Gamble, 1994). The formation of ketone bodies occurs when production of acetyl CoA exceeds the capacity of the citric acid cycle to metabolize acetyl CoA to CO_2 and H_2O as seen in the case of IDDM subjects with severe insulin lack (see below). DHA, oleic acid, and its *trans* isomer inhibit β oxidation in mitochondria because of the accumulation of 2, 4-di- or 2, 4, 7-trienoyl CoA. In diabetes, these fatty acids have less inhibitory effect on β oxidation, since 2, 4-dienoyl CoA can be readily oxidized, as mitochondria from diabetic rats have increased 2, 4-dienoyl CoA reductase activity (Osmundsen and Bjornstad, 1985).

In the heart of diabetic rats oxidation of fatty acids is increased more than glucose oxidation during heavy work load (Christe and Rogers, 1995) and in the absence of palmitate, both, TG lipolysis and endogenous palmitate oxidation rates are higher in the heart of diabetic rats (Saddik and Lopaschuk, 1994). The increased palmitate oxidation in diabetic rats may be due to decreased activity of PDH and acetyl CoA flux through the Krebs cycle (Abdel-aleem et al., 1997). In untreated diabetic rats, hepatic mRNA levels of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS)

are reduced. Vanadate treatment restores mRNA and the activities of these enzymes in liver but not in adipose tissue. The action appears to be via improvement of thyroid function (Brichard et al., 1994). In pancreatic islets ACC is abundant but FAS is poorly expressed. Fatty acid oxidation in islets appears to be regulated by malonyl CoA (Brun et al., 1996).

In addition to β oxidation, fatty acids can also be oxidized via ω oxidation in microsomes, though to a much lesser extent. In diabetic subjects (Lippe et al., 1987) as well as in experimental diabetic animals (Bjorkhem, 1973; Wada and Usami, 1977; Kam et al., 1978; Hemmelgarn et al., 1979), the rate of ω oxidation of fatty acids is increased compared to controls and accounts for almost 15% of overall fatty acid oxidation. The increased ω oxidation of fatty acids in diabetes can be advantageous for both carbohydrate and lipid metabolism. Dicarboxylic acids formed via ω oxidation are further metabolized by β oxidation to form succinyl CoA (Hemmelgarn et al., 1979), which leads to decreased glucose formation (Kam et al., 1978; Hemmelgarn et al., 1979). Second, increased ω oxidation also decreases the incorporation of fatty acids into phospholipids (Wada et al., 1971) and hence lowers lipid synthesis. The administration of long-chain dicarboxylic acids to diabetic rats also decreases the concentration of ketone bodies in the blood (Wada et al., 1971). Shimabukuro et al. (1997a) reported increased FFA oxidation in cultured islets by leptin. However, in islets of obese, mildly diabetic Zucker rat leptin had no effect possibly due mutation of leptin receptor. The Zucker diabetic fatty rats also have increased fatty acid transport by adipocytes as measured by the uptake of oleate (Berk et al., 1997).

It is not clear whether insulin or other hormones have significant effects on β or ω oxidation of fatty acids. However, increased ω oxidation in diabetes indicates that the lack of insulin may be responsible. By reducing lipolysis and glycogenolysis, insulin enhances the relative contribution of carbohydrate oxidation and reduces fat oxidation in resting state as well during exercise in diabetic rats (Houwing et al., 1995). The hormonal control of fatty acid oxidation in diabetes needs to be addressed.

D. Binding Proteins and Fatty Acid Oxidation

Fatty acid-binding proteins (FABP) play an important role in the fatty acid oxidation. In streptozotocin diabetic rat, fatty acid oxidation in liver homogenate increases significantly but the FABP content decreases. FABP also decreases in the aorta of streptozotocin diabetic rats and is reversed by insulin treatment (Sakai et al., 1995). In heart, fatty acid oxidation is unaltered but FABP content is increased (Glatz et al., 1994). Thus, changes in fatty acid oxidation capacity do not appear to correlate with FABP content during the development of diabetes (Veerkamp et al., 1996). This indicates that though FABP in different tissues are structurally identical, there is differential regulation of FABP in different tissues in diabetes. In muscle of streptozotocin diabetic rats, heart-FABP (H-FABP) level and mRNA are increased but the transcription rate was unchanged indicating that the regulation of expression of H-FABP in muscle may not be at the level of transcription (Carey et al., 1994). In streptozotocin diabetic rat fatty acid binding characteristics of myocardial plasma membrane FABP are altered in that there is a decrease in the affinity for the binding of saturated fatty acids but not for unsaturated fatty acids as indicated by the decreased affinity of *trans*-parinaric acid (Heylinger et al., 1995).

In Pima Indians, intestinal FABP with threonine at codon 54 instead of alanine has been shown to result in higher insulin resistance and increased fat oxidation *in vivo* and threonine-containing protein had twofold greater affinity for long-chain fatty acids than the alanine-containing protein indicating increased absorption and/or processing of dietary fatty acid by the intestine and thus increased fat oxidation (Baier et al., 1995; Tataranni et al., 1996).

In adipocytes, FABP has been shown to be a glycoprotein IV or CD36. CD36 is also responsible for fatty acid building in erythrocytes and platelets. It is strongly expressed in cardiac and skeletal muscle and adipose tissue but not in liver, pancreas, and brain (Greenwalt et al., 1995). It is up regulated in diabetes and by high fat feeling (Greenwalt et al., 1995). Type of fatty acids appears to modulate RNA for CD36 protein. In capillary endothelial cells CD36 expression appears to be

correlated with parenchymal cell fatty acid utilization (Greenwalt et al., 1995). CD36 transfers bound fatty acid to the fatty acid acyl-CoA enzyme on the inside of the membrane (Sfeir et al., 1997). Two other proteins have also been shown to have the same function, namely membrane-bound aspartate aminotransferase ($FABP_{pm}$) with a molecular weight of 43 kDa (Berk et al., 1990) and fatty acid transport protein (FATP) with a molecular weight of 63 kDa (Schaffer and Lodish, 1994). It is not clear whether FABP present in enterocytes, heart, mammary cells, adipocytes, and blood cells are same or different proteins. It has been suggested that FABP2, or a tightly linked gene may be associated with insulin resistance (Mitchell et al., 1995).

E. Conversion of EFAs to Eicosanoids

Formation of eicosanoids is another important biological function of EFAs. Eicosanoids can be formed only from fatty acids with 20-carbon atoms and at least three double bonds. There are three different pathways involving different enzyme systems. Microsomal cyclooxygenase converts fatty acids to prostanoids (prostaglandins, prostacyclins, and thromboxanes); lipoxygenase gives rise to hydroperoxyeicosatetraenoic acids, which are quickly converted to LTs, and cytochrome p450 monooxygenase which leads to formation of epoxides and hydroxyeicosatetraenoic acids. The three important fatty acids involved in eicosanoid production are DHLA, AA, and EPA. As they have different numbers of double bonds, they each give rise to a different series of eicosanoids. Thus, prostanoids of 1-series and LTs of 3-series are formed from DHLA. AA produces prostanoids of 2-series and LTs of 4-series, while EPA is converted to prostanoids of 3-series and LTs of 5-series. The two families of fatty acids, ω-3 and ω-6, compete for the same enzymes, and hence, depending on the availability of fatty acids, products of different series are formed. Thus, EPA inhibits the production of prostaglandins (cyclooxygenase pathway) from AA (Culp et al., 1979; Corey et al., 1983). The products formed from AA and EPA are biologically more active and more important. These fatty acids are usually derived from phospholipids by the action of phospholipase A_2. Prostanoids are produced in most tissues, whereas LTs are generally formed in different blood cells, pancreatic islets, and glomerular cells. Kinsella et al. (1990) have reviewed the conversion of fatty acids to eicosanoids.

In platelets, AA forms TXA_2 whereas EPA forms TXA_3. In endothelial cells of blood vessels, the major product of AA is prostacyclin PGI_2 and that of EPA is PGI_3. TXA_2 has potent platelet-aggregating activity; TXA_3 either has weak activity or is inactive. Both PGI_2 and PGI_3 have strong antiaggregating activity and are vasodilators. They prevent platelet clumping and increase bleeding time. PGE_1 derived from DHLA also has antiaggregating activity. LTs, on the other hand, cause chemotaxis in neutrophils and eosinophils, and stimulate cyclic adenosine monophosphate (cAMP) production and the release of lysosomal enzymes from polymorphonuclear cells. LTC_4 and LTD_4 are humoral agents and promote smooth muscle contraction.

In diabetes, AA in membrane phospholipids is decreased (see above). This is caused by decreases in Δ5 and Δ6 desaturases and in lipase activity. As a result there is decreased formation of TXA_2 and PGI_2 in diabetic subjects (Nordoy, 1981; Goodnight et al., 1984; Mikhailidis et al., 1986; Tilvis et al., 1987; Prisco et al., 1989b). There is also an increase in PGH_2 (Shimizu et al., 1993) and $PGF_{2\alpha}$ (Harrison et al., 1978) in vascular tissues. In IDDM subjects, DHLA is also decreased, leading to decreased PGE_1 formation (Mikhailidis et al., 1986). There is also a decrease in PGI_2 synthesis in IDDM as measured by the urinary 6-keto-$PGF_{1\alpha}$. These changes explain the increased adhesiveness and aggregation of platelets observed in IDDM subjects (Bern, 1978; Waitzman, 1979; Colwell and Halushka, 1982; Ewald et al., 1983; Mikhailidis et al., 1986). The role of different prostaglandins in platelet function in diabetes has been reviewed by Colwell and Halushka (1982).

In streptozotocin diabetic rats, PGI_2 synthesis in the aorta is decreased (Jeremy et al., 1987). The decrease in PGI_2 formation increases with the duration rather than the severity of diabetes. However, in the bladder there is increased PGI_2 formation, possibly due to distention (Jeremy et al., 1986). Fujii et al. (1986) observed a slight increase in PGI_2 and a greater increase in TXA_2

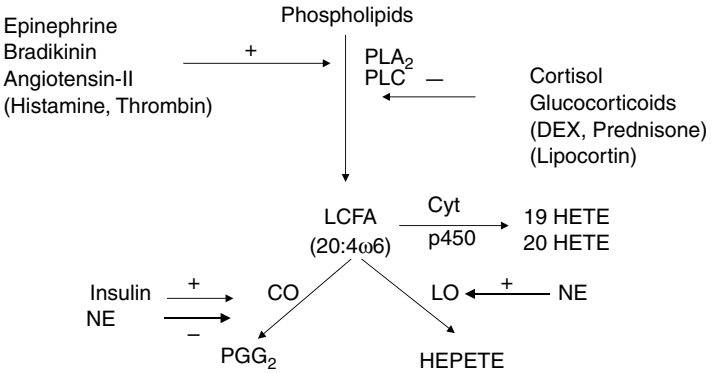


FIGURE 47.2 Role of hormones in eicosanoid formation. Most hormones affect eicosanoid formation by controlling lipolysis of phospholipids. Insulin and nor epinephrine act on the cyclooxygenase and lipooxygenase. The different PLA_2s involved are $cPLA_2$, cytosolic PLA_2 which is Ca^{2+} dependent; $IPLA_2$, cardiac PLA_2 which is Ca^{2+} independent; and $spPLA_2$, sarcoplasmic PLA_2. *Abbreviations:* PLA_2, phospholipase A_2; PLC, phospholipase C; Dex, dexamethasone; LCFA, long-chain fatty acids; cyt p450, cytochrome p450; CO, cyclooxygenase; LO, lipooxygenase; PGG_2, prostaglandin G_2; NE, norepinephrine; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

(as measured by its inactive metabolite TXB_2) and PGE_2 synthesis in the mesenteric vascular bed of streptozotocin-treated diabetic rats compared to control rats. Thus, there was a decrease in the PGI_2/TXA_2 ratio, indicating a tendency for increased platelet aggregation. In streptozotocin-treated diabetic rat, mRNA for cyclooxygenase is decreased in sciatic nerve and aorta but not in kidney and retina (Fang et al., 1997). Evening primrose oil increased mRNA for cyclooxygenase in nerve and retina indicating a tissue specific effect. Aldose reductase inhibitor, on the other hand, had no effect on cyclooxygenase expression (Fang et al., 1997).

Various hormones are involved in eicosanoid formation (Figure 47.2). The hormonal control occurs during the conversion of long-chain PUFA by phospholipases and also at the level of cyclooxygenase and lipooxygenase. Epinephrine, bradikinin, and angiotensin II stimulate phospholipase A_2 and phospholipase C (Vane, 1976; Van Den Bosch, 1980; Shukla, 1982) while cortisol and synthetic glucocorticoids, dexamethasone, and prednisone, inhibit the lipases (Blackwell et al., 1980). They appear to act via lipocortin. Histamine and thrombin also stimulate these lipases (Van Heugten et al., 1996). Insulin acts directly on cyclooxygenase and stimulates the conversion of AA to PGG_2, which is then converted to various prostanoids. TNF has also been reported to stimulate prostaglandin synthesis (Burch and Tiffany, 1989). The interactions between hormones and prostanoids have been reviewed by Myers et al. (1989).

Prostaglandins also play a role in insulin secretion. Sodium salicylate, which inhibits cyclooxygenase and thereby decreases prostaglandin synthesis, increases basal as well as glucose-stimulated insulin secretion in diabetic subjects indicating involvement of prostaglandin. Robertson and Chen (1977) infused PGE_2 in normal humans and inhibited acute insulin response to glucose. In addition, prostaglandins are also involved in abnormal collagen metabolism in diabetes (Yue et al., 1985). Bradykinin has been shown to stimulate phospholipids to release AA which is then metabolized via cyclooxygenase, lipooxygenase, and cytochrome p450 to yield vasoactive products (Quilley et al., 1994).

LTs formed by lipoxygenase are also biologically active. LT_5 formed from ω-3 fatty acids is more active in immune functions in humans (Kelley et al., 1991). Neutrophils and monocytes increase LT formation from EPA more than from AA (Lee et al., 1985). This explains the beneficial effects of fish oil on immune function in low-dose streptozotocin diabetic mice in which fish oil decreased the number of class II antigen-expressing cells in pancreatic islets (Linn et al., 1989).

F. Hormones, Eicosanoids, and Platelet Aggregation in Diabetes

Increased plasma glucose appears to be a factor in platelet aggregation in diabetes. In control animals, glucose infusion increases TXA_2 production and decreases the ratio of PGI_2/TXA_2. Insulin treatment reverses the ratio (Fujii et al., 1986). Glucose also decreases prostacyclin-stimulating activity in cultured aortic endothelial cells. This activity is increased by LDL and linoleic acid (possibly by conversion to AA), but insulin has no effect on the activity (Umeda et al., 1990). Thus, increased plasma glucose levels in diabetic subjects may be partly responsible for decreased PGI_2 levels and hence increased platelet aggregation. Alteration in platelet phosphoinositide has also been reported in streptozotocin diabetic rats (Jethmalani et al., 1994).

In diabetic subjects who consume large quantities of fish oil, the situation is different. There is an increase in TXA_3 and PGI_3 synthesis and decreased TXA_2 and PGI_2 owing to the decreased availability of AA and the competition of the ω-3 fatty acids from fish oil. Thus, because of the decreased TXA_2 and PGI_2 and the increased PGI_3 and inactive TXA_3, there is less platelet aggregation, which is beneficial for diabetic subjects with coronary artery disease and hypertension (Leaf and Weber, 1988; Knapp and Fitz-Gerald, 1989; Kasim, 1993; Nishikawa et al., 1997). Figures 47.3 and 47.4

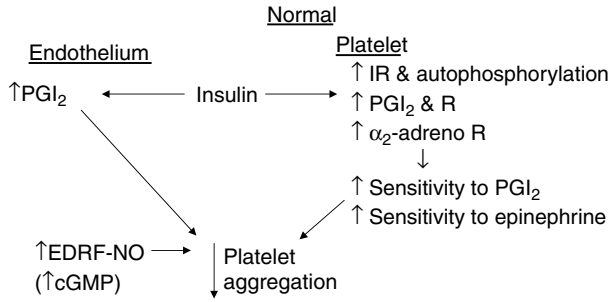


FIGURE 47.3 Role of insulin and prostanoids in platelet aggregation in normal state. Under physiologic (normal) condition, insulin stimulates prostacyclin in endothelium and in platelets which in turn inhibit platelet aggregation. *Abbreviations:* PGI_2, prostacyclin I_2; IR, insulin receptor; α_2-adreno R, α_2 adrenergic receptor; NO, nitric oxide; EDRF, endothelium-derived relaxing factor. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

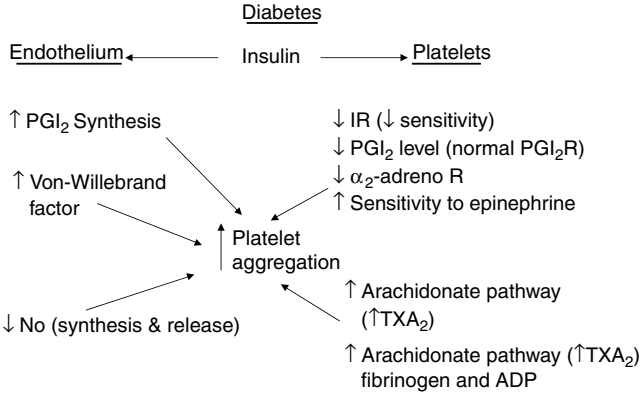


FIGURE 47.4 Role of insulin and prostanoids in platelet aggregation in diabetes. In diabetic state, decreased availability of insulin leads to decreased prostacyclin in endothelium and platelets which results in increase platelet aggregation. *Abbreviations:* PGI_2, prostacyclin I_2; IR, insulin receptor; α_2-adreno R, α_2 adrenergic receptor; NO, nitric oxide; TXA_2, thromboxane A_2; ADP, adenosine diphosphate. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

summarize the role of hormones, hormone receptors, and prostanoids in platelet aggregation in normal and diabetic states.

V. HORMONAL CONTROL OF FATTY ACID METABOLISM IN DIABETES

The hormonal control of fatty acid metabolism is summarized in Figure 47.5 and has been reviewed in normal and diabetic subjects (Liljenquist et al., 1974; Storlien et al., 1997; Saleh et al., 1999; Bhathena, 2006). The metabolic processes, lipogenesis and lipolysis, which control fatty acid concentration in plasma and tissues, are under hormonal control. The principal hormones involved in lipid metabolism are insulin, glucagon, catecholamines, cortisol, and growth hormone. Many other hormones also affect fatty acid metabolism as shown in Figure 47.5. The levels of these hormones are altered in diabetes, which explains altered lipid metabolism in diabetes. Insulin has multiple effects on lipid metabolism. Though insulin stimulates lipogenesis (Beynen et al., 1982; McTernan et al., 2002), its major effect is antilipolytic, especially the inhibition of hormone-stimulated lipolysis (Jungas and Ball, 1963; Mahler et al., 1964; Fain and Shepherd, 1979; Cavallo-Perin et al., 1992; Suda et al., 1993; Linfoot et al., 2005). Glucagon, catecholamines, cortisol, and growth hormone, which are counterregulatory hormones to insulin, have predominantly lipolytic activity (Ball and Jungas, 1963; Gerich et al., 1976; Rosen et al., 1981; Goodman and Grichting, 1983). However, glucagon (Wu et al., 1990a), growth hormone (Davidson, 1987), and catecholamines (Wahrenberg et al., 1989) also have antilipolytic activity. In humans, glucagon has only a marginal effect on LPL, but it has a much stronger effect in young animals. The lipolytic activity of catecholamine is mediated via β-adrenoreceptors and the antilipolytic effect is mediated through α-2-adrenoreceptors

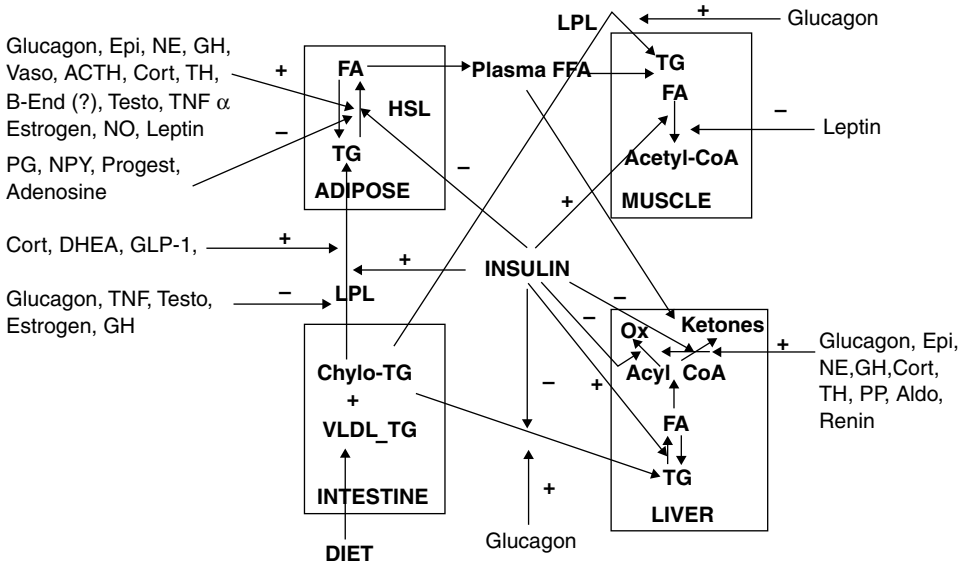


FIGURE 47.5 Hormonal control of fatty acid metabolism. Schematic diagram showing how different hormones control fatty acid metabolism in intestine, adipose tissue, muscle, and liver. "+" indicates stimulatory (positive) effect and "—" indicates inhibitory (negative) effect on different processes. *Abbreviations:* HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; Epi, epinephrine; NE, norepinephrine; GH, growth hormone; Vaso, vasopressin; ACTH, adrenocorticotropic hormone; Cort, cortisol; TH, thyroid hormone; β-end, β-endorphin; Testo, testosterone; TNF-α, tumor necrosis factor-α; NO, nitric oxide; PG, prostaglandins; NPY, neuropeptide Y; Progest, progesterone; DHEA, dehydroepiandrosterone; GLP-1, glucagon-like peptide 1; PP, pancreatic polypeptide; Aldo, aldosterone. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

(Leibel and Hirsch, 1987; Pecquery et al., 1988). The effects of these hormones on lipolysis occur through hormone-sensitive lipase that is activated by cAMP. Glucagon, catecholamine, cortisol, and growth hormone all increase cAMP formation by activating adenylate cyclase and hence stimulate lipolysis.

Insulin stimulates phosphodiesterase, which catabolizes cAMP and decreases cAMP level, thereby inhibits lipolysis. LPL present in adipose tissue, muscle, and heart, is responsible for the lipolysis of TG-rich lipoproteins (Nicoll and Lewis, 1980) and for the production of HDL-cholesterol (Nikkila et al., 1978), while TG lipase present in liver increases the catabolism of HDL-cholesterol (Kuusi et al., 1980). The effect of type of dietary fatty acids on insulin action is reviewed by Storlien et al. (1997). High intake of saturated fat appears to produce hyperinsulinemia and increases risk of diabetes. PUFAs appear to have the opposite effect. The role of insulin in controlling fatty acid metabolism is reviewed by Dutta-Roy (1994).

In diabetes, the counterregulatory hormones are generally elevated while either insulin is decreased or there is peripheral resistance to insulin. In IDDM, plasma norepinephrine is decreased. Also, the antilipolytic effect of catecholamines is normal, but the lipolytic effect is increased 10-fold (Wahrenberg et al., 1989). Thus, these changes in hormones favor increased lipolysis, and hence elevated levels of FFAs are observed in IDDM as well as NIDDM. Unsaturated fatty acids as well as glucose play an important role in these processes. Thus, the increased concentration of unsaturated FFAs stimulates the lipogenic effect of insulin on adipocytes (McTernan et al., 2002). In muscle, however, FFAs are antagonistic to insulin (Randle et al., 1963). In humans, glucose potentiates the antilipolytic effect of insulin on isolated adipocytes (Arner et al., 1983). In poorly controlled diabetic subjects, however, the antilipolytic activity of insulin is decreased (Trevisan et al., 1986; Jensen et al., 1989) because of the reduced insulin sensitivity. In rats, glucose has no effect on insulin-stimulated antilipolysis (Thomas et al., 1979) but inhibits the antilipolytic effect of catecholamine-stimulated lipolysis (Desai et al., 1973). In diabetic subjects, TG lipase is inversely proportional to HDL-cholesterol, being higher in subjects with low HDL-cholesterol and lower in those with elevated HDL-cholesterol (Kasim et al., 1987). In streptozotocin diabetic rats, TG lipase activity is decreased (Jansen and Hulsmann, 1975; Elkeles and Hambley, 1977), but this is reversed by PUFA (Hulsmann et al., 1977). Unlike TG lipase, insulin stimulates LPL (Garfinkel et al., 1976; Sadur and Eckel, 1982). In NIDDM subjects, LPL activity is decreased in adipose tissue and skeletal muscle (Taskinen and Nikkila, 1979). Insulin also stimulates phospholipase C in adipose tissue (Farese et al., 1986). In livers of diabetic mice insulin increases transcription of FAS (a key enzyme in lipogenesis) gene (Paulauskis and Sul, 1989). In human adipocytes, LPL is suppressed by TNF in the presence of insulin and dexamethasone (Fried and Zechner, 1989) but not in the absence of insulin (Kern, 1988). TNF decreases LPL mRNA levels, rates of LPL synthesis, and the LPL activity. Thus, TNF may be important in the pathogenesis of hypertriglyceridemia (Feingold et al., 1989; Fried and Zechner, 1989). It is important to note that TNF is involved in the pathogenesis of diabetes, and its administration to diabetic animals increase blood glucose levels without changes in insulin concentration (Feingold et al., 1989). Farese (1990) has reviewed the role of insulin in phospholipid metabolism. In line with its lipogenic activity, insulin stimulates phospholipid synthesis in adipocytes (Farese et al., 1982; Pennington and Martin, 1985), hepatocytes (Cooper et al., 1990), and diaphragm and skeletal muscle (Ishizuka et al., 1990). Various actions of insulin on fatty acid metabolism in adipose, liver, and muscle are summarized in Table 47.2.

Insulin also plays a role in cholesterol synthesis by controlling hydroxymethylglutaryl CoA reductase, a key enzyme in cholesterol biosynthesis. Insulin stimulates the activity of the enzyme *in vitro* and hence cholesterol synthesis (Bhathena et al., 1974; Geelen et al., 1980). It is important to note that owing to lack of insulin, lipogenesis in insulin-sensitive tissues is decreased but lipogenesis is normal in the intestine, which is insulin insensitive. Lipogenesis in the intestine is dependent on substrate availability, and in diabetes, because there is an increase in substrate availability, fatty acid and TG synthesis are elevated (Popper et al., 1985; Feingold et al., 1990). Thus, de novo intestinal synthesis partly accounts for the increased TG in VLDL in diabetes. However, Jiao et al. (1989)

TABLE 47.2
Insulin and Fatty Acid Metabolism

Adipose	
↓	HSL (↓ rate of lipolysis, ↓ plasma FFA)
↑	FA and TG synthesis (re-esterification)
↑	Fatty acyl-CoA and fatty acyl transferase
↑	LP lipase (↑ uptake of TG from plasma)
Liver	
↑	FA and TG synthesis (de novo lipogenesis from glucose)
↑	VLDL formation (↑ FAS and ACC)
↑	Cholesterol synthesis
↓	Rate of FA oxidation and ketone formation (↓CAT-1)
Muscle	
↓	Rate of FA oxidation (↓ malonyl CoA)
↓	Ketogenesis

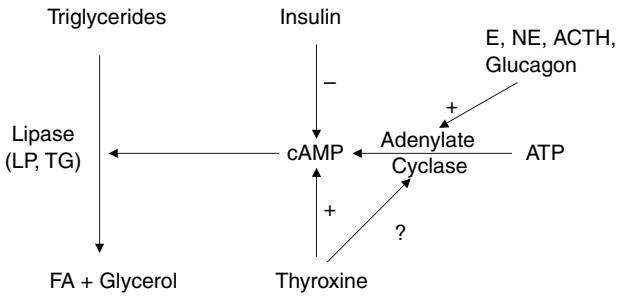


FIGURE 47.6 Hormonal control of fatty acid synthesis in adipose tissue. Note that insulin and counter-regulatory hormones control fatty acid synthesis via cAMP. "+" indicates stimulatory effect and "—" indicates inhibitory effect. "?" indicates the effect is not clearly established. *Abbreviations:* E, epinephrine; NE, norepinephrine; ACTH, adrenocorticotropic hormone; LP, lipoprotein lipase; TG, triglyceride lipase. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

reported inhibition of cholesterol synthesis and esterification in cultured human intestinal cell line Caco-2.

In diabetes there is an increase in lipid peroxidation as measured by free and total malondialdehyde (MDA). Insulin treatment reduces free MDA but not total MDA (Peuchant et al., 1997) indicating a partial protection against elevated lipid peroxidation in diabetes. Although insulin therapy in NIDDM subjects with secondary failure to oral agents increases GLA in TG and cholesterol esters, it does not alter the platelet function in these subjects (Rodier et al., 1995).

Figure 47.6 summarizes the hormonal control of fatty acid synthesis in adipose tissue. Glucagon has multiple effects on lipid metabolism. It stimulates lipolysis by activating lipase (see above). The action is via stimulation of adenylate cyclase. It also stimulates LPL in muscle but not in adipose tissue (Geelen et al., 1980). In liver, glucagon stimulates fatty acid oxidation (McGarry and Foster, 1981) and suppresses fatty acid synthesis (Allred and Roehrig, 1972; Bricker and Levey, 1972; Goodridge, 1973; Watkins et al., 1977). Glucagon also inhibits cholesterol synthesis (Geelen et al., 1980). In diabetes, increased lipolysis is also observed in the heart (Kenno and Severson, 1985) and is stimulated by epinephrine to the same extent as in controls (Rosen et al., 1981), but not by isoproterenol (Kenno and Severson, 1985). Many of the effects of hormones on lipogenesis have been studied in rats or in isolated cells *in vitro*. It is important to note that in humans lipogenesis occurs

predominantly in the liver, whereas in rats both liver and adipose tissue are involved. Although the intestine is insensitive to insulin *in vivo*, in isolated intestinal cells insulin has an inhibitory effect on cholesterol synthesis.

A major consequence of increased FFA in diabetes in the face of decreased insulin is ketoacidosis. Increased glucagon and decreased insulin will stimulate lipolysis in adipose tissue, producing FFA in plasma that is transported to liver. Decreased availability of insulin reduces lipogenesis, and hence FFAs are converted to ketone bodies (acetoacetate and β-hydroxybutyrate). Glucagon plays a critical role in ketoacidosis. In normal subjects, infusion of glucagon stimulates insulin secretion, and no rise in ketones occurs. However, in diabetic subjects deficient in insulin (IDDM), glucagon infusion produces a rise in ketones (Liljenquist et al., 1974). The role of glucagon in ketogenesis in diabetes has been shown by many others (Gerich et al., 1975; Schade and Eaton, 1975; McGarry and Foster, 1979). Ketogenesis by increased glucagon and decreased insulin has been shown to occur also in experimental animals and isolated cells (Keller et al., 1977; Woodside, 1979). McGarry and Foster (1981) suggested the following mechanism for ketogenesis in diabetes. Glucagon acutely suppresses fatty acid synthesis by blocking the formation of malonyl CoA (a key metabolite in lipogenesis from glucose), which in turn causes depression of carnitine acyltransferase I and activates fatty acid oxidation, leading to accelerated production of ketone bodies. Schade and Eaton (1979) also reported that other hormones counterregulatory to insulin, such as catecholamine, also stimulate ketogenesis. However, an elevated ratio of glucagon to insulin is the primary factor for accelerated ketogenesis in diabetes (McGarry and Foster, 1977, 1981) as shown in Figure 47.7. In diabetic ketoacidosis, saturated unbranched fatty acids, succinic and adipic, are predominantly excreted in the urine and can be used as a marker of the ketoacidotic state (Liebich et al., 1980).

Elevated FFA in plasma in diabetes and other disorders results in hypercoticoidism and initiates a positive feedback loop between adipocytes and hypothalamic-pituitary-adrenal axis (Widmaier et al., 1995). Abnormality in thyroid function and altered thyroid hormone levels have been reported in diabetic subjects and that dysfunction of the hypothalamic-pituitary-thyroid axis may be involved (Suzuki et al., 1994). Fluctuations in estrogens and insulin-like growth factor-II (IGF-II) have been reported to contribute to the pathogenesis of NIDDM and that IGF-II and insulin may be inversely regulated in NIDDM (Holden, 1995). Recently leptin has been reported to increase fatty acid oxidation and decrease fatty acid incorporation into TGs in soleus muscle and thus oppose the lipogenic effect of insulin (Muoio et al., 1997). Leptin also increases fatty acid oxidation in cultured pancreatic islets (Shimabukuro et al., 1997a). In healthy subjects no correlation was observed between plasma leptin levels and plasma glucose, insulin, TG or FFA (Pratley et al., 1997). Similarly, in obese, mildly diabetic Zucker rat, leptin had no effect possibly due to mutation of the leptin receptor.

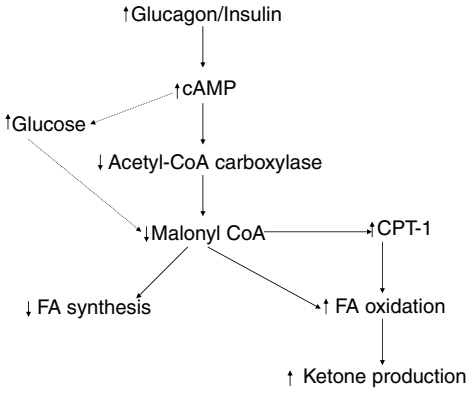


FIGURE 47.7 Hormonal control of fatty acid metabolism in liver in diabetes. Increased glucagon/insulin ratio in diabetic state leads to stimulation of cAMP in liver leading to increased fatty acid oxidation and increase ketone production. *Nutritional Neurosc*. 9:1–10, 2006. With permission.

VI. EFFECT OF TREATMENT OF DIABETES ON FATTY ACID METABOLISM

Hyperglycemia of diabetes is normally controlled by (1) diet and exercise, (2) oral agents, and (3) insulin. Diabetes in many cases is also associated with other complications such as hypertension, polyneuropathy, nephropathy, retinopathy, obesity, insulin resistance, and in most instances, hyperlipidemia, especially in NIDDM subjects. Until recently most drug therapy for diabetes was geared toward reducing hyperglycemia and correcting insulin responsiveness with less emphasis on correcting lipid abnormalities. Normalizing blood sugar levels by any means also partially lowers hyperlipidemia. However, more attention should be given to reduce lipid abnormalities in diabetes and the associated metabolic disorders. The reduction of elevated FFA levels and hyperlipidemia will help glucose-fatty acid cycle which is operative in many tissues by shifting the oxidation of FFA in favor of oxidation of glucose. The effects of insulin in the management of diabetes have been discussed throughout this chapter. The management of dyslipidemia of secondary complications of diabetes has been briefly reviewed by Garg and Grundy (1990). This section therefore deals primarily with the effect of diet, exercise, oral agents (hypoglycemic and hypolipidemic), and metal ions on fatty acid metabolism.

A. Diet and Exercise

The importance of dietary carbohydrate and lipid sources in the management of diabetes and their effects on fatty acid metabolism has been discussed throughout this chapter. Recently, several studies in humans and animals have shown beneficial effects of soybean and flaxseed meal in diabetes (Kaminskas et al., 1992; Nestel et al., 1997; Hermansen et al., 2001; Bhathena and Velasquez, 2002; Jayagopal et al., 2002; Torres et al., 2005). In addition to modulating hyperglycemia, soy protein reduces hyperlipidemia and hyperinsulinemia. In streptozotocin diabetic rats, soy protein compared to casein, lowered EPA and increased AA, which resulted in decreased ratio of aortic prostacyclin production to TXA_2 (Ikeda and Sugano, 1993). Soy protein along with its associated isoflavones and fiber reduces total cholesterol, LDL-cholesterol, VLDL-cholesterol, apolipoprotein B100, FFAs and TGs but has no significant effect on HDL-cholesterol, glucose or HbA1c (Hermansen et al., 2001). Zhan and Ho (2005) carried out meta-analysis of 23 human studies and found that soy protein with intact isoflavones was associated with significant decrease in serum total cholesterol, LDL-cholesterol, and TGs and significant increase in serum HDL-cholesterol. In another meta-analysis similar results were seen namely soy protein isolate with high isoflavones lowered serum LDL-cholesterol (Zhou et al., 2004). The hypocholesterolemic effect of isoflavones appears to be due in part to the modulation of steroid hormones involved in lipid metabolism (Ali et al., 2004). In contrast, recently, Sacks et al. (2006) analyzed data from 22 randomized trials and found no significant effect of soy protein with isoflavones compared to milk or other proteins on lipid parameters. Soy isoflavones also had no significant effect. It is possible that soy protein or isoflavones may not affect lipid parameters in normal subjects when lipid levels are in normal range, but may affect lipid levels when they are elevated as in diabetic subjects. Soy protein also reduces insulin/glucagon ratio which in turn down regulates the expression of hepatic sterol regulatory element-binding protein (SERBP-1) that results in decreased lipogenic enzymes leading to decreased LDL- and VLDL-cholesterol and TG (Torres et al., 2005). ω6 Fatty acids in soybean oil and ω3 fatty acids in flaxseed oil also activate SERBP-1 (Rodriguez-Cruz et al., 2005). They also activate alpha and gamma PPAR leading to increased lipid oxidation and hepatic steatosis.

The beneficial effects of flaxseed on lipid parameters in diabetes appear to be due to the presence of ω3 fatty acids. Flaxseed oil improves insulin sensitivity, decreases LDL oxidation and increases HDL-cholesterol (Nestel et al., 1997). In hypercholesterolemic rabbits, lignan present in flaxseed, secoisolariciresinol diglucoside, also lowers serum total- and LDL-cholesterol (Prasad, 1999). Secoisolariciresinol diglucoside also reduces the incidence of diabetes in both, type 1 and type 2

diabetic rats (Prasad, 2000, 2001; Prasad et al., 2000). The beneficial effects of isoflavones and lignans on tissue lipids may be due in part to their antioxidative actions since oxidative stress has been shown to be one of the causes of both type 1 and type 2 diabetes.

The beneficial effects of exercise on fatty acid metabolism, especially in NIDDM subjects, is primarily due to decreased body weight, increased insulin sensitivity, and lowered blood glucose level (National Institutes of Health, 1987). Improved insulin response to exercise is only acute and reverses after inactivity. Strenuous and prolonged exercise increases the rate of FFA metabolism in muscle and other tissues. There is a moderate fall in insulin, increased insulin sensitivity, increased insulin clearance and increases in glucagon, catecholamines, growth hormone, and cortisol (Keller et al., 1977; Schade and Eaton, 1979; Gray et al., 1980; Horton, 1988; Wasserman et al., 1989; Tuominen et al., 1997). As a consequence, there is an increase in lipolysis and increased ketone body production in the liver, which can be used for energy by muscle. In several prospective studies, exercise has been shown to reduce plasma TG and total and LDL-cholesterol and to increase HDL-cholesterol. The beneficial effects of light to moderate exercise on ischemic heart disease in diabetes are also suggested by epidemiological evidence (Leon, 1988). Exercise also improves altered immune function in obese diabetic rats (Plotkin and Paulson, 1996). The role of exercise training in NIDDM has been reviewed by Horton (1996) and Ivy (1997). Benefit of moderate exercise is also seen in IDDM subjects, where glucose uptake and oxidation is lower and fat oxidation is enhanced (Raguso et al., 1995). It is important to note that in poorly controlled IDDM subjects, strenuous exercise may not be beneficial because it can increase lipolysis, ketogenesis, and blood glucose levels due to insulin deficiency. Similarly, in well-controlled IDDM subjects, the dose of insulin should be reduced or extra carbohydrate should be given to prevent hypoglycemia.

In diabetic rats exercise causes increase in FFA more than in control rats. This mobilization appears to be due to reduced inhibition of lipolysis by the relative lack of insulin since insulin levels that are already low are unaltered (Houwing et al., 1997).

B. Oral Hypoglycemic Agents

The two classes of oral agents most frequently used in glycemic control are sulfonylureas and biguanides. A residual pancreatic insulin secretion is a prerequisite for the use of oral agents. The rationale for their use and the mechanisms of their actions have been extensively reviewed by Krall (1991), Lebovitz (2001, 2004), Ferner (1988), and others (Olefsky, 1985). The most widely used sulfonylureas are first-generation tolbutamide, second-generation glyburide (glibenclamide), and now third-generation glimepiride. The mechanisms of their action include increased glucose-stimulated insulin secretion and increased insulin responsiveness (decreased resistance) and action in target tissues due to increased insulin receptor number (Bhathena, 1987). They also decrease hepatic gluconeogenesis. In diabetic subjects, sulfonylureas also inhibit platelet aggregation *in vivo* (Sagel et al., 1975).

The commonly used biguanides are metformin and phenformin. Unlike sulfonylureas, they are not known to stimulate insulin secretion, but they do potentiate insulin action and increase insulin binding to hepatocytes and adipocytes and increases tyrosine kinase activity (Rossetti et al., 1990). More importantly, they inhibit glucose absorption and increase glucose utilization by the liver and muscle. They may also inhibit gluconeogenesis and hepatic glucose output in diabetic subjects thereby preventing hyperglycemia from occurring (Bailey, 1992). Phenformin causes increased lactic acidosis and is now used less frequently. Its use is banned in the United States but that of metformin has increased.

Both groups of compounds affect lipid metabolism. Stone and Brown (1966) reported that tolbutamide has antilipolytic activity. Thus, in IDDM subjects, injection of tolbutamide decreased plasma FFA concentration. *In vitro* studies in adipocytes from fasted nondiabetic rats, tolbutamide, but not phenformin, induced a significant decrease in FFA and glycerol release (Stone et al., 1966). This effect of tolbutamide may be related to decreased hyperglycemia, since sodium sulfadiazine,

another sulfonylurea that is nonhypoglycemic, had no effect on lipolysis. In NIDDM subjects, glibenclamide decreased plasma FFA and lactate concentrations (Jeng et al., 1989) but had no effect on apolipoproteins (Billingham et al., 1989). Insulin, however, reversed the apolipoprotein changes. In NIDDM subjects, metformin decreased serum total- and LDL-cholesterol. The effect persisted for a long time (Rains et al., 1989). Metformin also decreases fatty acid oxidation, reduces hepatic synthesis of VLDL TGs (Muntoni, 1974; Fedele et al., 1976) and reduces plasma FFA (Riccio et al., 1991; Perriello et al., 1994) and this may be in part responsible for its antidiabetic property (Gregorio et al., 1997). In short-term (21-day) treatment in IDDM subjects, metformin had no significant effect on plasma cholesterol, TG, glucose level, or glycosylated hemoglobin, but decreased maximum platelet aggregation stimulated by low-dose adenosine diphosphate (Gin et al., 1989). The site of action of metformin appears to at muscle, liver, and adipose tissue. It is important to note that insulin is more effective than oral agents in raising HDL-cholesterol levels in NIDDM subjects. In nondiabetic obese subjects, however, short-term (15-day) treatment with metformin decreased plasma TG and insulin concentrations. In muscle metformin has been shown to activate 5′-AMP-activated protein kinase which lowers lipid synthesis and increases oxidation of fatty acids. In type 2 diabetes atypical protein kinase C is defective and long-term metformin treatment in type 2 diabetic subjects improves basal as well as insulin-stimulated atypical protein kinase C in muscle (Luna et al., 2006). Metformin also lowered the elevated levels of plasminogen activator inhibition activity and increased the depressed euglobin fibrinolytic activity (Vague et al., 1987; Nagi and Yudkin, 1993). This indicates that biguanides may have a role in platelet function. The effects of oral hypoglycemic agents on platelet function in diabetic subjects needs to be explored further. The metabolic effects of metformin including its effect on fatty acid and lipid metabolism, have been reviewed by Wu et al. (1990b); Del Prato et al. (1995); Bailey and Turner (1996); Davidson and Peters (1997); and Raptis and Dimitriadis (2001).

C. Oral Agents to Treat Lipid Disorders

In the past two decades new classes of compounds has been introduced to treat lipid disorders of diabetes. The effects of some of them on fatty acid metabolism are briefly described. They are reviewed by Ilarde and Tuck (1994); Rachman and Turner (1995); Lefebvre and Scheen (1995); Dagogo-Jack and Santiago (1997); Raptis and Dimitriadis (2001); and Stumvoll (2003).

a. Insulin Sensitizers

Thiazolidinediones: The commonly used thiazolidinediones are rosiglitazone, pioglitazone, and troglitazone. They also affect fatty acid mobilization and oxidation. They lower blood glucose level without stimulating insulin secretion but increase insulin effectiveness (Chaiken et al., 1995) indicating that their action is peripheral. The effect appears to be due to lowering of plasma FFA levels (Miles et al., 1997). They also inhibit gluconeogenesis at the level of pyruvate carboxylase and glyceraldehyde 3-phosphate dehydrogenase reaction (Fulgencio et al., 1996) and stimulate glucose uptake by the muscle (Miles et al., 1997). Thiazolidinediones inhibit oxidation of long-chain fatty acid (18:1) but not the medium-chain fatty acid (octanoate). The inhibition of oxidation is via the inhibition of mitochondrial and microsomal long-chain acyl-CoA synthase activity but have no effect on mitochondrial CPT-I. In liver they increase insulin-stimulated conversion of glucose into fatty acids.

Thiazolidinediones are high-affinity ligands of PPAR-γ, a key factor for adipocyte differentiation. In preadipose cells they exert potent effect on the expression of genes encoding proteins involved in fatty acid metabolism such as FAS and phosphoenolpyruvate carboxykinase (Ibrahimi et al.,1994; Hallakou et al., 1997), LPL and hormone-sensitive lipase (Teruel et al., 2005). In adipose tissue thiazolidinediones act to conserve lipid by reducing lipid supply and subsequent utilization (Oakes et al., 1997). Troglitazone lowers serum FFA and TG concentrations by inhibiting TG synthesis and raises HDL-cholesterol level (Mimura et al., 1994; Kumar et al., 1996) but has no effect on

phospholipid synthesis. Plasma LDL-cholesterol increases with doses up to 600 mg/day but not at 800 mg/day of troglitazone. The possible side effect appears to be reduction in neutrophil counts at high doses of troglitazone (Kumar et al., 1996). In type 2 diabetic subjects rosiglitazone decreases postprandial FFA concentration (Boden et al., 2005; Van Wijk et al., 2005) and TG concentration (Tan et al., 2005) and increases oxidation of FFA in muscle (Wilmsen et al., 2003), total body fat, and oxidative phosphorylation (Boden et al., 2005). It also enhances downstream insulin receptor signaling in muscle (Miyazaki et al., 2003).

b. Fatty Acid Oxidation Inhibitors

Bromopalmitate and methylpalmoxirate (fatty acid derivatives) are fatty oxidation inhibitors. In 3T3-L1 adipocytes, 2-bromopalmitic acid, and 4-bromocrotonic acid inhibited basal as well as isoproterenol and dibutyryl cAMP-stimulated lipolysis (Fong et al., 1997). The effect of 4-bromocrotonic acid appears to be due to inhibition of hormone-sensitive lipase (Fong et al., 1997).

Dexfenfluramine, increase FFA turn over and oxidation rates in obese NIDDM subjects. It also reduces serum glucose but has no effect on insulin secretion (Greco et al., 1995).

c. Inhibitors of Lipolysis

Acipimox, a nicotinic acid analog, is an inhibitor of lipolysis. In lean and obese NIDDM subjects it decreases plasma FFA, glycerol and ketone levels and muscle lipid peroxidation, and increases insulin levels and insulin sensitivity (Vaag et al., 1991; Fulcher et al., 1992; Kumar et al., 1994; Piatti et al., 1996). In normal rats it decreases plasma FFA, inhibits lipolysis and hepatic gluconeogenesis and enhances the ability of insulin to suppress hepatic glucose production and peripheral glucose utilization (Al-Shurbaji et al., 1990; Lee et al., 1996). It may be used to treat lipid disorders in diabetes in combination with hypoglycemic agents.

Adenosine A1 agonist SDZ WAG944 is a potent inhibitor of adenosine deaminase-induced lipolysis. In diabetic rats it decreases plasma FFA and TG concentration. Antilipolytic agent *N*-{(1s, *trans*)-2 hydroxycyclopentyl} adenosine (GR 79236) reduces plasma FFA concentration and improves ketoacidosis in diabetic rats (Thompson et al., 1994). Inhibitor of hepatic fatty acid oxidation, B-aminobetaine is a carnitine analog and inhibits CPI-1 in hepatocytes but may induce fat deposition in the liver (Kashiwagi, 1995).

d. Lipid-Lowering Agents

Bezafibrate normalizes fatty acid changes in skeletal muscle TG of rats fed high fructose + lard (Matsui et al., 1997), which tend to produce insulin resistance. Whether bezafibrate has similar effect in diabetic animals or humans with insulin resistance and altered lipid and fatty acid composition needs to be evaluated. It also increases $\Delta 5$ desaturase activities.

Gemfibrozil decreases plasma FFA, TG, and phospholipids in elderly diabetic subjects. There is also a decrease in long-chain saturated fatty acids in phospholipids suggesting an impairment of chain elongation of fatty acid in liver microsomes (Brosche and Kipfmuller, 1996). However, in another study in NIDDM subjects it increased plasma FFA and LPL activity but it decreased insulin sensitivity (Ohrvall et al., 1995). It also decreased VLDL-cholesterol and TG.

Pravastatin, a HMG CoA-reductase inhibitor, is effective in patients with hypercholesterolemia secondary to diabetes and renal diseases (Haria and Mctavish, 1997). Hence it may be useful in combination therapy in diabetic patients who are at high risk of cardiovascular morbidity. Simvastatin, another HMG CoA-reductase inhibitor, also decreases LDL-cholesterol, and LDL/HDL ratio in NIDDM subjects with hyperlipoproteinemia (Ohrvall et al., 1995). Simvastatin has been shown to increase (Paolisso et al., 1991) as well as decrease (Ohrvall et al., 1995) insulin sensitivity. Its use in the treatment of NIDDM is not very effective, but may be used in conjunction with other treatments.

D. Metal Ions

The commonly used metal ions to treat diabetes and having an effect on fatty acid metabolism are chromium, vanadium, and molybdenum.

a. Chromium

The role of chromium in improving diabetes and its effect on lipid metabolism has been reviewed by Anderson (1995). In humans and diabetic animals chromium has been shown to have beneficial effects on glucose homeostasis and on lipid metabolism. Chromium appears to act via potentiating the effects of insulin by increasing insulin binding to cells (Anderson et al., 1987). Trivalent chromium in organic form appears to be a biologically active form. Chromium has been shown to decrease total cholesterol, LDL-cholesterol and TG, and increase HDL-cholesterol in subjects with IDDM as well as NIDDM (Canfield, 1979; Nath et al., 1979; Mossop, 1983; Evans, 1989; Anderson et al., 1997). In streptozotocin diabetic rats, glucose tolerance factor (GTF), a chromium-containing compound, reduces TG and FFA levels but has no effect on total cholesterol or HDL levels (Mirsky, 1993). In genetically diabetic mice, GTF decreased plasma TG and cholesterol levels (Tuman and Doisy, 1977).

b. Vanadium

In streptozotocin diabetic rats, vanadium compounds have beneficial effects on glucose and lipid metabolism. Vanadium lowers serum glucose level, decreases serum FFA and normalizes epinephrine-stimulated FFA release from adipose tissue (Brichard et al., 1994; Nakai et al., 1995; Sakurai et al., 1995). Vanadium appears to act via incorporation into adipose tissue (Nakai et al., 1995) and by improving low thyroid hormone status (Sakurai et al., 1995). Vanadium also partially increases mRNA and activities of key lipogenic enzymes, ACC and FAS in liver but not in adipose tissue (Brichard et al., 1994). Recently, new vanadium and zinc complexes have been synthesized (Yamaguchi et al., 2006). Vanadium compounds appear to have more insulin-mimetic activity than zinc compounds as measured by the effect on FFA release from rat adipocytes treated with epinephrine (Yamaguchi et al., 2006). Similarly, in streptozotocin diabetic rat macrocyclic binuclear oxovanadium complex lowered the elevated levels of lipids in plasma and tissue to near normal levels. It lowered the levels of LDL-cholesterol and increased the HDL-cholesterol levels and also normalized the altered fatty acid composition in liver and kidney (Ramachandran and Subramanian, 2005). The insulin-mimetic activity and the molecular mechanisms of various vanadium complexes had been reviewed by Scior et al. (2005). Wang et al. (2006) reported that in streptozotocin-treated mice, zinc supplement prevents diabetic cardiomyopathy via increased cardiac metallothionein.

c. Molybdenum

In streptozotocin diabetic rats, molybdenum decreased hyperglycemia and glucosuria and corrected the elevation of plasma FFA. Molybdenum also reversed low expression and activity of ACC and FAS in liver but not in adipose tissue (Ozcelikay et al., 1996). Thus, both vanadium and molybdenum, mimics certain insulin actions.

E. Other Agents

Probucol, an antioxidant, appears to decrease the oxidation of LDL in diabetic subjects. Addition of MUFA further decreases LDL oxidation, especially of dense LDL, which is more susceptible to oxidation (Reaven et al., 1996) and is more atherogenic.

Hydralazine, an antihypertensive drug, decreases serum TG and cholesterol in streptozotocin diabetic rats without affecting hyperglycemia. It also increases the binding of fatty acids by myocardial plasma membrane FABP (Heylinger et al., 1995).

Pyrazinoylguanidine reduces serum FFA, glucose and TG in hypertensive diabetic subjects and reduces glycosuria (Vesell et al., 1994). Thus, this drug appears to be beneficial for treatment of glucose as well as lipid abnormalities. Whether it is effective in treating diabetic subjects without hypertension remains to be studied.

β_3 *Adrenergic receptor* (β3AR) agonist treatment has been reported to normalize blood glucose, and decrease insulin and FFA levels in obese (ob/ob) mice (Arbeeny et al., 1995). Since similar defects are present in diabetes, β3AR agonists may also have therapeutic value in the treatment of NIDDM. Thus, a decrease in basal level of mRNA for the β3AR in brown adipose tissue in obese mice compared to lean controls has been observed.

Ascorbic acid supplementation alleviates hyperlipidemia in streptozotocin diabetic rats without affecting hyperglycemia or hypoinsulinemia (Dai and McNeill, 1995) suggesting that combination treatment of hypoglycemic and hypolipidemic agents may be beneficial.

Indobufen is an antiaggregatory agent and inhibits platelet aggregation by interfering with cyclooxygenase enzymes in platelets. In diabetic subjects it significantly lowers lipid peroxidation without affecting fatty acid composition of platelet phospholipids (Dmoszynska et al., 1995).

L-Propionylcarnitine (LPC) has beneficial effect in the heart of streptozotocin diabetic rats. It increases the rates of glucose and palmitate oxidation by heart (a favorable shift in glucose and fatty acid metabolism) and prevents the depression of cardiac mitochondrial respiration seen in diabetes (Broderick et al., 1996). LPC also has beneficial effect on neuropathy and retinopathy (Hotta et al., 1996).

Etomoxir, an inhibitor of CPT-I and fatty acid synthesis, has been reported to reduce high serum TG, FFA, and cholesterol in streptozotocin diabetic rats. It had no effect on low serum insulin or triiodothyronine (T_3) (Rupp et al., 1994). In NIDDM subjects it decreased blood glucose and improved lipid parameters, namely decreases in TG and cholesterol (Ratheiser et al., 1991).

Enprostil, a synthetic dehydoprostaglandin E_2, treatment for 1 week significantly reduced postprandial plasma FFA and TG and slightly decreased in cholesterol in NIDDM patients (Reaven et al., 1988). Thus, it may be useful in treating lipid disorders in type 2 diabetic subjects.

NO lowering agents such as nicotinamide and aminoguanidine may also be considered for diabetic treatment.

VII. CONCLUSIONS AND AREAS FOR FURTHER STUDY

In diabetes, fatty acid metabolism is altered qualitatively as well as quantitatively. Dyslipidemia appears to be due to altered lipogenesis as well as lipolysis, possibly due to hormonal imbalance. The desaturases that are responsible for synthesis of PUFAs are decreased in diabetes. This leads to more saturated fatty acid and less PUFA, especially AA in tissue phospholipids and other lipids. As a consequence, membrane fluidity is altered and eicosanoid production of 2-series is decreased. Dietary fish oils have several beneficial effects in normal and nondiabetic hyperlipidemic subjects, but in diabetic subjects the beneficial effects on lipid metabolism are offset by the deleterious effects on glucose homeostasis, and hence fish oils should be used with caution. Exercise has beneficial effects on carbohydrate and lipid metabolism in both NIDDM and IDDM, but in IDDM blood glucose levels and insulin dose requirements should be closely monitored during strenuous and/or prolonged exercise. Recently, soybean and flaxseed containing isoflavones and lignans have been found to have beneficial effects in lowering lipids in diabetic subjects.

Though we have gained significant knowledge on the fatty acid metabolism in diabetes, several areas need to be further explored.

1. The effect of ω-3 fatty acids, especially those from fish oils, on insulin has been studied in detail, but the effects on other hormones have not been investigated in detail in normal or diabetic subjects.
2. Lipid and carbohydrate metabolism are quantitatively different between males and females. In addition to pancreatic hormones, female sex hormones also play a role in lipid metabolism (Bhathena et al., 1989b). The differences in lipid metabolism between male and female diabetic subjects need to be studied in detail.
3. Recently, opiates and neuropeptides have been shown to be involved in the control of glucose and lipid metabolism. Limited data exist regarding the effect of dietary lipids on opiates and neuropeptides in diabetes.
4. The role of dietary fatty acids on insulin receptors in diabetes have been studied in detail, but the effects on the receptors of the counterregulatory hormones—glucagon, cortisol, growth hormone, catecholamines—need to be explored.
5. Diabetes, more often than not, is accompanied by increased risk and incidence of micro- and macroangiopathy, atherosclerosis, hypertension, nephropathy, peripheral polyneuropathy, and retinopathy. Though not discussed in this chapter, several abnormalities in the lipid metabolism have been well documented in these complications. However, the effects of dietary lipids on hormonal balance and metabolic fate of fatty acids—de novo synthesis, oxidation, desaturation, formation of eicosanoids—need to be further explored in these conditions.
6. Alteration in membrane fluidity has been clearly demonstrated in diabetic subjects. The effect of fatty acids on membrane fluidity in normal subjects has been studied in detail, and several of these studies have suggested the possible role of dietary fatty acids in the alteration in membrane fluidity in diabetic subjects. However, this needs to be demonstrated experimentally.
7. The TFAs appear to have more deleterious effects in humans than saturated fatty acids. No significant data exist on the metabolism of TFA in diabetes. Also the role of hormones on the metabolism of TFAs and the effects of TFAs on hormone levels in diabetes are not known.
8. The decrease in Δ9 and Δ4 desaturases and their regulation by dietary fatty acids and other factors remain unexplored.
9. The control of ω oxidation of fatty acids in diabetes also needs to be explored.
10. The role of oral hypoglycemic and lipid-lowering agents on platelet function and eicosanoid formation in diabetes is briefly reported here, but it needs further study.
11. Recently, amylin, interleukins and other cytokines, calcitonin gene-related peptide, and TNFs have been implicated in the pathogenesis of diabetes. Except for TNF's role in LPL modulation, not much is known about the role of these factors in lipid and fatty acid metabolism in diabetes. This is a new and exciting area of research.
12. Modified insulin (lispro insulin) where lysine and proline are exchanged at position B29 and B30, as well as acetylated insulin derivative (lys B29-tetradecanoyl des-[B30]) have been introduced to treat diabetes. Their effect on fatty acid and lipid metabolism needs to be evaluated.
13. Some of the advantages of breast feeding are attributed to the fatty acid composition of human milk (Lanting and Boersma, 1996). It is thus important to study long-term effect of fatty acids in breast milk compared to formula milk on the incidence of NIDDM and other chronic diseases in children.
14. Though progress has been made in the area of relationship between fatty acids and insulin resistance, the possible beneficial effects of lowering or preventing insulin resistance through increasing AA, EPA, and DHA and lowering linoleic acid and TFA on the incidence of NIDDM need to be further studied.
15. The studies on fatty acid metabolism in gestational diabetes are lacking and need to be carried out.

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48 Fatty Acid Metabolism in Skeletal Muscle and Nerve, and in Neuromuscular Disorders

Charles J. Rebouche and Jeffrey K. Yao

CONTENTS

I. INTRODUCTION

Fatty acids, mostly as their activated coenzyme A esters, participate in many metabolic processes in human nerve and skeletal muscle (Figure 48.1). They are broken down, primarily in mitochondria and peroxisomes, to release electrons to be used to drive ATP synthesis, or to remove species (e.g., phytanic acid) with no biological function in humans. They are used for synthesis of complex lipids that are incorporated into membranes, or that are storage vehicles (triacylglycerols). Fatty acids

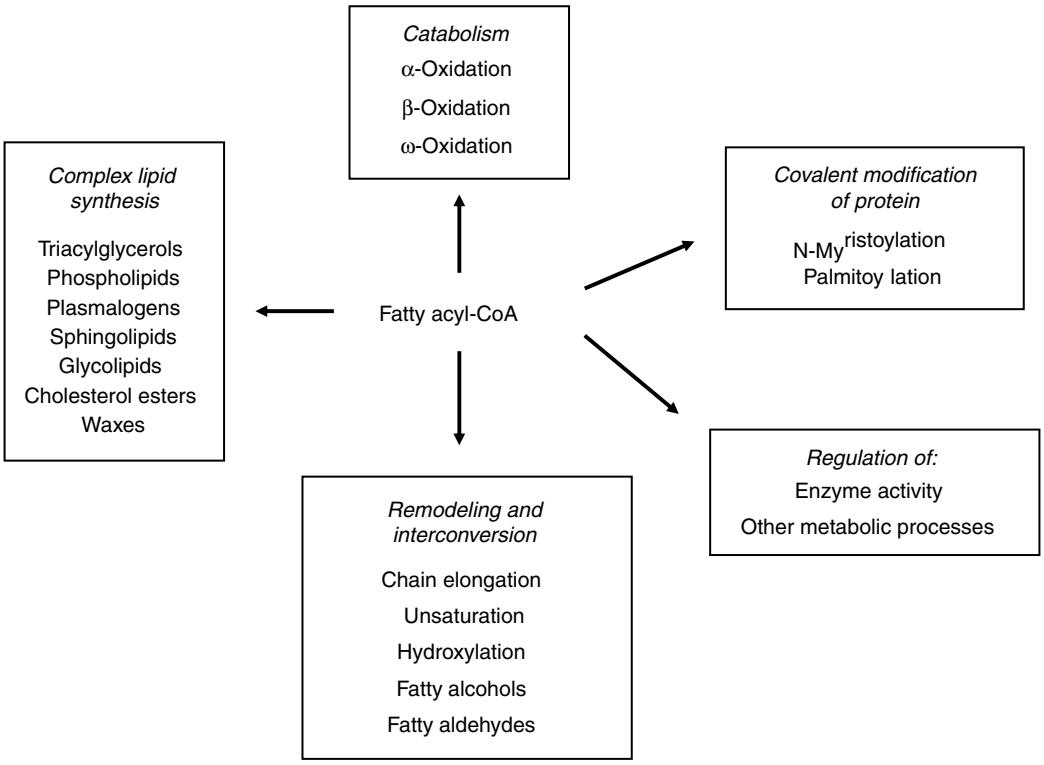

FIGURE 48.1 Metabolic roles of long-chain fatty acyl-CoA esters. (From Watkins, P.A. (1997). *Prog. Lipid Res. 36*:55–83. With modifications.)

contribute to covalent modification of proteins (N-myristoylation and palmitoylation), and participate in regulation of enzymatic activities and other metabolic processes.

Lipid abnormalities are often associated with neurologic and skeletal muscle dysfunction. Many disorders are due to single or multiple enzyme deficiencies, with resultant abnormal lipid storage in tissue, or abnormal lipid composition of membranes. For example, alterations of fatty acid metabolism are often associated with the process of demyelination in nervous tissues, because of their important role in the structure of membranes. In both nerves and skeletal muscle, enzyme deficiencies in pathways of fatty acid oxidation cause neuropathy, myopathy, muscle weakness, and abnormal lipid storage. Deficiency of dietary intake of fatty acids, as in protein-calorie malnutrition and anorexia nervosa, also may cause pathologic dysfunction of nerve and skeletal muscle. The purposes of this chapter are (1) to review fatty acid metabolism in nerve and skeletal muscle, and in other tissues where abnormal fatty acid metabolism impacts nerve and muscle function and (2) to review the current state of knowledge of some of the neuromuscular diseases caused by or are associated with abnormal fatty acid metabolism.

II. FATTY ACID METABOLISM IN MUSCLE AND NERVE

Skeletal muscle and nerve acquire free fatty acids from diet and from adipose tissue. Fatty acids derived from dietary triacylglycerols are reesterified and packaged into chylomicrons in enterocytes and released into the circulation. Fatty acid components of circulating chylomicrons are liberated by endothelial lipoprotein lipase. Fatty acids from triacylglycerols stored in adipose tissue are released by hormone-sensitive lipase. Nonesterified fatty acids bound to circulating albumin are the primary exogenous substrates for tissue fatty acid acquisition and subsequent storage, use in anabolic

processes, or oxidation. There is no general agreement as to whether exogenous fatty acids enter cells primarily by diffusion or by protein-mediated transport across the plasma membrane. Many long-chain fatty acids readily diffuse across model bilayer membranes (McArthur et al., 1999). However, the emerging view is that long-chain fatty acids cross plasma membranes via protein-mediated mechanisms, involving a number of fatty acid binding proteins, including fatty acid translocase (FAT/CD36), peripheral plasma membrane binding protein ($FABP_{pm}$), and possibly fatty acid transport protein 1 (FATP1) (Bonen et al., 2004; Koonen et al., 2005). Consistent with a role for diffusion, increased concentration of circulating fatty acids leads to increased accumulation of fatty acids in muscle. However, evidence for saturability of fatty acid uptake has been obtained in exercising human muscle, and correlations between abundance of FAT/CD36 and $FABP_{pm}$ in red oxidative skeletal muscle and white glycolytic muscle (both higher in red fibers) suggest an important role for protein-mediated uptake of fatty acids at the sarcolemmal membrane (Rasmussen and Wolfe, 1999). In the cytoplasm, long-chain fatty acids are transferred between intracellular membranes by binding to soluble fatty acid binding proteins ($FABP_c$). The predominant FABP isoform in muscle tissues, heart-type $FABP_c$ (H-$FABP_c$) is responsible for delivery of long-chain fatty acids from the sarcolemma through the cytoplasm to the outer mitochondrial membrane, the site of acyl-CoA synthetase (Koonen et al., 2005). This enzyme activates long-chain fatty acids to coenzyme A esters to make them available for triacylglycerol synthesis or mitochondrial β-oxidation.

A. Fatty Acid Metabolism in Peroxisomes

Peroxisomes utilize fatty acids in both catabolic and anabolic pathways. Peroxisomes catalyze α-oxidation and β-oxidation of activated fatty acids (as CoA-esters), and utilize fatty acids for synthesis of ether lipids. Although most β-oxidation of fatty acids occurs in mitochondria, peroxisomes are responsible for chain-shortening of very-long-chain fatty acids (C24 and C26), and long-chain dicarboxylic acids. Phytanic acid and 2-hydroxy fatty acids (as coenzyme A esters) undergo first α-oxidation and then β-oxidation in peroxisomes.

a. The Fatty Acid β-Oxidation Pathway in Peroxisomes

Peroxisomes contain at least 17 enzymes associated with the β-oxidation pathway. These include two acyl-CoA synthetases (Uchida et al., 1996), two acyl-CoA oxidases, two bifunctional proteins, two β-ketothiolases, α-methylacyl-CoA racemase, carnitine acetyl-transferase, carnitine octanoyl transferase, two enoyl-CoA isomerases, two enoyl-CoA reductases, and two acyl-CoA thioesterases (Wanders and Waterham, 2006). The peroxisomal membrane contains four half-ABC transporters, at least one of which is required for entry of activated fatty acids into peroxisomes.

Long-chain acyl-CoA synthetase (also known as palmitoyl-CoA ligase, and identical to mitochondrial and microsomal enzymes of the same name; Miyazawa et al., 1985) and very-long-chain acyl-CoA synthetase (also known as lignoceroyl-CoA ligase and cerotoyl-CoA synthetase) are associated with the peroxisomal membrane. The catalytic site of long-chain acyl-CoA synthetase is located on the cytoplasmic (external) side of peroxisomal membranes (Mannaerts et al., 1982; Lageweg et al., 1991). The catalytic site of very-long-chain acyl-CoA synthetase is located on the luminal (interior) side of peroxisomal membranes (Lazo et al., 1990; Steinberg et al., 1999). Thus, long-chain fatty acids are activated externally and are transported into peroxisomes as coenzyme A esters, but very-long-chain fatty acids enter peroxisomes as free acids. Short- and medium-chain acyl-CoA esters generated from chain-shortening of fatty acids are not transported out of peroxisomes as CoA esters, but rather, as esters of carnitine (Verhoeven et al., 1998a; Ramsay, 1999), or as acetate (Leighton et al., 1989). In mammals, peroxisomal acyl-CoA synthetases have mostly been studied with liver preparations. The presence, expression level, and existence of isozyme species in extrahepatic tissues have not been extensively characterized.

The enzymatic reaction sequence for β-oxidation in peroxisomes is similar but not identical to that in mitochondria. Two electrons are first extracted from activated fatty acids, by palmitoyl-CoA

oxidase (specific for very-long-chain fatty acids and straight chain mono- and dicarboxylic acids) and pristanoyl-CoA oxidase, which is active with activated branched-chain fatty acids (e.g., pristanoyl-CoA). Unlike the acyl-CoA dehydrogenases in mitochondria, peroxisomal acyl-CoA oxidases transfer electrons directly to molecular oxygen, producing hydrogen peroxide (Wanders et al., 2001). Peroxisomes contain two bifunctional proteins, each containing enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, but with distinct substrate-specific activities (Wanders and Waterham, 2006). Human peroxisomes contain two thiolases, one reactive with unbranched 3-ketoacyl-CoA esters, and one specific for 2-methyl (2S configuration) branched-chain ketoacyl-CoA esters. Auxiliary enzymes are required for metabolism of (2R)-methyl branched-chain fatty acids (2-methylacyl-CoA racemase), unsaturated fatty acids (2,4-dienoyl-CoA reductase and Δ^3, Δ^2-enoyl-CoA isomerase) and unsaturated fatty acids with a double bond at an odd-numbered position ($\Delta^{3,5}$,$\Delta^{2,4}$-dienoyl-CoA isomerase, $\Delta^{2,4}$-dienoyl-CoA reductase, and Δ^3,Δ^2-enoyl-CoA isomerase) (Wanders and Waterham, 2006).

b. The Fatty Acid α-Oxidation Pathway

The peroxisomal α-oxidation pathway provides for initial degradation of dietary phytanic acid, and dietary and endogenous α-hydroxy fatty acids (Figure 48.2). Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is derived from the phytol side chain of chlorophyll. Microorganisms in the rumen of domestic ruminant animals cleave the phytol side chain, and it is subsequently converted to phytanic acid (Verhoeven et al., 1998b). Phytanic acid is present in the human diet primarily in dairy products and ruminant fats, but is also found in lower concentrations at all levels of the food chain. Humans do not readily absorb chlorophyll from plant material; approximately 95% of ingested chlorophyll passes through the digestive system intact (van den Brink and Wanders, 2006). Chlorophyll may be degraded to a small extent by microorganisms in the large intestine of humans. Phytol released in this manner may be absorbed and metabolized to phytanic acid by a pathway present in microsomes and peroxisomes (van den Brink and Wanders, 2006). However, this pathway does not appear to be a significant source of phytanic acid in normal humans or in patients with Refsum disease (Wierzbicki, 2004).

Phytanic acid metabolism probably occurs mostly in the liver, but failure to degrade this fatty acid results in the serious neurological disorder, Refsum's disease (Refsum, 1981; Yao and Dyck, 1987). A major degradation product of phytanic acid, pristanic acid (2,6,10,14-tetra-methylpentadecanoic acid), is also present in the diet and is degraded by peroxisomal β-oxidation. α-Hydroxy derivatives of straight-chain C20 to C26 saturated or monounsaturated fatty acids are synthesized primarily in neural tissues for incorporation into sphingolipids, and are a major constituent of myelin (Alderson et al., 2004). Degradation of sphingolipids releases α-hydroxy fatty acids, which are degraded via the peroxisomal α-oxidation pathway.

Phytanic acid is activated to its coenzyme A ester by very-long-chain acyl-CoA synthetase. The activated fatty acid is then hydroxylated at the α carbon, by the catalytic action of phytanoyl-CoA hydroxylase, an α-ketoglutarate-requiring dioxygenase (Mihalik et al., 1995; McDonough et al., 2005). 2-Hydroxyphytanoyl-CoA is cleaved by 2-hydroxyphytanoyl-CoA lyase, producing pristanal and formyl-CoA (Foulon et al., 1999). This reaction requires the cofactor thiamine pyrophosphate. Long-chain fatty aldehydes are produced from long-chain α-hydroxy fatty acids by this same pathway (Foulon et al., 2005). Long-chain fatty aldehydes, including pristanal, are oxidized to the corresponding acids by aldehyde dehydrogenase (Jansen et al., 2001). These acids are subsequently activated to coenzyme A esters and are further metabolized by peroxisomal and mitochondrial β-oxidation.

c. Synthesis of Ether Lipids

Ether lipids differ from other phospholipids in that they contain an ether linkage at the *sn*-1 position instead of the more common ester linkage. Ether lipids, 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine,

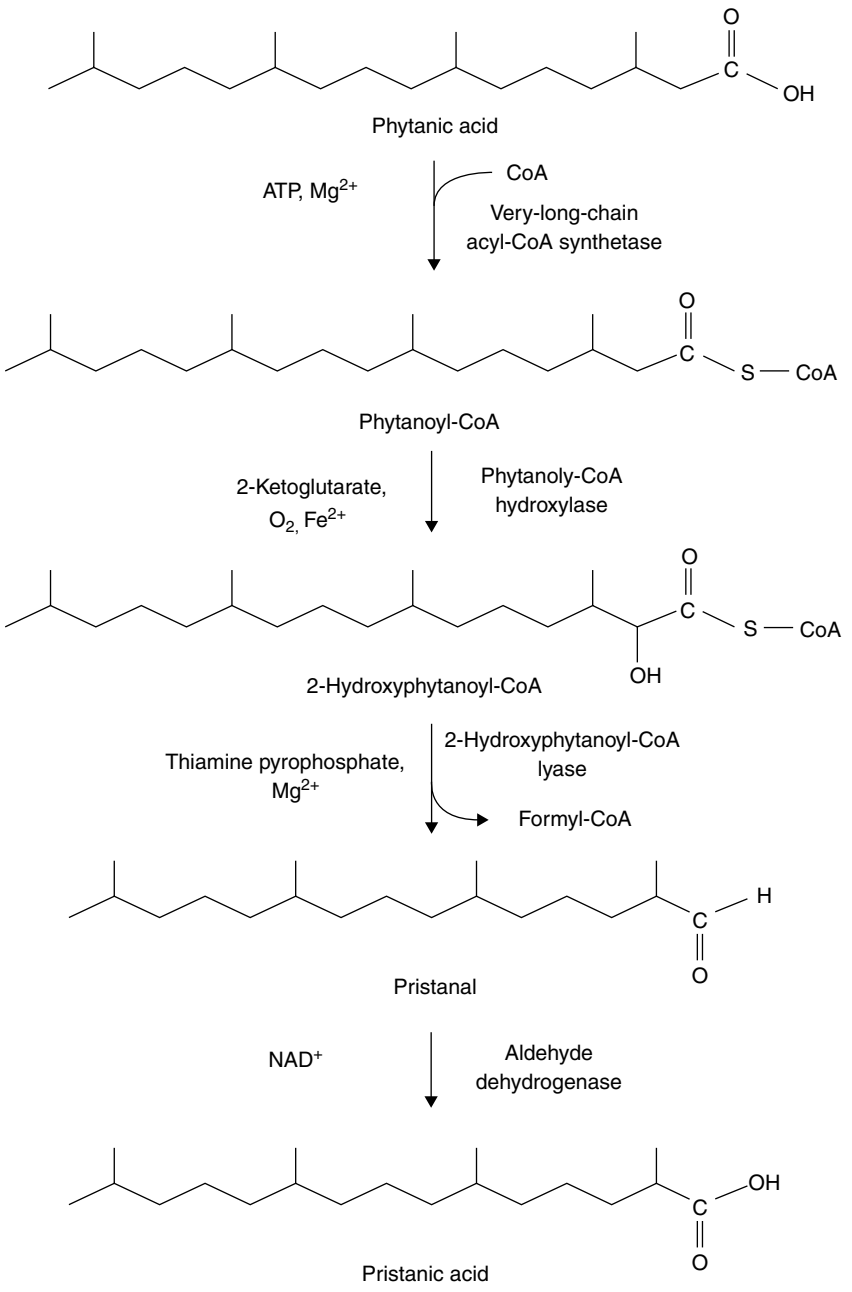

FIGURE 48.2 Pathway of α-oxidation of phytanic acid in peroxisomes.

1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine, and 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-ethanolamine, are synthesized by a series of enzymatic reactions that occur sequentially in peroxisomes and endoplasmic reticulum. 1-Alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine (plasmenylcholine) and 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-ethanolamine (plasmenylethanolamine) are called plasmalogens. In humans, plasmalogens make up about 18% of total phospholipid mass (Wanders and Waterham, 2006). Plasmenylethanolamine is an abundant component of the myelin membrane. Because of their surface charge and geometry,

plasmalogens reduce membrane fluidity and influence the temperature of phase transition (Thai et al., 1997). Plasmenylethanolamine in brain contains a high percentage of polyunsaturated fatty acids (PUFA) in the *sn*-2 position, including arachidonic acid (AA) and docosahexaenoic acid (DHA); it has been suggested that this molecule serves as a storage depot for these species (Brites et al., 2004). Plasmalogens may also have a role in signal transduction, cholesterol transport and metabolism, and oxidative stress (Brites et al., 2004; Wanders and Waterham, 2006). The importance of plasmalogens is underscored by the fact that single enzyme deficiencies in their biosynthesis have severe clinical consequences.

Synthesis of plasmalogens is a multistep process utilizing enzymes located in peroxisomes and endoplasmic reticulum. The committed step in this pathway is the condensation of a long-chain fatty alcohol with acyl dihydroxyacetone phosphate to form alkyl dihydroxyacetone phosphate and releasing the acyl group as a fatty acid (Wanders and Waterham, 2006). This reaction is catalyzed by peroxisomal alkyl-dihydroxyacetone phosphate synthase (Singh et al., 1993). Reactions catalyzing formation of substrates for this reaction also are peroxisomal. Long-chain fatty alcohol is formed by reduction of fatty acyl-CoA on the external surface of peroxisomes, catalyzed by the peroxisomal membrane protein acyl-CoA reductase (Burdett et al., 1991). Two molecules of NADPH provide four electrons for this two-step reduction. Acyl dihydroxyacetone phosphate is formed by the condensation of an acyl-CoA ester with dihydroxyacetone phosphate, with release of coenzyme A. The reaction is catalyzed by acyl-CoA:dihydroxyacetone phosphate acyltransferase. The active form of this enzyme is part of a heterotrimeric complex with alkyl-dihydroxyacetone phosphate synthase (Biermann et al., 1999). Alkyl dihydroxyacetone phosphate is reduced to form 1-alkyl glycerol-3-phosphate. This reaction is catalyzed by acyl-/alkyl-dihydroxyacetone phosphate reductase, an enzyme located in the peroxisomal membrane with its catalytic site on the external surface (Datta et al., 1990). NADPH is the preferred source of electrons for the reduction reaction.

The remaining reactions in the pathway of plasmalogens synthesis occur in the endoplasmic reticulum. 1-Alkyl glycerol-3-phosphate is acylated at the 2 position of glycerol. The phosphate is hydrolyzed producing 1-alkyl-2-acylglycerol. This molecule is condensed with CDP-choline or CDP-ethanolamine to produce 1-alkyl-2-acylglycerophosphocholine or 1-alkyl-2-acylglyceroethanolamine, respectively. The desaturase catalyzing formation of plasmalogens is similar to the enzyme that introduces double bonds into long-chain acyl-CoA esters (Berg et al., 2002). Co-substrates for the reaction are O_2 and NADH.

The physiological significance of segregation of the early steps of ether lipid synthesis in peroxisomes appears to be the need to overcome the inhibition by extraperoxisomal NADPH, which inhibits formation of ether lipids from dihydroxyacetone phosphate by reducing acyl dihydroxyacetone phosphate as soon as it forms (Hajra, 1995).

B. β-Oxidation of Fatty Acids in Mitochondria

In muscle and nerve, most fatty acid catabolism occurs in mitochondria. Long-chain fatty acids in the cytosol are first activated by esterification to coenzyme A (Figure 48.3). This reaction occurs on or in the outer membrane of mitochondria, and is catalyzed by long-chain acyl-CoA synthetase (Watkins, 1997). Activated fatty acids are transesterified to carnitine by the action of carnitine palmitoyltransferase I (CPT I), located on the inner aspect of the mitochondrial outer membrane. The fatty acyl carnitine esters are transported in the mitochondrial inner membrane by carnitine-acylcarnitine translocase (CACT). The activated fatty acids are then transesterified to intramitochondrial coenzyme A by the action of carnitine palmitoyltransferase II (CPT II) located on the matrix side of the mitochondrial inner membrane.

Activated long-chain fatty acids undergo successive rounds of mitochondrial β-oxidation. The first step in this process involves the loss of two electrons, and is catalyzed by one of three acyl-CoA dehydrogenases. Very-long-chain fatty acyl-CoA dehydrogenase (VLCAD; Izai et al., 1992) and acyl-CoA dehydrogenase-9 (ACAD-9, so-named because it was the ninth member of this enzyme

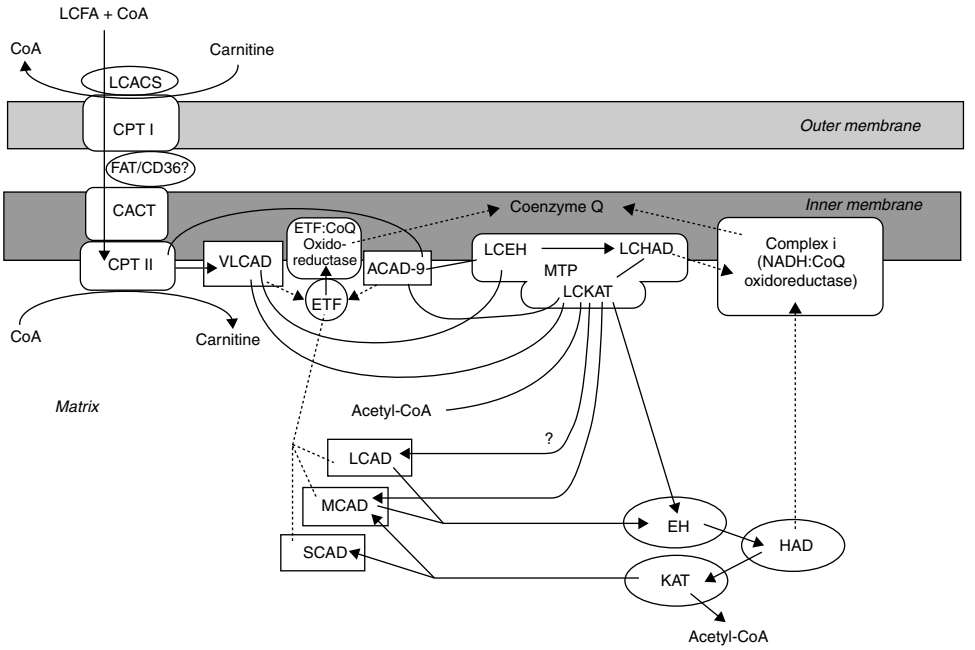

FIGURE 48.3 Schematic diagram of long-chain fatty acid β-oxidation in mitochondria. Solid lines indicate flow of carbon; dashed lines indicate flow of electrons. *Abbreviations:* LCFA, long-chain fatty acid; CoA, coenzyme A; LCACS, long-chain acyl-CoA synthetase; CPT, carnitine palmitoyltransferase; FAT, fatty acid transporter; CACT, carnitine-acylcarnitine translocase; VLCAD, very-long-chain acyl-CoA synthetase; ACAD-9, acyl-CoA synthetase-9; ETF, electron transfer flavoprotein; CoQ, coenzyme Q; MTP, mitochondrial trifunctional protein; LCEH, long-chain 2-enoyl-CoA hydratase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; LCKAT, long-chain 3-ketoacyl-CoA thiolase; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; EH, 2-enoyl-CoA hydratase; HAD, 3-hydroxyacyl-CoA dehydrogenase; KAT, 3-ketoacyl-CoA thiolase; and NADH, reduced nicotinamide adenine dinucleotide.

family to be identified; Zhang et al., 2002) are associated with the mitochondrial inner membrane. They catalyze electron transfer from activated long-chain saturated and unsaturated fatty acids, respectively, although there is some overlap in specificity (Ensenauer et al., 2005). VLCAD kinetics is most favorable with saturated, unbranched activated fatty acids 16–22 carbons in length (Izai et al., 1992). ACAD-9 has optimal activity toward C16:1-, C18:1-, C18:2-, and C22:6-CoA (Ensenauer et al., 2005).

Long-chain acyl-CoA dehydrogenase (LCAD) is located in the matrix of mitochondria. Before discovery of VLCAD and ACAD-9, LCAD was believed to be responsible for initiation of β-oxidation in mitochondria of all activated long-chain fatty acids. However, with the discovery of the two membrane-associated dehydrogenases, the role of LCAD has come into question. Its activity towards palmitoyl-CoA is tenfold lower than that for VLCAD (Izai et al., 1992). LCAD has been implicated in the initial cycles of β-oxidation of activated branched-chain fatty acids (Mao et al., 1995; Wanders et al., 1998) that are not substrates for VLCAD. Targeted disruption in mice of the gene coding for LCAD revealed profound abnormalities, including frequent gestational loss, severely reduced fasting tolerance with hepatic and cardiac lipidosis, hypoglycemia, elevated serum free fatty acids, and nonketotic hypoglycemia (Kurtz et al., 1998), indicative of an essential function for LCAD beyond its role in branched-chain fatty acid oxidation. The work of Eder et al. (1997) and Le et al. (2000) suggest that LCAD is important for oxidation of both saturated and unsaturated fatty acyl-CoA esters of 10–14 carbons chain length, which may be native or result from chain-shortening initiated by VLCAD, ACAD-9, or peroxisomal β-oxidation.

Fatty-acyl 2-enoyl-CoA esters generated by the action of acyl-CoA dehydrogenases are further metabolized by the mitochondrial trifunctional protein (MTP), located in or associated with the mitochondrial inner membrane (Uchida et al., 1992), or with the soluble enzymes enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase located in the mitochondrial matrix. Each of these three enzyme activities also resides on the MTP. Products of the long-chain acyl-CoA esters reactions with VLCAD or ACAD-9 may be channeled directly to MTP for efficient cycling without release of reaction products. Evidence from a number of experimental studies suggests that fatty acyl-CoA synthetase, CPT I, CACT, CPT II, VLCAD, and MTP are spacially organized in a "metabolon" to channel long-chain fatty acids from the cytosol to the mitochondrial matrix without release of intermediates (see Eaton, 2002, for a review of arguments both for and against supramolecular organization of these proteins).

Chain-shortened (≤ 14 carbon atoms) acyl-CoA esters undergo further β-oxidation cycles in the soluble matrix of mitochondria. Successive reaction cycles of acyl-CoA dehydrogenases [LCAD, medium-chain acyl-CoA dehydrogenase (MCAD), or short-chain acyl-CoA dehydrogenase (SCAD)], 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase reduce acyl-CoA esters to acetyl-CoA, which then feeds into the tricarboxylic acid cycle, or the activated acetyl is exported as acetyl-carnitine via CACT. Accessory enzymes (*cis*-Δ^3-enoyl-CoA isomerase, 2,4-dienoyl-CoA reductase, $\Delta^{3,5}$,$\Delta^{2,4}$-dienoyl-CoA isomerase, and short-branched-chain acyl-CoA dehydrogenase) metabolize unsaturated and branched-chain acyl-CoA esters (Berg et al., 2002; Di Donato and Taroni, 2004).

Efficient β-oxidation of activated fatty acids requires pathways for electron transfer to the electron transport chain. The acyl-CoA dehydrogenases transfer electrons from their associated flavin adenine dinucleotide (FAD) to electron transfer flavoprotein (ETF). ETF gives up its electrons to ETF:coenzyme Q oxidoreductase, which in turn reduces coenzyme Q (CoQ). ETF is a soluble protein in the mitochondrial matrix, and ETF:CoQ oxidoreductase is a component of the mitochondrial inner membrane. CoQ is linked to electron transport chain complexes I, II, and III. 3-Hydroxyacyl-CoA dehydrogenases (in the matrix and associated with MTP) transfer electrons to electron transport chain complex I via redox cycling of nicotinamide adenine dinucleotide.

C. Regulation of Fatty Acid Metabolism in Skeletal Muscle

Fatty acids are a major source of fuel for ATP production in skeletal muscle. At rest, fatty acid-triglyceride substrate cycling between adipose tissue and liver provides a relatively stable source of circulating fatty acids and at the onset of exercise. Free fatty acids from this substrate cycle can be used as a buffer to supply additional energy to skeletal muscle (Rasmussen and Wolfe, 1999). However, neutral lipid stored in muscle provides a ready source of fatty acids for β-oxidation and is perhaps quantitatively more important than exogenous fatty acids, at least in the initial phase of muscle activity (Dyck and Bonen, 1998). Indeed, the processes of intracellular fatty acid esterification and β-oxidation are dynamic and occur simultaneously. Hormonal influences also impact fatty acid metabolism in muscle. For example, insulin increases free fatty acid entry into skeletal muscle, and increases triacylglycerol synthesis while decreasing the rate of β-oxidation (Dyck et al., 2001). Adiponectin and leptin both increase the rate of fatty acid oxidation and decrease muscle lipid content (Dyck et al., 2006).

Control of β-oxidation flux appears to be largely at the level of entry of acyl groups to mitochondria. Although skeletal muscle has a relatively limited capacity for de novo lipogenesis, it does synthesize malonyl-CoA, a potent inhibitor of CPT I. Malonyl-CoA concentration rises in response to insulin, which may at least in part account for the shift from oxidation to triacylglycerol synthesis in skeletal muscle in response to insulin. The malonyl-CoA response to insulin is mediated by glucose binding to acetyl-CoA carboxylase. Glucose is an allosteric activator of this enzyme. Conversely, malonyl-CoA concentration decreases in response to adiponectin. A proteolytic cleavage product of adiponectin, gACRP30, activates AMP-activated protein kinase (AMPK), a fuel-sensing enzyme present in most mammalian tissues. AMPK phosphorylates and inhibits acetyl-CoA

carboxylase and phosphorylates and activates malonyl-CoA decarboxylase, leading to a decrease in the concentration of malonyl-CoA (Tomas et al., 2002).

The putative plasma membrane fatty acid transporter FAT/CD36 also is associated with the mitochondrial outer membrane (Bezaire et al., 2006), and coprecipitates with CPT I in human skeletal muscle (Schenk and Horowitz, 2006). Inhibition of FAT/CD36 translocase activity with the specific inhibitor sulfo-N-succimidyloleate reduced palmitate oxidation by 95% and palmitoylcarnitine oxidation by 92% in purified human skeletal muscle mitochondria (Bezaire et al., 2006). Octanoate oxidation and CPT I activity were not inhibited under the same experimental conditions. In normal humans, whole-body fat oxidation rates and palmitate oxidation rates in isolated mitochondria progressively increased during aerobic exercise, and the latter was positively correlated with a 63% increase in mitochondrial FAT/CD36 protein content in mitochondria (Holloway et al., 2006). These observations are consistent with a vital role for FAT/CD36 in entry of long-chain fatty acids into mitochondria; FAT/CD36 may provide an additional layer of regulation (in addition to malonyl-CoA concentration) for entry of long-chain fatty acids into mitochondria.

In addition to CPT I, the enzymes of the β-oxidation cycle may participate in regulation of flux through the pathway, through substrate availability and product inhibition. In particular, the redox state of nicotinamide adenine dinucleotide (NAD^+/NADH) and ETF may significantly influence the activities of the dehydrogenases of the cycle. The relative concentrations of acetyl-CoA and coenzyme A may alter the activity of the 3-ketoacyl-CoA thiolases, but the ratio of these metabolites also will be regulated by export of acetyl groups from mitochondria as esters of carnitine. Sharing of control between CPT I and other enzymes allows for flexible regulation of metabolism and the ability to rapidly adapt β-oxidation flux to differing requirements in skeletal muscle and other tissues (Eaton, 2002).

III. NEUROMUSCULAR DISORDERS ASSOCIATED WITH FATTY ACID METABOLISM

A. Peroxisomal Disorders

Peroxisomal disorders are divided into two major categories (Table 48.1), disorders of peroxisome assembly and disorders resulting from a single enzyme or transporter deficiency (Moser et al., 1995;

TABLE 48.1
Genetically Determined Peroxisomal Disorders Associated with Fatty Acid Metabolism

Category	Disorder
Peroxisomal assembly deficiency	Zellweger syndrome
	Infantile Refsum disease
	Neonatal adrenoleukodystrophy
	Rhizomelic chondrodysplasia punctata type 1
Single enzyme/transporter deficiency	Adult Refsum disease (phytanoyl-CoA hydroxylase deficiency)
	X-linked adrenoleukodystrophy
	Acyl-CoA oxidase deficiency
	D-Bifunctional enzyme deficiency
	3-Ketoacyl-CoA thiolase deficiency
	α-Methyl-CoA racemase deficiency
	Alkyl dihydroxyacetone phosphate synthase deficiency (rhizomelic chondrodysplasia punctata type 2)
	Dihydroxyacetone phosphate acyltransferase deficiency (rhizomelic chondrodysplasia punctata type 3)

Source: From Moser, A.B., et al. (1995). *J. Pediatr. 127*:13–22.

Watkins et al., 1995). In disorders of peroxisome assembly, the organelle fails to form normally and multiple functions are lost.

a. Peroxisome Assembly Disorders

This group of diseases is characterized by total absence of peroxisomes (Zellweger syndrome) or loss of partial functions of peroxisomes due to inability to import required proteins (neonatal adrenoleukodystrophy, infantile Refsum disease, and rhizomelic chondrodysplasia punctata type 1). These diseases are characterized by cellular accumulation of metabolites that are normally metabolized by peroxisomes (very-long-chain fatty acids, phytanic acid, and pristanic acid), or the inability to synthesize plasmalogens (rhizomelic chondrodysplasia punctata type 1 [RCDP1]).

Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease are referred to as the "Zellweger spectrum," and whose phenotypes represent a continuum of disease severity: Zellweger syndrome is the most severe phenotype, neonatal adrenoleukodystrophy is intermediate in severity, and infantile Refsum disease is the mildest form of the disease spectrum (Weller et al., 2003). Neurological symptoms in the Zellweger spectrum include severe hypotonia, seizures, developmental delay, and development of sensorineural hearing loss. Biopsy and autopsy findings include demyelination and loss of nerve fibers in infantile Refsum disease and diffuse white matter degeneration in neonatal adrenoleukodystrophy (Thomas and Goebel, 2005). The degree of neurologic impairment ranges from individuals with complete absence of psychomotor development to patients with the ability to learn to walk and communicate (Weller, 2003). The most severely affected patients die within the first months after birth, while patients at the mild end of the spectrum survive into the fourth or fifth decade of life. The milder forms of the disease may be misdiagnosed and only become apparent after careful genetic analysis.

The clinical phenotype of RCDP1 includes severe skeletal abnormalities characterized by rhizomelia with severe shortening and disturbed ossification of the proximal limbs. Neurologic symptoms include hypotonia and moderate-to-severe psychomotor retardation. Survival generally is up to several years.

Proteins necessary for peroxisomal biogenesis, including matrix protein import, membrane biogenesis, organelle proliferation and inheritance, are called peroxins, and the genes coding for these proteins are referred to as *PEX* genes (Distel et al., 1996). The known characteristics and functions of these proteins have been reviewed in detail by Weller et al. (2003). Mutations in 12 of these genes are known to cause defects in peroxisome biogenesis (Wanders and Waterham, 2006). Mutations in 11 of these genes (*PEX*1, 2, 3, 5, 6, 10, 13, 14, 16, 19, and 26) have been implicated in the Zellweger spectrum of peroxisomal biogenesis disorders.

RCDP1 results from a mutation in the *PEX*7 gene. Peroxisomal matrix proteins are synthesized on free cytosolic ribosomes and imported into peroxisomes. These proteins contain in their amino acid sequence a peroxisomal targeting sequence (PTS), of which there are two types, PTS1 and PTS2. Most imported proteins contain the PTS1 sequence, but a small number contain the PTS2 sequence. *PEX*7 encodes a peroxin protein required for import of proteins with a PTS2 sequence. In humans, alkyl dihydroxyacetone phosphate synthase, phytanoyl-CoA hydroxylase, and peroxisomal 3-ketoacyl-CoA thiolase contain a PTS2 sequence (Ofman et al., 1998). Thus, in RCDP1, these proteins cannot be imported into peroxisomes. Activity of acyl-CoA:dihydroxyacetone phosphate acyltransferase also is low in RCDP1. RCDP1 patients are deficient in plasmalogens and have an increased plasma phytanic acid concentration (de Vet et al., 1998).

b. Disorders Associated with Single Enzyme Deficiencies in Peroxisomes

i. Adult Refsum Disease

Adult Refsum disease (heredopathia atactica polyneuritiformis) is a rare autosomal recessive disorder of fatty acid oxidation characterized clinically by retinitis pigmentosa, blindness, anosmia,

deafness, cerebellar ataxia, and peripheral polyneuropathy (Weinstein, 1999; Wierzbicki et al., 2002). Accumulation of phytanic acid in adult Refsum's disease was first recognized by Klenk and Kahlke (1963). The age of onset varies from late childhood to late adulthood. The metabolic error in many cases of adult Refsum disease lies in a failure of the conversion of phytanoyl-CoA to α-hydroxyphytanoyl-CoA, which leads to a widespread tissue storage of phytanic acid (Steinberg et al., 1967, Herndon et al., 1969; Mize et al., 1969; Yao and Dyck, 1987). Missense mutations and deletions in the *PHYH* gene on chromosome 10p13 are responsible for absence of phytanoyl-CoA hydroxylase activity (Jansen et al., 1997; Mihalik et al., 1997). Some cases of adult Refsum disease also result from mutations in the *PEX*7 gene (van den Brink et al., 2003). Adult Refsum disease may also be caused by mutations in the gene coding for sterol carrier protein-2 (SCP-2; Mukherji et al., 2002). SCP-2 is a peroxisomal accessory protein that facilitates phytanoyl-CoA hydroxylation by maintaining its solubility and by retarding its hydrolysis (Mukherji et al., 2002). Adult Refsum disease is distinguished from infantile Refsum disease in that the latter is a peroxisomal biogenesis disorder (also called a multiple peroxisomal function disorder) involving abnormalities in phytanic acid and very-long-chain fatty acid metabolism and plasmalogen synthesis (Bader et al., 2000).

Treatment with diets low in phytanic acid reduces plasma phytanic acid concentration and brings about significant improvement in peripheral nerve function; however, full restoration of function is seldom achieved (Steinberg, 1989). Plasma exchange is an option when dietary control is inadequate (Winters et al., 2000; Wills et al., 2001). Plasma exchange has been shown to be particularly useful for rapidly lowering plasma phytanic acid levels during acute attacks and may be useful as a maintenance therapy (Weinstein, 1999).

In addition to the α-oxidation pathway for degradation in peroxisomes, phytanic acid also can be degraded to a limited extent by ω-oxidation in microsomes, followed by cycles of β-oxidation in peroxisomes. Degradation of phytanic acid by this pathway has been observed in normal humans and in patients with adult Refsum disease, with excretion of 3-methyladipic acid (Wierzbicki et al., 2003). Pharmacological stimulation of microsomal ω-oxidation conceivably could provide an alternate treatment option for patients with Refsum disease.

Mitochondria appear to be a major target of pathology associated with accumulation of phytanic acid. In isolated rat brain mitochondria, phytanic acid dissipated membrane potential and released cytochrome c (Reiser et al., 2006). Astrocytes from rat brain exposed to phytanic acid die within a few hours (Schönfeld et al., 2006). Death is preceded by depolarization of mitochondria, and an increase in cytosolic Ca^{2+} concentration. In both isolated mitochondria and intact astrocytes phytanic acid increased generation of reactive oxygen species. Bunik et al. (2006) observed that phytanoyl-CoA is a potent inhibitor of purified mitochondrial pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Indeed, phytanoyl-CoA was seen to be ten times more potent an inhibitor than palmitoyl-CoA. These authors suggest that the neuropathology seen in Refsum disease may be at least in part due to inhibition of mitochondrial metabolism at the level of these enzymes. They further point out, however, that *in vivo* these enzymes are located in the mitochondrial matrix, and that a means to achieve a sufficiently high concentration of phytanoyl-CoA in this organelle has not been demonstrated.

ii. Deficiency of Enzymes of Very-Long-Chain Fatty Acid Oxidation

Complementation analysis has identified patients with three distinct genotypes associated with deficiency of acyl-CoA oxidase, D-bifunctional protein, or 3-ketoacyl-CoA thiolase in the pathway of very-long-chain fatty acyl-CoA oxidation (Watkins et al., 1995). The clinical manifestations of these disorders are similar to those of the peroxisome assembly disorders (Zellweger syndrome and neonatal adrenoleukodystrophy), but differ from those of X-linked adrenoleukodystrophy (Watkins et al., 1995).

iii. X-Linked Adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD) is an inherited neurodegenerative disease with variable phenotypes. The most frequent phenotypes are childhood cerebral X-ALD with inflammatory cerebral

demyelination, and adult-onset adrenomyeloneuropathy, a slowly progressive form that affects mainly spinal cord and peripheral nerves of the limbs (Oezen et al., 2005). Adreno-cortical insufficiency is common to both forms. The childhood form is more severe. It affects primarily young males, is rapidly progressive, leading to death resulting from the central nervous system lesions (Powers et al., 2005).

Biochemically, the hallmark of X-ALD is increased presence of saturated, unbranched, and very-long-chain fatty acids (C26:0 and C24:0) in plasma and tissues, particularly in the cholesterol ester and ganglioside fractions of brain white matter and adrenal cortex. Powers et al. (2005) suggest that pathogenesis occurs in two stages: a primary defect in myelination (due to incorporation of very-long-chain fatty acids into myelin gangliosides and phospholipids and the proteolipid protein) that destabilizes myelin and leads to its spontaneous breakdown, and a secondary inflammatory demyelination process. X-ALD is caused by mutations in the *ABCD1* gene that encodes the peroxisomal membrane protein ALDP (Mosser et al., 1993). This protein belongs to a family of ATP-binding cassette membrane transport proteins, of which four are present in the peroxisomal membrane. X-ALD was initially thought to be due to a deficiency of very-long-chain acyl-CoA synthetase, because this enzyme activity is low or absent in ALD patients. However, recent evidence suggests that normally there is a physical interaction between very-long-chain acyl-CoA synthetase and ALDP in the peroxisomal membrane (Makkar et al., 2006). Loss of this interaction may cause dysfunction of very-long-chain acyl-CoA synthetase in X-ALD.

Dietary therapy has been considered in treatment of X-ALD. Kishimoto et al. (1980) have shown that hexacosanoic acid (C26:0) given orally appeared in brain cholesterol esters of an X-ALD patient, demonstrating that diet can contribute to the very-long-chain fatty acid load of X-ALD patients. Very-long-chain fatty acids are for the most part minor components of dietary lipids and usually are not reported separately in fatty acid analyses of foods. However, Brown et al. (1982) found microgram quantities of C26:0 in single serving amounts of a variety of common foods such as French fries, orange juice, bananas, low-fat chocolate milk, and yogurt. Peanut oil had the highest concentration at 10.42 μg/tsp. Although dietary therapy may be useful, a diet that sharply reduced the intake of saturated very-long-chain fatty acids did not alter plasma C26:0 concentrations (Brown et al., 1982).

Dietary supplement therapy with Lorenzo's Oil, a 4:1 mixture of glyceryl trioleate and glyceryl trierucate, has shown some benefit in neurologically asymptomatic patients (Moser et al., 2005). Erucic acid (C22:1) inhibits chain elongation of saturated fatty acids. Lorenzo's Oil was shown to normalize the concentrations of saturated very-long-chain fatty acids in plasma in most patients with X-ALD (Rizzo et al., 1989). In the study by Moser et al. (2005), asymptomatic patients treated with Lorenzo's Oil and a restricted fat diet showed a reduction of plasma C26:0, that correlated with reduced risk of developing brain structural abnormalities identified by magnetic resonance imaging. Earlier single-arm clinical trials, however, led to the consensus that Lorenzo's Oil did not alter the rate of progression significantly in patients who were already neurologically symptomatic when therapy was initiated (Moser et al., 2005). Lorenzo's Oil therapy may slow the progression of X-ALD patients with the milder adrenomyeloneuropathy form of the disease (Moser, 2006).

Very-long-chain fatty acids are substrates for hepatic microsomal ω-oxidation. ω-Oxidation is catalyzed by cytochrome P450 enzymes. Two P450 enzymes, CYP4F2 and CYP4F3B participate in ω-oxidation of very-long-chain fatty acids, and the hydroxylated acids are further metabolized to dicarboxylic acids by cytochrome P450-mediated reactions (Sanders et al., 2006). Thus, an alternative or adjunct treatment for X-ALD may involve pharmacological stimulation of the microsomal ω-oxidation pathway as a means to reduce the accumulation of very-long-chain fatty acids.

iv. α-Methylacyl-CoA Racemase Deficiency

Two patients with adult-onset sensory motor neuropathy and one child diagnosed with Nieman–Pick type C were examined for biochemical abnormalities and found to have elevated plasma pristanic acid (Ferdinandusse et al., 2000). Pristanoyl-CoA, whether derived from dietary pristanic acid or

phytanic acid, occurs in two stereochemical configurations, 2R and 2S. The 2R configuration must be converted, by α-methylacyl-CoA racemase (AMACR), to the 2S configuration before it can be further metabolized in peroxisomes. None of these patients had detectable α-methylacyl-CoA racemase activity in cultured fibroblasts. Sequence analysis of *AMACR* cDNA from the patients identified two different mutations that are likely to cause the disease.

v. Rhizomelic Chondrodysplasia Punctata Types 2 and 3

Rhizomelic chondrodysplasia punctata types 2 (RCDP2) and 3 (RCDP3) are caused by mutations in genes coding for acyl-CoA:dihydroxyacetonephosphate acyltransferase and alkyl-dihydroxyacetonephosphate synthase, respectively (Ofman et al., 1998; de Vet et al., 1998). Patients with these disorders were identified as not belonging to the same complementation group as those with RCDP1. However, patients with RCDP2 and RCDP3 display severe clinical abnormalities similar to those seen with RCDP1 (de Vet et al., 1998).

B. Genetic Disorders of Mitochondrial Fatty Acid Oxidation

Efficient mitochondrial fatty acid oxidation is dependent on proper functioning of three general pathways, the carnitine cycle (including carnitine entry into cells), the β-oxidation spiral (including electron transfer to the respiratory chain), and the respiratory chain. Mutations in nuclear genes coding for transport proteins and enzymes of the carnitine cycle and β-oxidation have been identified that cause metabolic diseases of skeletal muscle and/or nerve (Table 48.2). Abnormalities in the respiratory chain are due to defects in either the nuclear or the mitochondrial genomes.

a. Disorders of the Carnitine Cycle

This group of disorders includes primary systemic carnitine deficiency, CACT deficiency, and CPT II deficiency. Liver CPT I deficiency also belongs to this group of disorders, but has no muscle or nerve involvement and will not be considered here. No defects in the gene coding for the muscle form of CPT I have been identified at this time.

TABLE 48.2
Genetic Diseases Associated with Mitochondrial Fatty Acid Metabolism in Skeletal Muscle and Nerve

Type	Deficient Protein or Enzymatic Activity
Transporters	Carnitine transporter (OCTN2)
	Carnitine-acylcarnitine translocase (CACT)
Enzymes of the mitochondrial inner membrane	Carnitine palmitoyltransferase II (CPT II)
	Very-long-chain acyl-CoA dehydrogenase (VLCAD)
	Mitochondrial trifunctional protein (MTP)
	Electron transfer flavoprotein (ETF)/ETF:coenzyme Q (CoQ) oxidoreductase
Enzymes of the mitochondrial matrix	Medium-chain acyl-CoA dehydrogenase (MCAD)
	Short-chain acyl-CoA dehydrogenase (SCAD)
	Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD)
	Medium-chain 3-ketoacyl-CoA thiolase (MCKAT)

Source: Modified from Vockley, J., and Whiteman, D.A.H. (2002). *Neuromuscul. Disord. 12*:235–246; Gregersen, N., et al. (2004). *Eur. J. Biochem. 271*:470–482.

i. Primary Systemic Carnitine Deficiency

A sufficiently high concentration of intracellular carnitine is necessary for efficient entry of activated long-chain fatty acids into mitochondria. Primary systemic carnitine deficiency is a rare disorder resulting from molecular defects in the gene coding for the high-affinity carnitine transporter OCTN2. OCTN2 is the primary mediator of carnitine transport into most tissues, including skeletal muscle and nerve. Typically, carnitine concentrations are much higher in tissues such as skeletal muscle and heart than in the circulation, owing to the highly efficient concentrating power of OCTN2. Carnitine is synthesized in mammals at a rate that is considered adequate (e.g., it is not considered an essential nutrient) for normal metabolic function. Carnitine is synthesized primarily in liver and kidney, but is not synthesized in skeletal muscle and heart. Carnitine balance is maintained by dietary intake and endogenous synthesis and active renal reabsorption of carnitine, such that modest loss by renal excretion equals synthesis plus dietary accrual. Renal reabsorption of carnitine is mediated at the apical membrane by OCTN2. Typically 90%–98% of filtered carnitine is reabsorbed by normal humans. In the absence of OCTN2 carnitine is not effectively reabsorbed, leading to depletion of circulating carnitine. In addition, tissues dependent on OCTN2 for carnitine transport, including skeletal muscle and heart, fail to concentrate carnitine from the circulation. The combination of low circulating carnitine concentration (typically 5%–20% of normal) and the inability to concentrate carnitine in tissues leads to a severe impairment of long-chain fatty acid oxidation, particularly in skeletal muscle and heart.

Patients typically present early in life with hypoketotic hypoglycemia, hepatic encephalopathy, skeletal and cardiac myopathy (Longo et al., 2006), and more rarely with peripheral neuropathy (Makhseed et al., 2004). Treatment includes oral carnitine supplementation, low-fat diet, and avoidance of fasting.

ii. Carnitine-Acylcarnitine Translocase (CACT Deficiency)

CACT deficiency is a severe, life-threatening disease that manifests in early infancy with hypoketotic hypoglycemia, cardiomyopathy, liver failure, and muscle weakness (Iacobazzi et al., 2004a). Diagnostic features that distinguish CACT deficiency from other disorders of fatty acid oxidation include a normal ketone response after administration of medium-chain triglycerides (indicating that the disease is specific for long-chain fatty acids), relatively few fatty acid metabolites in urine during fasting (unlike in defects of the β-oxidation spiral), and an elevation of plasma long-chain acylcarnitine ester concentrations (consistent with a defect distal to CPT I) (Hsu et al., 2001). Relatively few of the reported cases have had a mild phenotype compatible with long-term survival; the majority of cases have had a severe phenotype with cardiomyopathy and death in early childhood. Treatment with carnitine supplementation and formula containing medium-chain triacylglycerols (with minimal long-chain triacylglycerols) has been effective in some cases (Iacobazzi et al., 2004b).

iii. Carnitine Palmitoyltransferase II (CPT II) Deficiency

CPT II deficiency occurs in three forms: neonatal, infantile, and adult. The neonatal and infantile forms are severe and mainly involve liver and heart but without skeletal muscle involvement. The adult form is primarily associated with skeletal muscle symptoms, including recurrent attacks of myalgias and muscle stiffness and weakness, and often associated with myoglobinuria (Bonnefont et al., 1999). Attacks last from several hours to several weeks. Rhabdomyolysis may be severe enough to precipitate renal failure or respiratory insufficiency. There is no liver or heart involvement in the adult form of CPT II deficiency. Symptoms usually appear after prolonged exercise, fasting, or other metabolic stress. Age of onset is usually 6–20 years. Remarkable clinical signs or markers of the disease (elevated serum creatine kinase and transaminase) only appear during episodic attacks. Effective prevention of attacks may be accomplished with a high-carbohydrate, low-fat diet with frequent and regularly-scheduled meals, and by avoiding metabolic stress associated with, for example, cold exposure or prolonged exercise (Di Donato and Taroni, 2004).

b. Disorders of the β-Oxidation Spiral and Electron Transfer to the Respiratory Chain

Generally, mitochondrial fatty acid oxidation disorders have several common clinical features, including metabolic decompensation during fasting, hypoketotic hypoglycemia, and acute or chronic dysfunction of one or more fatty acid-dependent tissues, and in particular, skeletal muscle, heart and liver (Kamijo et al., 1997). Disorders with skeletal muscle and/or nervous system involvement are summarized below.

i. Very-Long-Chain Acyl-CoA (VLCAD) Deficiency

Three phenotypes of VLCAD deficiency have been described: (1) a severe form presenting in infancy or early childhood with high incidence of cardiomyopathy, hypoglycemia, and mortality; (2) a milder childhood form characterized by hypoketotic hypoglycemia and low mortality; and (3) a milder adult form with isolated skeletal muscle involvement, rhabdomyolysis, and myoglobinuria usually triggered by exercise or fasting (Andresen et al., 1999; Bartlett and Pourfarzam, 2002; Gregersen et al., 2004). In the milder childhood and adult forms, some residual enzyme activity is present and in the adult form this activity is sufficient to maintain normal oxidation of fatty acids at rest, but cannot increase the rate of fat oxidation above basal levels during exercise (Ørngreen et al., 2004). Evidence of a clear correlation of genotype with disease phenotype has been demonstrated (Andresen et al., 1999). Management strategies for VLCAD deficiency are similar to those for CPT II deficiency, and include avoidance of fasting, dietary cornstarch and/or medium-chain triacylglycerol and carnitine supplementation (Nyhan and Ozand, 1998).

ii. Mitochondrial Trifunctional Protein (MTP) Deficiencies

The MTP is a hetero-octamer consisting of four α subunits and four β subunits. These subunits are coded by two contiguous, coordinately expressed genes (*HADHA* and *HADHB*) on chromosome 2p23 (Bartlett and Pourfarzam, 2002). Long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity resides on the C-terminal end of the α subunits. A common mutation in the gene coding for the α subunit, resulting in substitution of glutamine for glutamic acid at position 474 of the protein, is responsible for deficiency of this enzymatic activity, with no effect on the other two MTP activities. In some patients, LCHAD deficiency was associated with impaired respiratory chain activity, thought to be due to inhibition particularly of respiratory chain complex I enzymes by fatty acid oxidation intermediates that accumulate due to the primary LCHAD deficiency (Tyni and Pihko, 1999). The *HADHB* gene encodes long-chain 3-ketoacyl-CoA thiolase (LCKAT). Two patients have been reported with isolated LCKAT deficiency (Das et al., 2006). Molecular analysis revealed compound heterozygosity for 185G→A (R62H) and 1292T→C (F431S) mutations in the *HADHB* gene in these patients. Loss or deficiency of all three enzyme activities are due to mutations in either the α or β subunits, resulting in MTP complex instability (Spiekerkoetter et al., 2004b). Clinically, the combined deficiency is similar to but more severe than the isolated LCHAD deficiency. Like CPT II deficiency and VLCAD deficiency, clinical presentation is heterogeneous. Patients identified in the neonatal period present with hypoglycemia, whereas the adolescent or adult variant of the disease is characterized predominantly by myopathy. Patients presenting during adolescence or adulthood generally have some residual enzyme activity. A small number of patients with adult-onset, relatively benign disease, present with recurrent exercise-induced rhabdomyolysis similar to that seen in adult form of CPT II deficiency (Schaefer et al., 1996). An additional feature of both isolated LCHAD deficiency and the combined deficiency is peripheral neuropathy (Schaefer et al., 1996; Tyni and Pihko, 1999; Di Donato and Taroni, 2004; Spiekerkoetter et al., 2004a).

Treatment of MTP deficiency includes avoidance of fasting and a high-carbohydrate diet with medium-chain triacylglycerols replacing all but essential long-chain triacylglycerols (Bartlett and Pourfarzam, 2002; Angdisen et al., 2005).

iii. Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

MCAD deficiency was the first acyl-CoA dehydrogenase deficiency to be discovered (in 1983) and is the most common of the fatty acid oxidation disorders. The phenotypic expression of this disease includes intermittent hypoketotic hypoglycemia, myopathy, cardiomyopathy, sudden infant death syndrome, and hyperammonemia. (Nyhan and Ozand, 1998; Vockley and Whiteman, 2002). Muscle weakness and lipid accumulation in muscle, C6 to C10 dicarboxylic aciduria during recurrent hypoglycemic attacks, and mild carnitine deficiency have been observed. Attacks generally occur in response to fasting or infections associated with reduced food intake and increased energy requirements (Rinaldo et al., 2002). The disease is often recognized within the first two years of life, although some affected individuals may remain asymptomatic into adulthood. The most common genetic alteration responsible for MCAD deficiency is the 985A→G mutation of the MCAD gene (*ACADM*), which changes a highly conserved lysine at position 304 into a glutamate, impairing assembly of the tetrameric mature protein and decreasing its stability (Yokota et al., 1992). Eighty percent of patients are homozygous for this mutation, 18% are compound heterozygous with this mutation on one allele and a rare disease-associated sequence variation on the other allele, and only about 2% carry other sequence variations on both alleles (Gregersen et al., 2004). The outcome for these patients is very good once the correct diagnosis is made. Treatment regimens include foremost avoidance of fasting. Carnitine supplementation may be beneficial.

iv. Medium-Chain 3-Ketoacyl-CoA Thiolase (MCKAT) Deficiency

MCKAT catalyzes cleavage of medium- to short-chain 3-ketoacyl-CoA esters with release of acetyl-CoA in the mitochondrial matrix. To date only one case, a neonate who died shortly after presentation at 2 days of age, has been reported (Kamijo et al., 1997). Clinical features of this infant included vomiting, dehydration, metabolic acidosis, liver dysfunction, and terminal rhabdomyolysis with myoglobinuria. Laboratory analyses revealed ketotic lactic acidosis and C_6 to C_{12} dicarboxylic aciduria, and reduced MCKAT protein concentration in fibroblasts (to 60% of normal), as determined by immunoprecipitation.

v. Short-Chain Acyl-CoA Dehydrogenase (SCAD) Deficiency

SCAD catalyzes the first step in mitochondrial β-oxidation of activated fatty acids four to six carbons in length. Patients present with a wide range of clinical symptoms, including hypoglycemia, metabolic acidosis, vomiting, drowsiness, myopathy, hypotonia, and seizures (Pedersen et al., 2003; Seidel et al., 2003). Axonal neuropathy was reported in two unusual cases (Kurian et al., 2004). Some individuals bearing the genetic alterations associated with SCAD deficiency remain asymptomatic (Bok et al., 2003). Ethylmalonic aciduria is a common finding in this disease, but is nonspecific (Seidel et al., 2003).

Molecular genetic analysis has uncovered several rare pathogenic mutations that result in undetectable enzymatic activity in homozygous patients (Corydon et al., 2001; Seidel et al., 2003). In addition, two common susceptibility gene variations [625G→A (G185S) and 511C→T (R147W)] are associated with varying degrees of decreased enzyme activity when present as double heterozygotes (a pathogenic mutation in one allele and susceptibility gene variation in the other allele) or with one susceptibility gene variation in each allele (Corydon et al., 2001). Molecular biochemical analysis of mutant proteins has led to the conclusion that some variants of SCAD (R22W, G68C, W153R, and R359C) were susceptible to severe misfolding, whereas others (R147W, G185S, and Q341H) were only mild folding variants (Pedersen et al., 2003). These mutant proteins were susceptible to proteolytic degradation by mitochondrial proteases, and formed aggregates of nonnative conformers.

vi. Short-Chain L-3-Hydroxyacyl-CoA Dehydrogenase (SCHAD) Deficiency

SCHAD deficiency is a rare disorder with highly variable presentation. Of the few cases reported, diagnosis was made in infancy, early childhood, or adolescence. Those identified early in life presented with typical liver-associated abnormalities including fasting-induced vomiting, ketosis, and hypoglycemia (Bartlett and Pourfazam, 2002). Hyperinsulinism was noted in one infant (Clayton et al., 2001). One adolescent presented with recurrent myoglobinuria, hypoketotic hypoglycemic encephalopathy, and hypertrophic cardiomyopathy (Tein et al., 1991). In this case, skeletal muscle SCHAD activity was approximately 35% of normal, with normal activity in cultured fibroblasts. Mutation analysis in the infant with hyperinsulinism revealed a homozygous mutation (773C→T) resulting in a P258L enzyme with no catalytic activity (Clayton et al., 2001). Mutation analysis was not done or was inconclusive in the other reported cases (Rinaldo et al., 2002).

vii. Electron Transfer Flavoprotein (ETF)/ETF:Coenzyme Q (ETF:CoQ) Oxidoreductase Deficiencies

Molecular defects in either the α or β subunits of ETF or in ETF:CoQ oxidoreductase are responsible for the group of diseases commonly referred to as glutaric aciduria type 2 or multiple acyl-CoA dehydrogenase deficiency. At least, nine mitochondrial acyl-CoA dehydrogenases transfer electrons to the respiratory chain via ETF and ETF:CoQ oxidoreductase. Three different clinical phenotypes have been described (Schiff et al., 2006). Two present in the neonatal period and are most severe. Congenital abnormalities, dysmorphic features reminiscent of peroxisomal disorders, and acute metabolic decompensation are characteristics of the first type. Patients of the second type lack congenital anomalies and may survive beyond infancy. The third type, also referred to as ethylmalonic-adipic aciduria, includes patients with a milder form of the disease, in which residual enzyme activity is present. These patients have muscle weakness and may not be diagnosed until early adulthood. They are characterized by recurrent, potentially life-threatening episodes of vomiting, hypoglycemia, hepatomegaly, and myopathy (Rinaldo et al., 2002). A relationship between genotype and clinical phenotype has been demonstrated (Olsen et al., 2003). Treatment of these disorders includes restriction of dietary fat and protein, and supplementation with glycine and carnitine.

A variant of glutaric aciduria type 2 is a true multiple acyl-CoA dehydrogenase deficiency that is responsive to riboflavin supplementation. The vitamin riboflavin is a precursor for the electron acceptors/donors FAD and flavin mononucleotide (FMN). FAD is a tightly-bound cofactor for the mitochondrial acyl-CoA dehydrogenases, ETF, and ETF:CoQ oxidoreductase. This disorder, called riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency, apparently is due to malabsorption of riboflavin, a defect in synthesis of FAD from riboflavin, or a defect in import of FAD into mitochondria. It is thought that deficient mitochondrial FAD causes an accelerated breakdown of flavin-dependent proteins. The two major phenotypes are the infantile form with nonketotic hypoglycemia, hypotonia, failure to thrive, and acute metabolic episodes similar to Reye syndrome, and a juvenile form that includes progressive proximal myopathy with lipid storage in muscle fibers (Di Donato and Taroni, 2004). This disease is not associated with defects in genes coding for the acyl-CoA dehydrogenases, ETF or ETF:CoQ oxidoreductase. The genetic and molecular etiology is unknown, but perhaps is due to poor absorption of riboflavin, impaired delivery to tissues, or a defect in the synthesis of FAD. Oral supplementation with riboflavin dramatically reverses the course of this disease and normalizes activities of the flavin-dependent mitochondrial enzymes (Russell et al., 2003).

C. Chanarin–Dorfman Disease

Chanarin-Dorfman disease, also known as multisystem triglyceride storage disease or neutral-lipid-storage disease with ichthyosis, is a rare genetic disease characterized by ichthyosis, neutral lipid storage (as triacylglycerol droplets) in many tissues and cells, including skeletal muscle, and

a variety of neurologic manifestations, including psychomotor delay in childhood, nystagmus, ataxia, neurosensory hearing loss, microcephaly, and myopathy (Di Donato and Taroni, 2004). The disease has been found mostly in Middle Eastern, Mediterranean populations.

Mutations specific for this disease have been localized to the gene *CGI-58* (comparative-gene-identification-58) (Lefèvre et al., 2001). The protein product of this gene, CGI-58, interacts with proteins on the surface of lipid droplets that are associated with esterification and hydrolysis of fatty acids in lipid droplets, including perilipin and ADRP (Yamaguchi et al., 2004). CGI-58 recently was shown to be required for activation of adipose triglyceride lipase (ATGL) (Lass et al., 2006), itself a recently identified enzyme important for mobilization of triglycerides in adipose tissue (Zimmermann et al., 2004). ATGL is highly expressed in adipose tissue, but only weakly expressed in skeletal muscle (Zimmermann et al., 2004). Thus, it remains speculative whether lack of CGI-58-mediated activation of ATGL in skeletal muscle is responsible specifically for lipid droplet accumulation in this tissue.

D. Barth Syndrome

Characteristics of Barth syndrome include mitochondrial myopathy, dilated cardiomyopathy, cyclic neutropenia, and short stature (DiMauro and Bonilla, 2004). The disease can be fatal in infants and children due to cardiac failure or sepsis (van Werkhoven et al., 2006). Barth syndrome is caused by abnormalities in cardiolipins, acidic phospholipids highly enriched in the mitochondrial inner membrane. Cardiolipins are essential for the proper assembly and function of the respiratory chain. Cardiolipins contain four fatty acids, two each in two phosphatidylglycerol moieties. Cardiolipin biosynthesis occurs via the cytidine diphosphate-diacylglycerol pathway. Newly formed cardiolipins undergo extensive remodeling by deacylation and reacylation to produce mature forms with three or four linoleic acid moieties (Schlame et al., 2000). Tetralinoleoyl-cardiolipin (Figure 48.4) is specifically enriched in skeletal muscle and heart (Schlame et al., 2002). Synthesis of cardiolipins in fibroblasts from patients with Barth syndrome was observed to be normal, but the cardiolipin content of these cells was reduced (Vreken et al., 2000). Moreover, incorporation of linoleic acid into cardiolipins was abnormally low. A subsequent study found tetralinoleoyl-cardiolipin was very

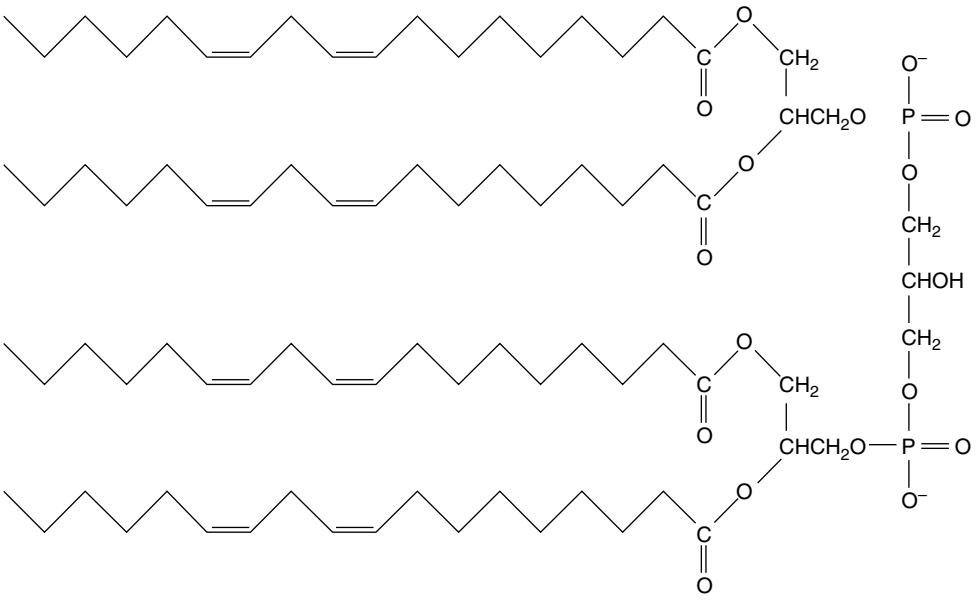

FIGURE 48.4 Structure of tetralinoleoyl-cardiolipin.

low or absent in skeletal muscle, heart, and platelets of children with Barth syndrome (Schlame et al., 2002).

The gene responsible for Barth syndrome (*TAZ*) encodes the protein tafazzin (Claypool et al., 2006). Tafazzin is thought to be involved in remodeling of cardiolipins, specifically adding linoleic acid to lysocardiolipins to produce mature and functional tetralinoleoyl-cardiolipin (Chicco and Sparagna, 2006). Lysocardiolipin has been found to be increased in cultured fibroblasts from Barth syndrome patients (van Werkhoven et al., 2006). Incubation of Barth syndrome fibroblasts with linoleic acid led to a time- and dose-dependent increase in cardiolipin concentration (Valianpour et al., 2003). Whether or not dietary treatment with linoleic acid will be useful in patients with Barth syndrome remains to be determined.

E. Diabetes and Obesity

a. Diabetic Neuropathy

One of the major complications of diabetes mellitus is peripheral neuropathy, which occurs to some degree in up to 60% of diabetic patients (Pirart, 1978; Thomas and Tomlinson, 1993). Main pathologic features of diabetic polyneuropathy include loss of myelinated and unmyelinated fibers; clusters of regenerating fibers; thickening of endoneurial blood vessels; and increased durability and rigidity of Schwann cell basal laminae (Llewelyn et al., 2005). Hyperglycemia is probably the most important initiator of the process that leads to neuropathy. Hyperglycemia causes secondary biochemical changes in the polyol pathway, nonenzymatic glycation of macromolecules, and oxidative stress. Tricyclic antidepressants are the most commonly prescribed symptomatic treatments for diabetic neuropathy, but these have little if any impact on the progression of the disease. Experimental treatments to address progression have included aldose reductase inhibitors (ARI) (to inhibit accumulation of polyols; Greene et al., 1999; Misawa et al., 2006), various botanicals and dietary supplements, including evening primrose oil, capsaicin, acetyl-L-carnitine and α-lipoic acid (Halat and Dennehy, 2003), and neurotrophic peptides (Tam et al., 2006).

Beneficial effects of dietary treatment with γ-linolenic acid (GLA) in both streptozotocin-induced diabetes and patients with distal diabetic polyneuropathy have rekindled interest in the role of essential fatty acids in the prevention and treatment of diabetic neuropathy. A defect in the pathway for synthesis of PUFA (Figure 48.5) in experimental diabetes was first recognized by Peifer and Holman in 1955. They found that diabetic animals required much higher amounts of linoleic acid to compensate their deficiency of essential fatty acids than those of normal controls. Later, an altered fatty acid profile showing increased linoleic acid and DHA as well as decreased AA was also found in central and peripheral nervous systems of diabetic animals (Holman et al., 1983; Lin et al., 1985). In human diabetes, Horrobin (1988, 1992) further demonstrated that levels of dihomo-γ-linolenic acid (DGLA) and AA were substantially reduced in patients with type 1 diabetes. These findings were confirmed in a large-scale study comprised of 319 normal controls, 224 type 1 and 364 type 2 diabetic patients (Horrobin, 1997a). As expected, the ratio of linoleic acid to DGLA + AA was elevated in plasma and RBC lipids of type 1 patients, but to a lesser degree in those of type 2 patients. Moreover, there was a marked reduction of PUFA (both n-6 and n-3 types) in RBC membranes of all patients.

Characteristic fatty acids of peripheral nerve myelin are mainly saturated and monounsaturated (Yao, 1984). A marked increase of PUFA, particularly AA, was found in endoneurial phosphatidylethanolamine of both developing and regenerating rat sciatic nerve (Yao, 1982). Furthermore, the cyclooxygenase derivatives of PUFAs have been shown to be required for normal nerve conduction (Horrobin et al., 1977). Inhibition of prostaglandin biosynthesis can slow nerve conduction and decrease action potential amplitudes. These observations suggest an important role of PUFA in peripheral nerve metabolism and function. There is ample evidence demonstrating that PUFA metabolism is altered in diabetes, possibly due to a deficiency of 6-desaturase (Figure 48.5) and partial loss of 5-desaturase (Eck et al., 1979; Poisson, 1985; Horrobin, 1988; Dang et al., 1989).

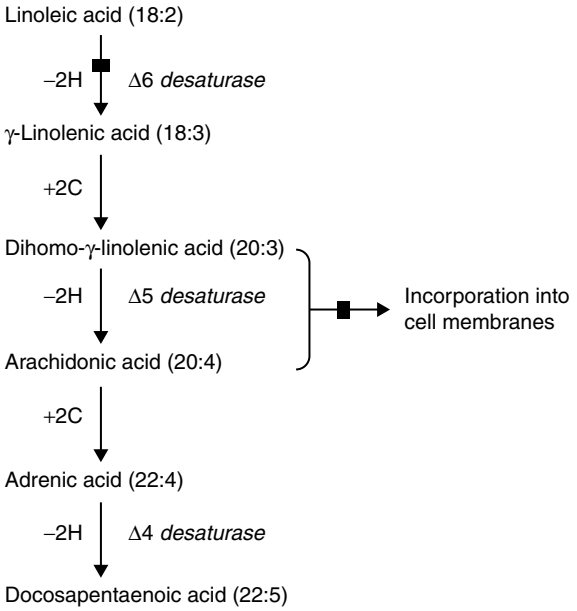

FIGURE 48.5 Proposed metabolic blocks in the elongation pathway of n-6 family of essential fatty acids in patients with diabetic neuropathy.

The accumulation of polyols in the lens is postulated to increase osmotic pressure and overhydration, leading to lens fiber disruption and to the formation of cataracts (van Heyningen, 1962; Chylack and Kinoshita, 1969). Interestingly, high-fat diets (rich in maize oil) can inhibit or delay the development of cataracts in streptozotocin-induced diabetic rats (Patterson, 1955; Patterson et al., 1965; Hutton et al., 1976). Sorbitol levels in the lenses of treated diabetic rats, however, were still substantially higher than those of normal rats, which raise doubt that sorbitol is the only cause of diabetic cataracts.

To correct the abnormal linoleic acid desaturation pathway, evening primrose oil enriched with GLA has been used to treat diabetic animals. GLA supplementation may prevent as well as normalize the impaired nerve functions (i.e., nerve blood flow, nerve conduction, and microcirculation) in streptozotocin-induced diabetic rats (Julu, 1989; Tomlinson et al., 1989; Cameron and Cotter, 1993; Stevens et al., 1993; Omawari et al., 1996). The efficacy of GLA treatment, however, depends on the source of natural oils. Evening primrose oil appears to be the choice of treatment as compared to blackcurrant, borage, and fungal oils (Dines et al., 1996). Alternatively, diabetic rats treated with AA-enriched oil also showed improvements of nerve conduction and perfusion deficits (Cotter and Cameron, 1997).

Jamal and Carmichael (1990) conducted a 6-month randomized, double-blind, parallel, placebo-controlled study with evening primrose oil in humans. When patients with distal diabetic polyneuropathy were treated with 8×500 mg twice daily (providing a daily dose of 360 mg of GLA), their clinical, neurophysiological, and thermal threshold parameters were significantly improved as compared to those treated with a placebo. A similar dietary treatment with GLA supplementation was subsequently conducted in two multicenter trials. The first trial involving 111 patients in seven centers demonstrated more favorable responses to GLA diet than that with a placebo (Keen et al., 1993). The second trial involving 293 patients in ten centers (Horrobin, 1997b) confirmed the findings of the first trial. To date, no serious adverse effects have been reported, suggesting a high degree of safety of GLA treatment. Collectively, these findings support a beneficial effect of GLA supplementation in the management of diabetic neuropathy (Horrobin, 1997a,b), but the body of evidence remains inconclusive (Halat and Dennehy, 2003).

There are multiple defects involving metabolic functions associated with diabetic neuropathy. As a result of increased flux in polyol pathway, ARIs have been applied effectively to restore nerve blood flow and conduction velocity in experimental diabetes (Cameron et al., 1994, 1996). Cameron and Cotter (1997) demonstrated that the combined ARI and evening primrose oil treatment produced a tenfold amplification of nerve conduction velocity and blood flow responses. In human trials, however, clinical improvements with ARIs have been less successful. Aldose reductase pathway inhibition in diabetic patients with moderate glycemic control resulted in small improvements in nerve conduction velocity but did not improve clinical symptoms (Greene et al., 1999; Misawa et al., 2006). Combined therapy in humans with evening primrose oil and ARIs, and/or longer-term therapy with high-dose, potent ARIs such as zenarestat may provide better outcomes.

b. Fatty Acids and Insulin Resistance in Skeletal Muscle

Insulin resistance in skeletal muscle is a major feature of obesity and type 2 diabetes mellitus. Accumulation of intracellular triacylglycerol and a low capacity to oxidize fatty acids are correlated with the presence of insulin resistance in skeletal muscle. Elevated intramuscular triacylglycerol stores may be only a marker of abnormal fatty acid metabolism. Accumulation of more reactive lipids derived from fatty acids, including long-chain acyl-CoA, diacylglycerol, and/or ceramides, may actually be responsible for impaired insulin signaling (Dyck et al., 2006).

Kelley (2005) has described an elegant model of metabolic flexibility of skeletal muscle with regard to utilization of glucose and fatty acids. During fasting, the lean, aerobically fit (metabolically flexible) individual displays a robust preference for fat oxidation in muscle with suppressed glucose oxidation, whereas in the obese, aerobically unfit (metabolically inflexible) individual, glucose oxidation is less suppressed and the preference for fat oxidation is blunted. During insulin-stimulated conditions, the metabolically flexible individual will suppress fat oxidation and utilize glucose for energy, whereas the metabolically inflexible individual is less able to stimulate glucose oxidation and thus does not substantially suppress fatty acid oxidation. Thus, the "metabolically flexible" individual has considerably more latitude to utilize glucose when it is abundant, and spare glucose when it is not. In type 2 diabetes and obesity, skeletal muscle manifests inflexibility in the transition between lipid and carbohydrate fuels (Kelley et al., 2002).

Insulin sensitivity and metabolic flexibility may result from neuroendocrine and environmental factors, or could be due to genetic, epigenetic or other mechanisms intrinsic to muscle cells. Ukropcova et al. (2005) have demonstrated that insulin sensitivity and metabolic inflexibility are at least in part programmed within muscle cells, and are retained when those cells are removed from their *in vivo* neuroendocrine environment. Skeletal muscle insulin resistance in type 2 diabetes and obesity is caused at least in part by accumulation of intracellular triacylglycerols and long-chain acyl-CoA esters. These accumulations result from an increased rate (approximately threefold) of intracellular fatty acid esterification, normal or reduced rate of fatty acid oxidation, and an increased rate (approximately fourfold) of fatty acid transport into muscle cells (Bonen et al., 2004). The increased rate of fatty acid transport is associated with an increase in sarcolemmal FAT/CD36 that resulted from increased translocation of sub-sarcolemmal protein to the membrane, and not increased expression of the FAT/CD36 gene. One alteration that may contribute to insulin resistance in skeletal muscle of obese individuals and patients with type 2 diabetes is a concurrent increase in translocation of FAT/CD36 and decrease of translocation of the glucose transporter GLUT4 to the sarcolemma.

FAT/CD36 also is present in the mitochondrial membranes and has a role in activated long-chain fatty acid translocation into mitochondria. Exercise training and diet intervention, but not diet intervention alone, increased the amount of FAT/CD36 that coprecipitated with CPT I from mitochondria of obese women, and this increase was strongly correlated with an increase in whole-body fatty acid oxidation (Schenk and Horowitz, 2006). Thus, it is conceivable that skeletal muscle insulin resistance is at least in part due to misdirection of FAT/CD36 to the sarcolemma and away from mitochondria, and that aerobic exercise could lead toward normalization of FAT/CD36 distribution,

with concomitant increase in fatty acid oxidation and decrease in triacylglycerol and acyl-CoA accumulation.

CACT activity may be a mitigating factor in insulin resistance in susceptible individuals. In obese, insulin-resistant humans, mitochondrial carnitine-carnitine and carnitine-acylcarnitine exchange rates were shown to be significantly lower than in mitochondria from normal subjects (Peluso et al., 2002). CACT mRNA levels were reduced by about 75% in affected subjects compared to controls, and the exchange rate was reduced by 50%–75%. Thus, CACT expression and activity, in addition to FAT/CD36 activity, may contribute to cytosolic accumulation of long-chain fatty acids, acyl-CoA esters, and triacylglycerols in insulin-resistant individuals. Insulin resistance in obese individuals and patients with type 2 diabetes may be at least partially overcome by administration of carnitine or acetyl-L-carnitine (Mingrone, 2004). Administration of either of these compounds increases nonoxidative glucose disposal in normal and affected humans. Carnitine and acetyl-L-carnitine may regulate trafficking of fatty acids to correctly meet energy demand, and/or may regulate synthesis of transporters, including CACT, and key enzymes involved in glycolytic and gluconeogenic pathways.

F. Nutritional Deficiencies

a. Protein-Energy Malnutrition

A reversible neuromuscular syndrome has been described in malnourished children (Dastur et al., 1979; Donley and Evans, 1989). Hypotonia, weakness, and absent stretch reflexes, and electromyographic abnormalities were observed. Histologic examination revealed decreased muscle fiber diameter, group atrophy suggestive of denervation, and variation in fiber size and rounding, suggestive of myopathic change (Dastur et al., 1979). Clear differences in this syndrome and specific vitamin deficiencies (e.g., thiamine and vitamin D deficiency) were found (Donley and Evans, 1989). Improvement in strength and reflexes was observed after adequate nutrition was restored.

b. Anorexia Nervosa

Anorexia nervosa is a psychological disorder characterized by an obsession for weight loss. Patients develop bradycardia, hypotension, hypothermia, a depressed metabolic rate, and effort intolerance (Essen et al., 1981). It is most common in adolescent and young adult females. During the early stages of anorexia nervosa, avoidance of carbohydrate and lipid leads to negative energy balance and depletion of body stores of lipids. Eventually, undernutrition becomes severe and leads to protein-energy malnutrition. Muscle mass in addition to fat stores becomes reduced. Patients with anorexia nervosa may develop a reversible metabolic myopathy with type 2 muscle fiber atrophy (McLoughlin et al., 1998). Individuals with severe weight loss (40%) develop proximal muscle weakness and a diminished lactate response to ischemic exercise (McLoughlin et al., 1998).

c. Myopathy, Wasting Syndrome, and Lipodystrophy Associated with HIV Infection

Wasting syndrome is a significant cause of morbidity and mortality in patients with type 1 human immunodeficiency virus (HIV) infection, and is considered an AIDS-defining illness (Miró et al., 1997). Clinical findings include extreme fatigue and marked amyotrophy involving proximal and distal segments of the limbs (Bélec et al., 1992). Malnourishment is an early feature of HIV infection. Wasting syndrome seems to be closely related to the reduced nutritional status. In addition to anorexia and malabsorption (Sharkey et al., 1992), intermediary metabolism is deranged. Oxidation of carbohydrates is suppressed and sensitivity to insulin increased, plasma triacylglycerols are increased, and the turnover of protein may be increased, the net result of which is a redirection of absorbed substrates into lipid rather than lean tissue (Macallan et al., 1995). Inflammatory myopathy

often accompanies the wasting syndrome. HIV-related muscle wasting is a cachectic process that affects type 2 muscle fibers, and is separate from HIV myositis and myopathy associated with AZT (zidovudine) therapy (Dalakas, 2004).

Lipodystrophy syndrome encompasses both fat accumulation and wasting. Changes include abdominal fat accumulation with fat wasting of the face, buttocks, and extremities. Both fat accumulation and loss may be present in the same individual, or occur independently. Thus, these possibly are distinct pathological processes, rather than components of a whole-body maldistribution syndrome (Day et al., 2004). Both appear linked to antiretroviral therapy and are seen in association with metabolic abnormalities in glucose and lipid metabolism.

IV. SUMMARY

Defects in metabolic pathways of fatty acid utilization or disposal can lead to many profound functional disturbances of nerve and muscle tissues. In addition to those diseases mentioned in the text, there are many other neurological disorders (e.g., hereditary motor and sensory neuropathies, Friedreich's ataxia, multiple sclerosis, epilepsy) showing fatty acid and phospholipid abnormalities. These changes are probably secondary to primary defects and the attendant secondary pathology, including oxidative stress, inflammation, and tissue degeneration/regeneration. On the other hand, proper dietary treatment with specific or restricted fatty acid(s) can be beneficial to some patients with a primary fatty acid disorder (e.g., Refsum's disease). Dietary therapy, however, mainly prevents or arrests the progress of the disease, and does not necessarily correct or replace the enzyme defect or fully restore the neurological and/or muscular functions.

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49 Fatty Acids and Psychiatric Disorders

Sarah M. Conklin, Ravinder D. Reddy, Matthew F. Muldoon, and Jeffrey K. Yao

CONTENTS

I. INTRODUCTION

Research suggests that fundamental aspects of brain function depend on the adequate consumption and thus membrane levels of polyunsaturated fatty acids (PUFAs), particularly, eicosapentaenoic acid [EPA, 20:5(n-3)], docosahexaenoic acid [DHA, 22:6(n-3)] and also arachidonic acid [AA, 20:4(n-6)]. It has been shown that by increasing the incorporation of these fatty acids into membrane phospholipids several basic properties of the membrane are improved. PUFAs are integral

components of cell membrane lipids, primarily phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol comprising 80% of total membrane phospholipids. The central nervous system is highly enriched in PUFAs, primarily AA and DHA (Salem et al., 2001). In addition to helping maintain normal membrane structure and function, PUFAs also are critical in all aspects of normal brain development and neurotransmission (Das, 2003; Casper, 2004).

Since the dynamic state of the neuronal membranes is dependent on its composition, small changes in particular PUFAs that make up phospholipids, can lead to altered receptor binding, neuro-transmission, signal transduction, and prostaglandin synthesis (Clandinin et al., 1992; Hulbert et al., 2005). Given the fact that AA and EPA function as a second messengers, deficits in membrane PUFAs may underlie many biological, physiological, and clinical phenomenon observed in psychiatric disorders. In humans, relative membrane deficiency of dietary n-3 and n-6 PUFAs has been implicated in schizophrenia (Yao, 2003; Mahadik and Yao, 2006) and in major depressive disorder (Hibbeln and Salem, 1995; Freeman, 2000; Logan, 2003; Peet and Stokes, 2005). PUFA deficiencies have also been linked to aggressive behavior and disorders of impulse control (Hallahan and Garland, 2004) and impaired cognitive function. In this chapter, our goal is to review literature linking PUFA deficits to psychiatric disorders, with particular emphasis on schizophrenia and depression as clinical research is most developed for these diagnoses. However, as research extends the relevance of PUFAs to other areas in psychiatry, we will conclude by discussing how these fatty acids are implicated in other syndromes and symptoms with psychiatric relevance.

II. SCHIZOPHRENIA

Numerous theories have been proposed aiming to conceptualize the pathophysiology of schizophrenia, focusing on neuronal maldevelopment, impaired neurotransmission, viral infections, and autoimmune dysfunction, among others. A point of convergence for these proposed mechanisms occurs at the level of the neuronal membrane, which is the structural and functional site of neurotransmitter receptors, ion channels, signal transduction, and most drug effects. The membrane also is a point where there is a natural intersection between genetic and environmental factors (Horrobin et al., 1995). Two lines of evidence demonstrate the presence of membrane fatty acid defects in schizophrenia: (1) levels of phospholipid and fatty acids in red blood cell (RBC) membranes, platelets, and postmortem brain tissue; and (2) functional measures of cerebral phosphorus metabolism.

A. Membrane Lipid Defects

a. Erythrocytes and Platelets

i. Phospholipids

Stevens (1972) first reported that, compared to that of healthy controls, the RBC membranes of schizophrenic patients is characterized by a relative excess of PS and a relative deficiency of PC and PE. These results were later replicated by Henn and Henn (1982) as well as by Tolbert et al. (1983). On the other hand, Hitzemann and Garver (1982), and Hitzemann et al. (1984) found that the deficiency in RBC-PC that was compensated by an increase of sphingomyelin (SM) or ethanolamine plasmalogens, not PS. In contrast, Sengupta et al. (1981) showed that the percentages of both PC and PS were relatively increased in RBC and platelets from schizophrenic patients. Finally, Cordasco et al. (1982), and Lautin et al. (1982), failed to show any significant changes of PC, PE, and SM.

Results of the above studies are difficult to interpret due to differences in lipid methodology, in diagnostic criteria, and current or prior treatment. Since PUFA's are major constituents of membrane phospholipids, the discrepancies of RBC membrane phospholipid abnormalities obtained from patients with schizophrenia in different studies may be explained in part by the finding (Glen et al., 1994) of a bimodal distribution of RBC PUFAs in patients, in contrast to the unimodal distribution seen in normal controls. Since treatment can affect neurochemistry it is notable that

phospholipid abnormalities have been found in medication-free schizophrenic patients (Keshavan et al., 1993a), and decreases in all four key membrane phospholipids were found in fibroblasts from neuroleptic-naive patients with schizophrenia (Mahadik et al., 1994). These latter findings suggest that phospholipid and fatty acid abnormalities may be disease related. At the very least, they are present early in the course of illness, prior to initiation of treatment. Other factors likely contribute to these discrepancies, one being that fatty acids from schizophrenic patients appear to be hydrolyzed more rapidly during freezer storage at –20°C than those of nonpatient controls. Fox et al. (2003) reported that the decay rates of schizophrenic patients' RBC PUFAs were nearly twice that of nonpatient control participants when stored at –20°C. The authors proposed that elevated phospholipase A_2 (PLA_2) activity in the patients was likely responsible for the increased degradation. Consequently, additional *in vivo* measurements of fatty acids with magnetic resonance spectroscopy may be useful to avoid the problems associated with freezer storage (Glen and Glen, 2004).

ii. Fatty Acid Composition

Fatty acid abnormalities have been found in the plasma and RBC membranes of psychotic patients. Horrobin et al. (1989) reported a significant decrease of both LA [18:2(n-6)] and AA [20:4(n-6)], and an increase of total n-3 fatty acids in the plasma of three schizophrenic patient groups from England, Scotland and Ireland (Table 49.1). Similarly a decrease of 18:2(n-6), but not of 20:4(n-6) was found in plasma of schizophrenic patients from Japan (Kaiya et al., 1991). In RBC membranes, both 20:4(n-6) and 22:6(n-3) were also significantly reduced in schizophrenic patients (Vaddadi et al., 1989; Glen et al., 1994; Peet et al., 1995). In cultured skin fibroblasts, only 22:6(n-3) and the total n-3 PUFAs were found significantly lower in schizophrenic patients than in normal subjects and bipolar patients (Mahadik et al., 1996).

The above studies were mainly carried out in patients with schizophrenia on neuroleptic treatment. We (Yao et al., 1994b) have reported a significant decrease of PUFA's, particularly 18:2(n-6) and 20:4(n-6), in the RBC ghost membranes of schizophrenic patients on and off haloperidol (HD) treatment (Table 49.2). Such a decrease remained in those patients withdrawn from HD for >5 weeks. Similarly, decreased 18:2(n-6) in RBC membranes of schizophrenic patients was reported in a double-blind trial of EFA supplementation in patients with tardative dyskinesia (Vaddadi et al., 1989). Such a decrease was persistent in schizophrenic and schizoaffective patients followed for four and half years (Vaddadi, 1996). A decrease of RBC 18:2(n-6) was also present in the first degree relatives of patients with schizophrenia (Peet et al., 1996), as well as patients with first-episode neuroleptic-naive schizophrenia (Table 49.3) (Reddy et al., 2004), suggesting a familial tendency of membrane PUFA differences in schizophrenia. Similarly, Sobczak et al. (2004) reported that the first degree relatives of patients with bipolar spectrum disorders had lower total n-3 and increased total n-6 relative to a nonpatient control group.

To test whether the levels of PUFAs and the degree of fatty acid unsaturation in RBC ghost membranes of schizophrenic patients were related to their severity of psychopathology, we (Yao et al., 1994a) compared the levels of 18:2(n-6), 20:4(n-6), total PUFAs, and the fatty acid unsaturation index (FAUI) with various behavioral ratings. The usual complexity of natural mixtures makes it difficult to understand the overall unsaturation. To obtain the average number of double bonds per fatty acid molecule, the FAUI was thus calculated as $\Sigma N_i L_i / M_i$, where N_i is the number of double bonds in the fatty acid, "i" in L_i is the percentage by weight of the fatty acid in the given sample, and M_i is the molecular weight (Dole et al., 1959). It was shown that decreases in both RBC 18:2(n-6) and FAUI were significantly correlated to the increases in psychosis ratings (Figure 49.1), suggesting a close association between fatty acid abnormality and the severity of symptomatology.

Glen et al. (1994) reported that patients with prominent negative symptoms (e.g., poverty of thought and anhedonia) were associated with high levels of saturated fatty acids and low levels of PUFA's in RBC membranes, while patients with positive symptoms (hallucinations and delusions) showed the opposite association. Interestingly, schizophrenic patients RBC membrane unsaturated fatty acids with 20 and 22 carbons appeared to have a bimodal distribution. Such a bimodal distribution

TABLE 49.1
Distribution of Polyunsaturated Fatty Acids (PUFAs) in Plasma of Normal and Schizophrenic Subjects

Subject Origin	PUFAs	Normals	Schizophrenics	*p*
Scotland[a]	18:2(n-6)	24.4 ± 3.6[d]	21.4 ± 3.3	<.01
	20:3(n-6)	2.7 ± 0.6	2.3 ± 0.6	<.02
	20:4(n-6)	9.2 ± 1.7	8.2 ± 1.3	<.01
	20:5(n-3)	1.4 ± 0.7	1.5 ± 0.5	
	22:6(n-3)	4.2 ± 1.1	4.5 ± 0.9	
	Total n-6[c]	36.7 ± 3.9	32.5 ± 3.0	<.01
	Total n-3	6.9 ± 1.7	7.6 ± 1.1	
England[a]	18:2(n-6)	25.3 ± 4.1	21.6 ± 3.4[e]	<.001
	20:3(n-6)	2.8 ± 0.6	2.2 ± 0.6	<.001
	20:4(n-6)	10.6 ± 1.7	8.7 ± 2.3	<.001
	20:5(n-3)	1.4 ± 0.7	1.7 ± 0.8	
	22:6(n-3)	4.2 ± 0.9	5.1 ± 1.7	<.01
	Total n-6	39.3 ± 4.8	33.3 ± 5.0	<.001
	Total n-3	6.9 ± 1.4	8.4 ± 2.5	<.01
Ireland[a]	18:2(n-6)	25.3 ± 3.5	22.0 ± 3.7	<.01
	20:3(n-6)	2.3 ± 0.4	2.4 ± 0.6	
	20:4(n-6)	11.3 ± 2.0	10.4 ± 1.2	
	22:5(n-3)	1.8 ± 0.6	2.0 ± 1.5	
	22:6(n-3)	4.0 ± 1.0	5.0 ± 1.7	<.02
	Total n-6	38.9 ± 3.7	35.2 ± 3.1	<.02
	Total n-3	7.1 ± 1.4	8.4 ± 2.8	
Japan[b]	18:2(n-6)	25.3 ± 3.3	23.5 ± 3.5	<.02
	20:3(n-6)	1.9 ± 0.1	2.3 ± 0.7	<.02
	20:4(n-6)	9.8 ± 2.2	10.0 ± 2.2	
	22:5(n-3)	1.0 ± 0.5	1.3 ± 0.4	<.02
	22:6(n-3)	8.2 ± 1.3	8.6 ± 1.4	

[a] From Horrobin et al. (1989).
[b] From Kaiya et al. (1991).
[c] The total is greater than the sum of listed fatty acids in each case because some small amounts of PUFAs were omitted from the final tabulation.
[d] Results are expressed as mean ± SD mg/100 mg total lipid present.
[e] Values are from schizophrenic patients with tardive dyskinesia.

was later confirmed by Peet et al. (1996), although psychiatric rating scales were not determined to differentiate positive from negative symptoms patients in their study.

Hibbeln et al. (2003) failed to replicate such a bimodal distribution but pointed out that PUFA abnormalities, especially in schizophrenic patients, may be an artifact of smoking status. Following n-3 supplementation for 16 weeks, patients with schizophrenia who smoked had the lowest EPA. However, it remains unclear whether smoking causes reduction of RBC PUFA levels since there was not a normal control group. On the other hand, Yao et al. (2001) indicate that the altered antioxidant defenses, as well as PUFA levels were not associated with the degree of smoking in patients with schizophrenia.

b. Brain

^{31}P magnetic resonance spectroscopy (^{31}P MRS) permits noninvasive study of cell membrane phosphorus metabolism in humans. Phosphomonoesters (PMEs) are precursors, and phosphodiesters (PDEs)

TABLE 49.2
Quantitative Determination of Polyunsaturated Fatty Acids (PUFAs) in Red Blood Cells of Normal, Haloperidol (HD)-Treated- and Drug-Free (DF) Schizophrenic Subjects[a]

		Schizophrenics		
PUFAs	**Normals (22)[b]**	**HD-Treated (24)**	**<5 Weeks DF (19)**	**>5 Weeks DF (10)**
18:2(n-6)	218 ± 69[c]	167 ± 77[d]	164 ± 93[d]	165 ± 42[d]
20:3(n-6)	33 ± 16	25 ± 12	26 ± 17	26 ± 11
20:4(n-6)	301 ± 125	190 ± 97[d]	208 ± 135[d]	216 ± 87[d]
22:4(n-6)	70 ± 35	46 ± 25[d]	51 ± 31	56 ± 23
22:5(n-3)	36 ± 17	21 ± 11[d]	29 ± 20	28 ± 13
22:6(n-3)	58 ± 33	32 ± 19[d]	39 ± 27[c]	47 ± 29
Total[e]	733 ± 282	493 ± 231[d]	535 ± 324[d]	557 ± 190[d]
Unsaturation index[f] (%)	41 ± 4	33 ± 6[d]	36 ± 8[d]	35 ± 8[d]

[a] From Yao, J.K., and van Kammen, D.P. (1994). *Schizophr. Res*. 11:209–216. With permission.
[b] Figures in parentheses indicate the number of subjects examined.
[c] Values are expressed as mean and standard deviation in nmol/mL of packed red blood cells.
[d] Values of schizophrenic patients are significantly ($p < .05$) different from those of normal controls.
[e] The total is greater than the sum of listed fatty acids in each case because some small amounts of PUFAs were omitted from the final tabulation.
[f] Unsaturation index represents average number of double bonds per fatty acid molecule.

TABLE 49.3
Fatty Acids and Essential PUFA Distribution in Red Blood Cell Membranes of Normal Controls and First-Episode Neuroleptic-Naïve Schizophrenic Patients[a]

Fatty Acids	**Normal Controls (n = 31) Mean ± SD**	**Patients (n = 24) Mean ± SD**	***p* (Two-Tailed *t* Tests)**
18:2(n-6)	317 ± 712[b]	311 ± 106	0.810
20:3(n-6)	40 ± 12	39 ± 17	0.747
20:4(n-6)	339 ± 61	278 ± 69	0.001
22:4(n-6)	60 ± 18	59 ± 26	0.816
22:5(n-3)	69 ± 37	44 ± 17	0.002
22:6(n-3)	61 ± 24	45 ± 16	0.003
Saturated	1243 ± 287	1280 ± 303	NS
Monounsaturated	471 ± 97	477 ± 121	NS
Polyunsaturated[c]	896 ± 148	782 ± 193	0.020
Total fatty acids	2611 ± 451	2538 ± 519	NS

[a] From Reddy, R.D., et al. (2004). *Schizophr. Bull.* 30:901–911. With permission.
[b] Sum of fatty acids with more than one double bond.
[c] nmol/mL packed RBC.

the breakdown products of membrane phospholipids. PME and PDE concentration reflect membrane turnover and may differ between healthy and pathological states. In fact, phospholipids themselves constitute a large part of the broad resonance underlying the PDE and PME peaks. Pettegrew et al. (1991, 1993) demonstrated a significant reduction of PMEs and significantly increased levels of PDEs in the dorsolateral prefrontal cortex of neuroleptic-naïve first-episode schizophrenic patients

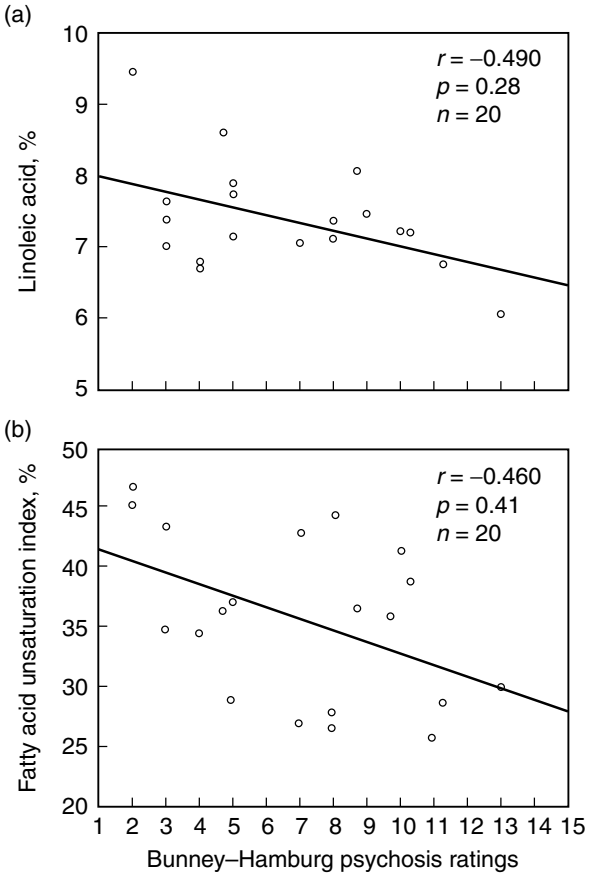

FIGURE 49.1 (a) Relationships of linoleic acid and (b) fatty acid unsaturation index of red blood cell membrane phospholipids to Bunney–Hamburg psychosis ratings (3-day mean) in drug-free schizophrenic patients. *Source:* Adapted from Yao, J.K., et al. (1994a). *Schizophr. Res*. 13:227–232.

as compared to control participants. In addition, an increased level of ATP and decreased inorganic orthophosphate were also found in the frontal cortex of schizophrenic patients. Pettegrew et al. (1993) suggested that changes in membrane phospholipids may be related to molecular changes that precede the onset of clinical symptoms and brain structural changes in schizophrenia, while changes in high energy phosphate metabolism may be state dependent. Other groups (Williamson et al., 1991; Fukazako et al., 1992, 1996; Deicken et al., 1993; Stanley et al., 1994) reported similar perturbations in membrane phospholipids in both acutely and chronically ill patients. Based on ^{31}P MRS findings, Keshavan et al. (1993b) suggested a possible familial basis for membrane phospholipid changes in schizophrenia. Post mortem studies have provided direct evidence of decreased fatty acid levels in the frontal cortex (Horrobin et al., 1991) as well as the caudate nuclei (Yao et al., 2000) of patients with schizophrenia relative to normal controls (Table 49.4). These studies demonstrated significant reductions of AA in phosphatidylethanolamine (PE; which contains the highest concentration of EFA among the phospholipids), which are consistent with findings in plasma (Horrobin et al., 1989; Kaiya et al., 1991) and RBC lipids (e.g., Glen et al., 1994; Yao et al., 1994b).

Furthermore, a significant correlation was found between RBC phospholipid PUFAs and ^{31}P MRS measures of phospholipid metabolites in the combined right and left frontal lobe, but not other brain regions including caudate, occipital, parietal, and temporal areas (Yao et al., 2002). Specifically, both the total PUFA and the individual 20:4(n-6) contents were significantly correlated with the freely-mobile phosphomonoester [PME(s-τ_c)] levels (Figure 49.2). The 18:2(n-6) PUFA

TABLE 49.4
Quantitative Determination of Polyunsaturated Fatty Acids (PUFAs) in Postmortem Brain Tissues of Normal and Subjects with Schizophrenia

Brain Region	PUFAs	Normal Controls	Schizophrenics	Psychiatric Controls
Frontal cortex[a]	18:2(n-6)	0.8 ± 0.5[b]	0.4 ± 0.2[c]	Not available
	20:4(n-6)	15.2 ± 1.1	13.8 ± 1.7[c]	Not available
	22:4(n-6)	7.1 ± 0.4	7.5 ± 1.1	Not available
	22:6(n-3)	34.0 ± 3.5	34.2 ± 4.4	Not available
Caudate[d]	18:2(n-6)	3.0 ± 1.3[e]	2.1 ± 0.8[c]	2.7 ± 0.8
	20:2(n-6)	1.6 ± 0.2	1.3 ± 0.5	1.8 ± 0.5
	20:4(n-6)	17.9 ± 2.1	15.4 ± 3.0[f]	18.9 ± 3.1
	22:4(n-6)	5.4 ± 0.6	4.8 ± 1.1	5.1 ± 0.5
	22:5(n-3)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	22:6(n-3)	9.7 ± 1.6	8.6 ± 2.8	10.5 ± 2.3

[a] From Horrobin et al. (1991). PUFAs were isolated from phosphatidylethanolamine.
[b] Values from frontal cortex are expressed as mean and standard deviation of mg/100 mg total lipids.
[c] Values of schizophrenic patients are significantly lower than normal controls.
[d] From Yao et al. (2000). PUFAs were isolated from total phospholipids.
[e] Values from caudate are expressed as mean and standard deviation of nmol/mg dry weight.
[f] Values of schizophrenic patients are significantly lower than both control groups.

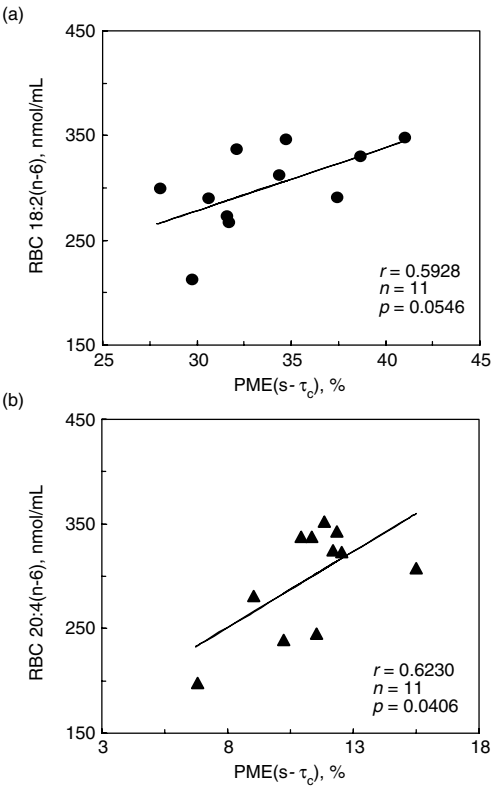

FIGURE 49.2 (a) Relationships of phosphodiester (PDE) and RBC linoleic acid concentration and (b) phosphomonoester (PME) and RBC arachidonic acid. *Source:* Adapted from Yao, J.K., et al., (2002). *Biol. Psychiatry* 52:823–830.

content correlated positively with freely-mobile PDE [PDE(s-τ_c)] levels (Figure 49.2). The above correlations were present in the combined right and left prefrontal region of the brain while other regions including the basal ganglia, occipital, inferior parietal, superior temporal, and centrum semiovale yielded no significant correlations. Our data thus support the association between the decreased RBC membrane phospholipid PUFA content and the decreased building blocks [PME (s-τ_c)] and breakdown products [PDE(s-τ_c)] of membrane phospholipids in the prefrontal region of patients with first-episode neuroleptic-naive schizophrenia.

Thus, there is ample evidence for the existence of phospholipid and fatty acid pathology, in both peripheral and central nervous system membranes, in early and chronic, and medicated and unmedicated, schizophrenia. As is known from membrane physiology, changes in the composition of membranes lead to a number of functional disturbances, and such pathological changes of relevance to schizophrenia are reviewed below.

B. Pathological Consequences of Impaired Membrane Dynamics

The membrane is a complex structure that provides scaffolding for many key functional systems, including transmembrane ion channels, neurotransmitter receptor binding, signal transduction, immune-inflammatory process, and in the case of mitochondrial membranes the electron-transport chain. Alterations due to changes in membrane composition can lead to pathological alterations in a number of these systems, some of which have been found in schizophrenia.

a. Decreased Membrane Fluidity

Biological membranes are highly dynamic structures composed predominantly of lipids and proteins. The dynamic state, or fluidity, of the cell membrane is dependent on its composition and, in the intact cell, is also influenced by motion imposed by the cytoskeleton. Small changes in membrane fluidity have been shown to have considerable effects on a wide variety of membrane functions. Specifically, alterations in membrane fluidity have been implicated in the regulation of the densities and binding affinities of neurohormone and neurotransmitter receptors, including dopamine (De la Pressa Owens and Innis, 1999; Zimmer et al., 2000), serotonin (Heron et al., 1980a,b; De la Pressa Owens and Innis, 1999), norepinephrine (Hirata and Axelrod, 1980), and opiates (Heron et al., 1980a,b). Therefore, the observed changes in membrane phospholipid composition associated with schizophrenia may result in altered neurotransmission by virtue of a perturbation of membrane fluidity. Hitzemann et al. (1986) demonstrated a significant increase in the steady-state anisotropy (r_s) of DPH-labeled RBC ghost membranes from patients with schizophreniform disorder that pointed out that five of eight schizophreniform patients had values higher than the highest control value. Later, Pettegrew et al. (1990) reported an increased molecular motion on the RBC membrane surface and in the phospholipid head group region in the unmedicated schizophrenic patients. No significant motional differences, however, were found in the membrane hydrocarbon core of the same unmedicated patients. We (Yao and van Kammen, 1994) have reported a significant increase in r_s values from schizophrenic patients withdrawn from HD. Furthermore, changes in r_s values of drug-free patients were significantly correlated with their increase in psychosis ratings as well as decreases in the FAUI (Figure 49.3). Therefore, decreased RBC membrane fluidity in schizophrenia is consistent with decreased PUFA's in RBC membranes (Vaddadi et al., 1986; Glen et al., 1994; Yao et al., 1994b).

b. Increased Free Radical Production

It is well known that elevations in free AA are associated with oxidative stress (Yao et al., 2001; Yao and van Kammen, 2004). Metabolism of AA by oxygenases yields superoxide and hydroxyl radical production (Chan and Fishman, 1980). Increased superoxide production is seen with application of

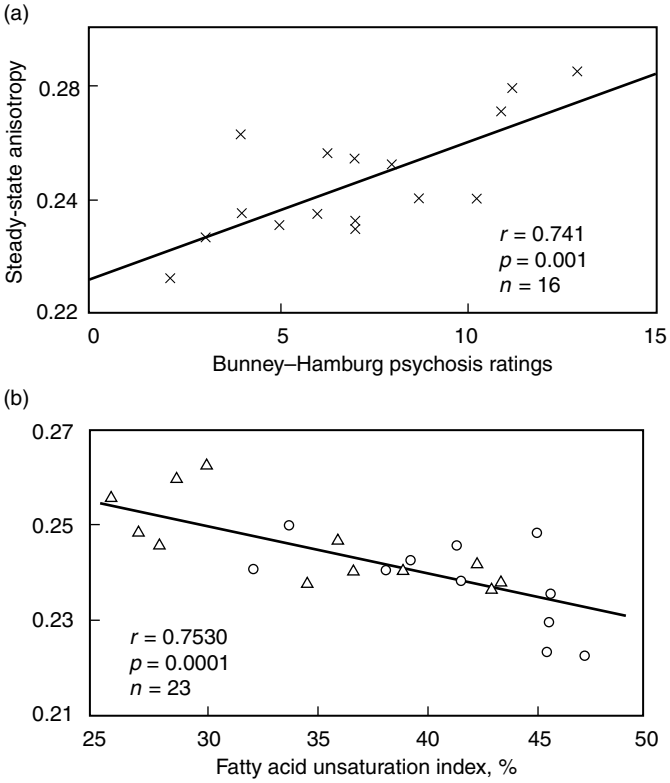

FIGURE 49.3 (a) Relationships of the steady-state anisotropy (r_s) of 1,6-diphenyl-1,3,5-hexatriene-labeled RBC ghost membranes to 3-day mean Bunney–Hamburg psychosis ratings from drug-free schizophrenic patients (x) and (b) between the fatty acid unsaturation index of RBC membrane phospholipids and steady-state anisotropy of normal subjects (O) and drug-free schizophrenic patients (Δ). *Source:* Adapted from Yao, J.K., and van Kammen, D.P. (1994). *Schizophr. Res*. 11:209–216; Yao, J.K., et al. (1994b). *Schizophr. Res.* 13:217–226.

exogenous AA, and indomethacin (blocks cyclooxygenase metabolism of AA) inhibits AA-induced free radical generation and subsequent lipid peroxidation (Hall et al., 1993). Lipid peroxidation, in turn, causes significant membrane damage (reviewed in Reddy and Yao, 1996; Yao and van Kammen, 2001; Mahadik and Yao, 2006), and may lead to further depletion of AA. There is independent evidence for the presence of oxidative stress in first-episode and chronic schizophrenia, which may be closely linked to phospholipid and fatty acid deficits seen in schizophrenia. For example, high levels of hydrogen peroxide and lipid peroxides lead to decreased synthesis of prostaglandins (Deby and Deby-Dupont, 1980), which also have been reported in schizophrenic patients (Rotrosen and Wolkin, 1987; van Kammen et al., 1989). There are a variety of adverse effects on neurotransmitter binding (dopamine, GABA, serotonin) that are induced by oxidative stress, likely mediated by alterations in membrane composition and function (Yao et al., 2001; Skosnik and Yao, 2003; Yao and van Kammen, 2004; Hulbert et al., 2005).

c. Decreased Dopamine Transporter Receptor (DATR)

Changes in membrane dynamics can affect the transmembrane processes that are mediated through the conformational changes of the membrane-bound proteins (Hitri et al., 1994). Maguire and Druse (1989) have demonstrated an inhibition of transmembrane process of DA uptake by increasing the ratio of cholesterol to phospholipid (C/PL) in the synaptic plasma membrane. Thus, the function

of the transporter is highly influenced by the lipid composition of membrane environment. Given a DAT model that mediates the transport of DA through a series of sequential conformational changes of the transporter protein (Hitri et al., 1994), it is likely that the conformational state of DAT is regulated by the synaptic membrane fluidity. Moreover, both n-6 and n-3 series of PUFAs may be involved in the presynaptic receptor control of dopamine release (Davidson et al., 1988). It is therefore possible that an elevated ratio of C/PL and/or a reduced degree of fatty acid unsaturation may be associated with a decrease in the density of DATR in the cortical areas of the schizophrenic brain with high metabolic activity. In laboratory animals, Zimmer et al. (2000) reported the effects of chronic PUFA deprived diet on DA transmission in the nucleus accumbens. Remarkably, chronic deprivation of n-3 dietary PUFA lead to a 60% reduction in DA transporter sites.

d. Serotonin Dysfunction

5-HT_2 receptors in the brain are thought to play a regulatory role in behavior (Leysen and Pauwels, 1990). However, conflicting findings had left unresolved the role of abnormal serotonin (5-hydroxytryptamine; 5-HT) function in schizophrenia. The development of serotonin-dopamine antagonists (atypical) antipsychotic drugs that potently block 5-HT_2 receptors has renewed interest. 5-HT stimulates the release of AA in hippocampal neurons through the activation of PLA_2 that is independent of inositol-phospholipid hydrolysis (Felder et al., 1990). Thus, 5-HT may potentially mediate some pathophysiological processes through receptor-stimulated AA or eicosanoids.

Previously, we demonstrated that drug-free schizophrenic patients exhibited reduced physiologic responsivity mediated through the platelet 5-HT_2 receptor complex, which can be modified by HD treatment (Yao et al., 1996b). Similarly, blunted platelet serotonergic responsivity was also demonstrated in first-episode treatment-naive patients with schizophrenia, but not with mood disorders (Reddy et al., in press). It has been observed that n-3 fatty acids such as DHA can influence the turnover rate of 5-HT in the brain (Hibbeln and Salem, 1995), and that cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA, metabolite of 5-HT) and plasma DHA levels are correlated in the healthy volunteers (Hibbeln et al., 1998). Thus, it is plausible that membrane PUFA deficits may lead to the blunted serotonergic platelet responsivity in patients with schizophrenia.

C. Biochemical Mechanisms Underlying Decreased Membrane PUFAs

There are several known mechanisms that can lead to altered membrane fatty acid composition that characterizes schizophrenia, and include: (1) low dietary intake or synthesis of essential PUFAs; (2) decreased fatty acid incorporation into phospholipids; (3) increased phospholipid degradation; and (4) increased lipid peroxidation. The most compelling evidence in schizophrenia exists for increased phospholipid degradation and decreased incorporation of AA, which are discussed below.

a. Low Intake or Synthesis of Arachidonic Acid

Unlike saturated/monounsaturated fatty acids, long-chain PUFAs cannot be synthesized de novo by humans. Therefore, we must obtain the long-chain PUFAs directly in the diet or consume their 18 carbon precursors linoleic acid (18:2n-6 or LA) and (18:3n-6 or ALA). Increased intake of n-6 fatty acids or reduced intake of both n-6 and n-3 can cause deficits in brain and behavioral development (Simopoulos, 1999). Substantial cultural differences exist in the quantity of EFA, particularly n-3 EFA consumption, primarily due to the local availability of these substances in the diet, in addition to effects of lifestyle and consumption of antioxidants (Peet et al., 1995; Mahadik et al., 2001). Christensen and Christensen (1988) found that geographic differences in the clinical outcome of patients with schizophrenia, studied by the WHO, were related to differences in the patients' dietary intake of oils from fish and vegetables, the primary sources of n-3 EFAs, ALA, and DHA. In addition, the increased incidence of schizophrenia seen in children born during the Dutch famine

(Hoek et al., 1999) may have resulted from reduced consumption of fresh fruits and vegetables (primary sources of antioxidants and precursor EFAs).

Other lifestyle characteristics of individuals with schizophrenia may affect brain PUFAs through prooxidant effects such as high intake of calories, fat, alcohol, and little exercise (Brown et al., 1999). Ecological studies of patients with schizophrenia have found that these patients consumed higher amounts of saturated fats and sugars compared to intakes for the U.S. population (Peet, 2003). High caloric intake has been reported to cause peroxidative brain damage and cognitive deficits in animals (Bruce-Keller et al., 1999).

b. Decreased Fatty Acid Incorporation

Demisch et al. (1987, 1992) have shown that incorporation of [^{14}C]AA into platelet phospholipids was significantly lower in untreated patients (>6 months) with a schizophreniform or schizoaffective disorder than in normal control subjects. The incorporation rates were only slightly but not significantly reduced in chronic schizophrenic patients. This suggests that the rate of AA incorporation was related to the type and time course of the disorder. We (Yao et al., 1996a) demonstrated that the total incorporation of [^{3}H]AA in drug-free patients was significantly lower than in the same individuals on HD treatment as well as that of normal controls. No significant difference was demonstrated between relapsed and nonrelapsed drug-free patients. Thus, it is unlikely that changes in AA incorporation are related to a specific syndrome or its intensity. More importantly, these two studies suggest that neuroleptic treatment ameliorate symptoms while normalizing AA incorporation into phospholipids, indicative of a connection between these two phenomena.

c. Increased Phospholipids Degradation

PLA_2 is enriched in neuronal membranes and is a key enzyme responsible for the breakdown of membrane phospholipids. Importantly, this enzyme regulates the production of prostaglandins, or hormones involved in inflammatory processes (du Bois et al., 2005). Increased cytoplasmic PLA_2 activity has been observed in serum of drug-free schizophrenic patients (Gattaz, 1992; Noponen et al., 1993; reviewed in Law et al., 2006). Such increases in serum PLA_2 activity, however, were also found in patients with other psychiatric disorders (Noponen et al., 1993), including bipolar disorder (Ross et al., 2006), questioning the specificity of this finding to schizophrenia. Albers et al. (1993) found no significant differences of serum PLA_2 activity between neuroleptic-naive schizophrenics and normal controls. These discrepancies may be due to the differences in assay procedure and the heterogeneous class of extracellular PLA_2 (Ross et al., 1997). Gattaz et al. (1995) showed that the intracellular membrane-bound PLA_2 activity was significantly higher in the platelets of schizophrenic patients than in normal and psychiatric controls, with no significant differences between normal and psychiatric controls. It is thus unlikely that increased platelet PLA_2 activity in schizophrenia results from nonspecific stressors. Furthermore, HD treatment reduced platelet PLA_2 activity to control levels. Other neuroleptics also inhibit PLA_2 activity (Aarsman et al., 1985; Taniguchi et al., 1988).

Further support of elevated PLA_2 activity in schizophrenia comes from the findings of increased lysophosphatidylcholine (LPC; the break-down product of PLA_2 reaction) found in platelets of schizophrenic patients (Pangterl et al., 1991; Steudle et al., 1994). The increased LPC was significantly correlated with duration of illness in neuroleptic-naive but not previously treated patients, suggesting an accelerated breakdown of membrane phospholipids after the onset of disease. Such a correlation is consistent with the findings that duration of illness prior to first-episode treatment in drug-naive patients was significantly associated with time to remission (Wyatt, 1986; Loebel et al., 1992).

d. Increased Lipid Peroxidation

PUFAs are highly susceptible to free radical insult and autoxidation to form peroxyradicals and lipid peroxide intermediates, the existence of which within cell membranes result in unstable membrane

TABLE 49.5
Association of RBC-PUFA Levels with Plasma Lipid Peroxides[a]

Biochemical Measures	Normal Controls	FEP	CSP-CT
RBC-20:4(n-6), %	14.52 ± 2.00	3.12 ± 2.55	6.68 ± 3.60
RBC-22:6(n-3), %	2.99 ± 0.79	0.25 ± 0.71	1.08 ± 0.90
Plasma TBARS, nmol/mL	5.67 ± 2.11	24.67 ± 4.87	16.23 ± 3.95

Abbreviations: RBC, red blood cells; PUFA, polyunsaturated fatty acid; TBARS, thiobarbituric acid-reactive substance; FEP, first-episode drug-naïve psychotic patients; CSP-CT, chronic schizophrenic patients with clozapine treatment.

[a] From Khan, M.M., et al. (2002). *Schizophr. Res.* 58:1–10. With permission.

structure, altered membrane fluidity and permeability, and impaired signal transduction. The brain has a higher concentration of long-chain PUFAs than any other organ and, is particularly vulnerable to free radical-mediated damage (reviewed in Yao et al., 2001; Skosnik and Yao, 2003). Thus, lipid peroxidation can lead to both increased free radical production and decreased membrane AA content. For example, Arvindakshan et al. (2003) reported that never medicated patients with schizophrenia had reduced RBC membrane AA levels and were negatively associated with greater levels of oxidative stress, reflected in plasma thiobarbuturic acid reactive substances (TBARS). This association was only apparent in never-medicated schizophrenic patients and not in a well-matched normal control group. Considering the observed negative association, the authors interpreted these findings as evidence that reduced membrane AA and DHA levels were the result of increased lipid peroxidation in the schizophrenic group. In other work, higher plasma TBARS were associated with greater severity of negative symptoms in schizophrenic patients (Mahadik et al., 1998). Similarly, lower erythrocyte membrane AA and DHA were associated with significantly higher levels of TBARS in first-episode drug-naive schizophrenic patients than matched normal controls (Table 49.5) (Khan et al., 2002). Interestingly, a third group of chronically medicated patients showed higher AA and DHA relative to the drug-naive group suggesting that the PUFA membrane deficits may potentially contribute to illness onset and that antipsychotic treatment may increase PUFA levels; although TBARS remained elevated relative to the control group. As suggested by the authors, future research should continue to explore the effect of simultaneous supplementation with antioxidants as a method to reduce lipid peroxidation.

D. Therapeutic Effects

a. Dietary Essential Fatty Acid Supplementation

A dose-ranging study with adjunctive ethyl-EPA (placebo, 1, 2, or 4 g/day for 12 weeks) in patients with persistent schizophrenic symptoms inadequately responsive to clozapine, atypicals (olanzapine, risperidone, or quetiapine), or one of the typical antipsychotic drugs has been reported (Peet and Horrobin, 2002). Placebo was 4 g/day of liquid paraffin that has been used up to 15–30 g/day as a laxative. It was concluded that 2 g/day of ethyl-EPA had the maximum therapeutic effect. However, patients on typical antipsychotics and new atypical drugs did not show improvement with the adjunctive ethyl-EPA over patients on placebo, which showed significant improvements from baseline. The clozapine group had very little placebo effect and therefore responses to 2 g dose of ethyl-EPA were substantially better than placebo. However, changes in RBC membrane EPA and DHA did not correlate with clinical response, whereas clinical improvement was proportional to changes in membrane AA. In any case, the therapeutic benefit of EPA in this small trial warrants further investigation.

Since increased oxidative stress-mediated PUFA peroxidative degradation as well as defective phospholipid-PUFA metabolism exists in schizophrenia, the use of a combination of PUFAs and antioxidants (e.g., vitamins E and C) for supplementation may be preferable (Mahadik et al., 2001). Antioxidants have been found to be very effective in protecting membrane PUFAs, in addition to preventing oxidative damage of vital cellular proteins, mitochondria, and DNA. Earlier studies have also used only vitamin E; supplementation with vitamin C, an effective intracellular antioxidant, has not yet been tried.

One study has reported the effects of 4 months supplementation of a combination of EPA:DHA (360 mg:240 mg) and vitamins E:C (800 IU:1 g) per day in two equal doses in 34 chronic schizophrenic patients on stable antipsychotic medications (Arvindakshan et al., 2003). Patients showed over 25% reductions in most of the psychopathological scores, and these effects were sustained up to 4 months after termination of supplementation. However, this study did not have a placebo group. RBC membrane EPA and DHA concentrations rose from baseline, to those of matched normal controls without any change in the plasma lipid peroxides. This indicated that such a low dose PUFA treatment may be adequate to correct the preexisting membrane PUFA deficits when combined with antioxidants. Pretreatment AA levels were similar to normal controls, fell during treatment, and returned to pretreatment levels by 4 months posttreatment. This suggests that supplementation of n-3 PUFAs (Peet and Horrobin, 2002), may reduce AA incorporation in membranes by competition. Finally, two small double-blind placebo-controlled pilot studies of EPA and DHA for improvement of schizophrenia related symptoms yielded promising results (both reported in Peet et al., 2003). In the first study, following 3 months of supplementation with EPA, a significant reduction of positive symptoms was observed. No reduction was seen with DHA or the placebo. In the second study, treatment with EPA ($n = 14$), compared to placebo, lead to significantly lower scores on a positive symptom measure.

Recently, we examined whether supplementation with the n-3 fatty acid eicosapentaenoic acid (EPA) modified 5-HT amplified ADP-induced platelet aggregation in patients with schizophrenia (Yao et al., 2004). Two grams of ethyl-EPA was administered daily for 6 months adjunctively to ongoing antipsychotic treatment in 12 patients with chronic schizophrenia, using an open-label design. RBC membrane fatty acids and platelet functions (platelet aggregation and dense granule secretion) were monitored at baseline, 1-, 3-, and 6-months. The EPA levels were elevated more than fivefold in RBC membranes of all patients after 3 months of supplementation, indicating a high degree of compliance. Consistent with previous reports (Kristensen et al., 1989), there was inhibition of ADP-induced platelet aggregation by EPA supplementation. Moreover, EPA markedly enhanced the 5-HT responsivity as measured by the magnitude of 5-HT amplification on ADP-induced platelet aggregation (Figure 49.4). Previously, we demonstrated a significant inverse correlation between 5-HT responsivity and psychosis severity in unmedicated patients with schizophrenia (Yao et al., 1996b). Taken together, the present data support the notion that EPA may be mediating its therapeutic effects in schizophrenia via modulation of the 5-HT_2 receptor complex.

More recently, Kemperman et al. (2006) assessed the nutritional status and effects of subsequent supplementation with n-3 fatty acids in schizophrenic patients. As a group, the schizophrenic patients had elevated RBC saturated and monosaturated fatty acids but low PUFA (RBC 20:3n-9 >0.46 mol %), as well as increased homocysteine levels probably due to poor B-vitamin status. Following a 100-day supplementation period in the patients low PUFA status with an EPA/DHA combination of 310 mg and 200 mg, respectively, the PUFA nutritional deficits were improved, although, changes in symptomatology were not assessed.

b. Atypical Antipsychotic Drugs

Apolipoprotein (ApoD) is a member of the lipocalin superfamily of transporter proteins that bind small hydrophobic molecules, including AA. The ability of apoD to bind AA implicates it in

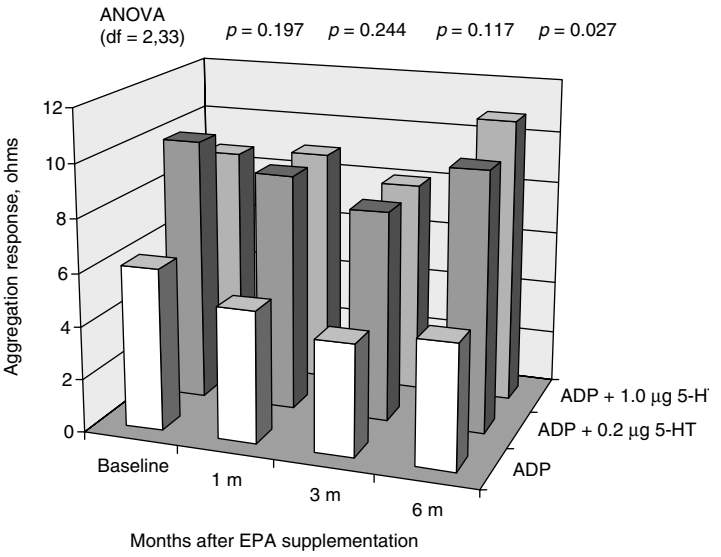

FIGURE 49.4 Increased platelet serotonin responsivity in clinically stable patients with chronic schizophrenia (n = 12) following ethyl-EPA supplementation. *Source:* Adapted from Yao, J.K., et al. (2004). *Prostaglandins Leukot. Essent. Fatty Acids* 71:171–176.

pathways associated with membrane phospholipid signal transduction and metabolism. An increased expression of apoD in the mouse brain after clozapine treatment suggested a role for apoD in the pharmacological action of clozapine (Thomas et al., 2003). Recently, we have demonstrated that plasma apoD levels were significantly correlated with RBC-AA and DHA in first-episode neuroleptic-naive patients with schizophrenia (Yao et al., 2005). Moreover, Thomas and Yao have further collaborated to study membrane phospholipids and fatty acids in brains from apoD knock-out (KO) mice and wild-type (WT) controls that were treated with clozapine, olanzapine, or saline. Preliminary data (Thomas and Yao, in press) indicate significant decreases in the levels of AA, its precursors, 18:2(n-6) and 20:3(n-6), and its elongated product, 22:4(n-6); in response to clozapine treatment in the brains of apoD KO mice, but not WT mice. Clozapine treatment also caused a reduction in other fatty acids and in the membrane phospholipids, phosphatidylinositol, and phosphatidyl-serine in both apoD KO and WT mice, and a reduction in PE in apoD KO mice only. Taken together with other *in vitro* studies, these results support the view that an increased expression of apoD such as the one induced by atypical antipsychotic drugs may facilitate incorporation of AA into membrane phospholipids by its selective binding to AA.

E. Summary

The above findings reported in the literature and those from our laboratory indicate in patients with schizophrenia exhibit differences in membrane PUFAs in a variety of peripheral cell types and brain tissues (see recent reviews by Skosnik and Yao, 2003; Law et al., 2006; Mahadik and Yao, 2006). These differences appear to be independent of neuroleptic treatment (based on findings from drug-free and neuroleptic-naive patients), and is associated with illness severity. The key mechanisms that may lead to reduced membrane PUFA are increased phospholipid hydrolysis and/or decreased incorporation. The accelerated breakdown of membrane phospholipids is supported by the ^{31}P MRS findings in brains of schizophrenic patients. Supplementation of PUFAs may improve clinical state, indicating that behavioral changes parallel membrane PUFA changes. A proposed model of relations between membrane AA and treatment outcome is illustrated in Figure 49.5.

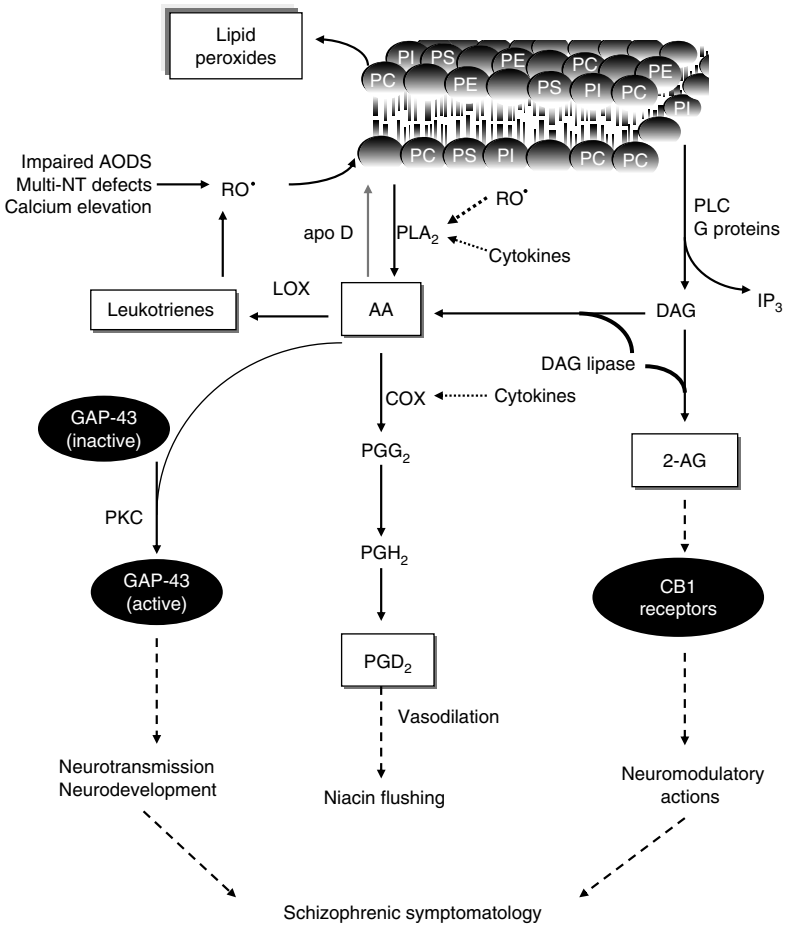

FIGURE 49.5 An overview of phospholipids turnover, arachidonic acid signaling and schizophrenic symptomatology. *Abbreviations:* AODS, antioxidant defense system; NT, neurotransmitters; RO, reactive oxygen species; apoD, apolipoprotein D; PLA_2, phospholipase A_2; PLC, phospholipase C; LOX, lipooxygenase; AA, arachidonic acid; DAG, diacylglycerol; COX, cyclooxygenase; 2-AG, 2-arachidonoyl glycerol; GAP, growth-associated protein; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PGD_2, prostaglandin D_2; CB, cannabinoid. *Source:* Adapted from Skosnik, P.D., and Yao, J.K. (2003). *Prostaglandins Leukot. Essent. Fatty Acids* 69:367–384.

III. MAJOR DEPRESSION

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), depression is one of the major mood disorders and is characterized by loss of interest or pleasure, and disturbances of affect severe enough to alter cognition, judgment, and interpersonal relationships. As with schizophrenia the molecular basis of major depression remains unclear. Considerable data point to several neurobiological mechanisms in depression, including norepinephrine (NE) and serotonin (5-HT) dysregulation, corticotropin-releasing factor (CRF) stress response abnormalities, as well as differences in neurotrophic factors and cellular transduction factors (Ressler and Nemeroff, 2000; Trivedi, 2003; Duman and Monteggis, 2006). A deficiency of 5-HT activity in brain appears to increase vulnerability to depression (Maes and Meltzer, 1995). Decreased 5-HT uptake and 5-HT transporter binding in platelets (Kaplan and Mann, 1982; Nemeroff, 1998) and in brain (Malison et al., 1998) has been reported in patients with depressive disorders. Thus, changes in the NE and 5-HT systems

may mediate many of the signs and symptoms in depression. It is generally agreed that long-term antidepressant administration is associated with a decreased neurotransmission of NE system and increased 5-HT transmission (Owens, 1997; Delgado and Moreno, 2000). Selective serotonin reuptake inhibitors (SSRIs) are effective treatments for depression (Vaswani et al., 2003).

A. Reduced Membrane PUFAs

Since the early 1900s, there has been an increased incidence rate of major depression. One hypothesis is that this is secondary to a dramatic increase in the ratio of n-6/n-3 PUFAs in the Western diet (Smith, 1991). This association was in general agreement with recent data that such an imbalanced diet can led to high levels of n-6 PUFAs in tissues and low levels of n-3 PUFAs in plasma and membranes of Western population (Sinclair et al., 1994). Moreover, an increased incidence of cardiovascular and inflammatory disorders has also coincided with an increased ratio of n-6/n-3 PUFAs (Smith, 1991). Studies by Maes et al. (1996, 1997) have shown that an increased ratio of 20:4(n-6) to 20:5(n-3) is not only related to depression but also to a higher risk of cardiovascular disease. Among patients with recent acute coronary syndromes, major depression was associated with low levels of n-3 PUFAs (Frasure-Smith et al., 2004).

We recently investigated these potential associations in a convenience sample of 105 adult community volunteers, with untreated hypercholesterolemia (Conklin et al., unpublished data). Subjects completed the Beck Depression Inventory, the NEO-Five Factor Inventory and the Barratt Impulsiveness Scale, and their fasting serum fatty acid composition was determined. Controlling for gender, race and age, we found that a 1 SD increase in DHA reduced by approximately 50% the odds of mild or greater depressive symptomatology (Beck score ≥ 10, $p < .02$) (Figure 49.6). EPA and DHA also correlated negatively with both neuroticism and cognitive impulsiveness (p value range 0.04–0.002) (Table 49.6). These data suggest that n-3 fatty acid status contributes to the normative variability in affective regulation, personality, and impulse control.

As previously discussed, there are several mechanisms that may contribute to the depletion of membrane PUFA's. When RBC membranes from normal controls were exposed to hydrogen peroxide, the resulted changes in fatty acid composition closely resembled to those found in depressive patients (Peet et al., 1998). This finding suggests that differences in the n-3 PUFA pathway in

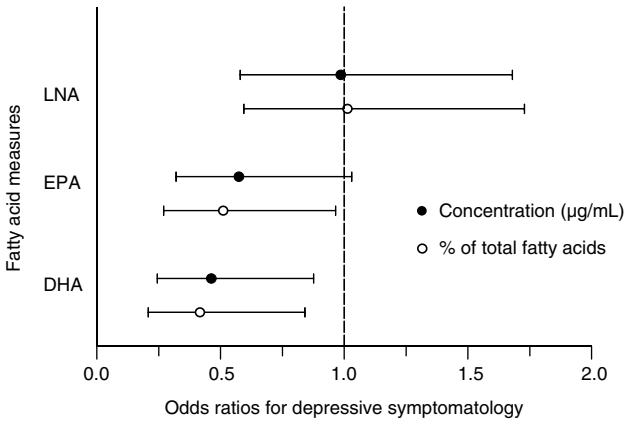

FIGURE 49.6 Odds ratios and 95% confidence intervals for depressive symptomatology (BDI-II ≥ 10) for a one SD increase in fatty acid measures. The figure illustrates a significant reduction in the odds for scoring ≥10 on the BDI-II with each SD increase in EPA expressed as a percentage of the total serum fatty acids (OR = 0.51, 95% CI = 0.27–0.97) and for a 1 SD increase in DHA expressed as a concentration (OR = 0.46, 95% CI = 0.24–0.88) or as a percentage of the total fatty acids (OR = 0.42, 95% CI = 0.21–0.84). *Source:* Adapted from Conklin, S.M., et al. *Psychiatry Res.*, in press.

TABLE 49.6
Multiple Linear Regression Analyses Assessing the Relationship between Fatty Acids and Measures of Impulsivity and Personality[a]

Behavioral Measurements[b]	EPA[c]		DHA	
	β[d]	ΔR^{2e}	β	ΔR^2
Serum Concentration (μg/mL)				
BIS Cognitive	–.236	.054[f]	–.214	.045[f]
NEO-FFI				
Neuroticism	–.206	.042[f]	–.247	.061[f]
Agreeableness	.138	.018	.217	.046[f]
Percentage of Total Fatty Acid Pool				
BIS Cognitive	–.279	.075[f]	–.240	.054[f]
NEO - FFI				
Neuroticism	–.282	.042[f]	–.318	.095[f]
Agreeableness	.135	.017	.199	.038[f]

[a] $n = 105$.
[b] Covariates were age, race, and gender entered simultaneously on the first step.
[c] PUFA values were log transformed.
[d] β = standardized value.
[e] ΔR^2 reports the proportion of variance accounted for by each fatty acid.
[f] $p < .05$.

Source: Adapted from Conklin, S.M., et al. *Psychiatry Res*., in press.

patients with major depression may be due to oxidative membrane damage, possibly resulting from an increased oxidative stress load or decreased antioxidant defense mechanisms.

Another mechanism may be reduced dietary intake of the n-3 PUFAs. For example, global ecological studies have found that low dietary intake of n-3 and high n-6: n-3 ratios are associated with increased rates of depression (Figure 49.7), (Hibbeln, 1998, 2002; Tanskanen et al., 2001a), whereas populations with higher fish consumption have lower rates of depression (Hibbeln, 2002) and also suicide (Tanskanen et al., 2001b). Other observational studies conducted within single populations links low n-3 and/or high n-6 intake or blood levels with depressive symptomatology (Adams et al., 1996; Mamalakis et al., 2002; Timonen et al., 2004), major depressive disorder (Edwards et al., 1998; Peet et al., 1998; Maes et al., 1997; Frasure-Smith et al., 2004), and other forms of negative affect, including hostility (Iribarren et al., 2004) and social anxiety disorder (Green et al., 2006).

B. Pathological Consequence of Reduced Membrane n-3 PUFAs

As mentioned earlier, small changes in membrane fatty acid composition can alter the dynamic state of cellular membranes and, subsequently, a variety of membrane functions. It has been shown that depressive patients have a decreased serotonin uptake (Mellerup and Plenge, 1988; Ellis and Salmond, 1994). We (Yao and van Kammen, 1994) have previously demonstrated that decreases in the degree of fatty acid unsaturation was significantly correlated to the increases in "structure order" of RBC ghost membrane as measured by the steady-state fluorescence anisotropy. Thus, the observed decrease of RBC n-3 PUFAs may lead to decreased membrane fluidity, and consequently, reduced serotonergic transport. Such an effect of plasma membrane fluidity on serotonin transport

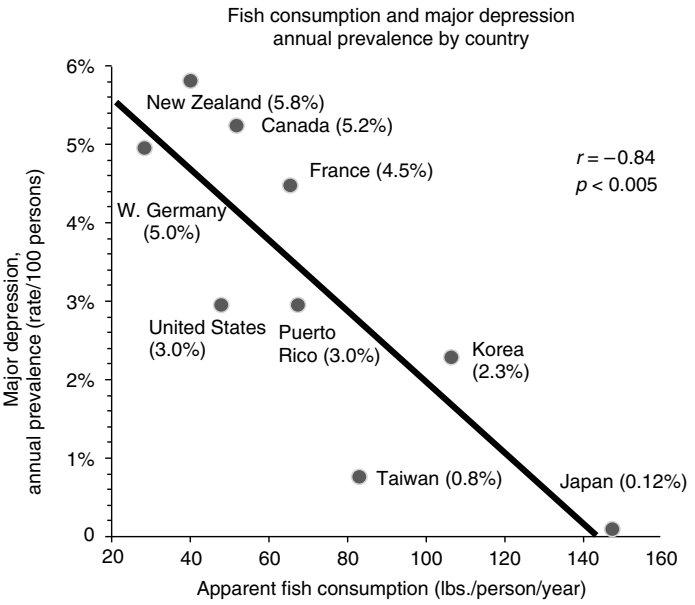

FIGURE 49.7 Relationship between national fish consumption and annual prevalence of major depression by country. *Source:* Adapted from Hibbeln, J.R. (1998). *Lancet* 351:1213.

has been previously demonstrated in endothelial cells (Block and Edwards, 1987). In addition, increased membrane fluidity has been associated with an improved serotonin binding in mouse brain membranes (Heron et al., 1980b). This is due to the biophysical effects of changes in membrane fluidity on receptor binding sites (Heron et al., 1980b). Basic neurotransmission appears to depend on the integrity of membrane structure and receptor function, which in turn provides a theoretical model relating the membrane PUFA deficits to the traditional monoaminergic hypotheses of major depression (Maes et al., 1996, 1997; Peet et al., 1998).

Decreased levels of serum brain-derived neurotrophic factor (BDNF) were demonstrated in clinically depressed patients (Shimizu et al., 2003; Aydemir et al., 2006). In addition to physical and psychological stress, diets with high saturated fat and sucrose can inhibit BDNF expression, an effect that is reversed by supplementation or consumption of n-3 fatty acids (Wu et al., 2004). By contrast, antidepressants can promote neurogenesis (Vaidya and Duman, 2001) and enhance BDNF expression (Russo-Neustadt, 2003). Depletion of cell membrane n-3 PUFAs, particularly DHA, has been postulated to play an etiologic role in major depression (Hibbeln and Salem, 1995). There is also an increasingly recognized role of proinflammatory cytokines in the pathophysiology of depression. Below, we discuss the evidence that supports the role of membrane PUFA and immune alterations, in depression.

C. PUFAs and Immune Function

A high n-6/n-3 PUFA ratio in the diet can also lead to an overproduction of inflammatory cytokines (Endres et al., 1993), since PUFAs are precursors of eicosanoids that play a pivotal role in the inflammatory process. Supplementation of fish oil (rich in the n-3 PUFAs) substantially reduces cytokine production while linoleic acid (the n-6 PUFA) increases proinflammatory cytokine secretion (Meydani et al., 1991; Endres et al., 1993; Caughey et al., 1996). These findings support an association between increased ratio of 20:4(n-6) to 20:5(n-3) and increased production of proinflammatory cytokine in major depression (Maes et al., 1996).

Both n-6 and n-3 PUFAs are involved in the regulation of inflammatory responses. The n-6 PUFAs, particularly AA, have proinflammatory features, since AA is the precursor of proinflammatory

eicosanoids, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) and increase production of interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and IL-6 (Soyland et al., 1994; Tashiro et al., 1998). On the other hand, the n-3 PUFAs, EPA, and docosahexaenoic acid (DHA) suppress the production of AA-derived eicosanoids, thus having comparatively anti-inflammatory and immunosuppressive effects (Tashiro et al., 1998). Several groups have reported that n-3 PUFA-enriched diets (e.g., fish or fish oil) can lead to partial replacement of AA by EPA in cell membranes and significantly reduce the *ex vivo* production of proinflammatory cytokines (Calder, 1998; De Caterina et al., 2004; Mori and Beilin, 2004). Therefore, an imbalance of n-6/n-3 PUFAs may result in an increased production of proinflammatory cytokines. Smith (1991) proposed that abnormal fatty acid composition might be related to the inflammatory response system underlying pathophysiology of major depression. Maes et al. (1997, 1998) have further substantiated the role of n-3 PUFAs in predicting the response of proinflammatory cytokines to psychological stress.

D. Dietary Effects of PUFAs Supplementation

In some but not all preliminary clinical trials, supplementation with the long-chain n-3 PUFAs have significantly improved mood in patients with major depression and bipolar disorder (Stoll et al., 1999; Peet and Horrobin, 2002; Nemets et al., 2002; Emsley et al., 2003; Marangell et al., 2003; Ness et al., 2003; Su et al., 2003). The effect appears to be specific to EPA, and not other n-3 fatty acids, that is, DHA (Marangell et al., 2003). There also appears to be dose specific effects; as higher doses of EPA may not be effective (EPA 6 g/day) (Post et al., 2003). Of particular note is the onset of response with EPA. Peet and Horrobin (2002) found a significant reduction in severity of depressed mood as early as 2 weeks and maximally at 4 weeks. Emsley et al. (2003) found significant treatment response at 4 weeks. Thus, response to EPA occurs relatively rapidly. This may be important in managing patients who are not responding to conventional treatments and remain at risk for complications of depression, such as suicide. In the above placebo-controlled trials, there were no dropouts due to EPA-related side effects. An additional advantage is that EPA is not known to alter levels of psychotropic drugs used in treatment of depression.

IV. OTHER PSYCHIATRIC DISORDERS

The relevance of PUFAs in psychiatry is not limited to schizophrenia and depressive disorders. In fact, low serum levels and/or dietary intake of n-3 PUFA have been implicated in psychopathologies for which problems of impulse control figure prominently, including substance dependence (Buydens-Branchey et al., 2003), borderline personality disorder (Stoll et al., 1999; Zanarini and Frankenburg, 2003), suicide (Huan et al., 2004), and ADHD (Richardson and Puri, 2002; Richardson and Montgomery, 2005). Increasing evidence suggests that such disorders may involve essential fatty acid nutritional deficiencies or imbalance (Richardson and Ross, 2000; Hallahan and Garland, 2004). For example, compared to healthy controls, both children and adults with ADHD have lower levels of n-3 PUFAs in their plasma and RBC phospholipids (Taylor et al., 2000; Stevens et al., 1995; Young et al., 2004). The clinical trial evidence, albeit limited, suggests that supplementation with DHA alone is not effective (Voigt et al., 2001; Hirayama et al., 2004). However, combined EPA and DHA, benefits children with ADHD, dyslexias, or dyspraxias (Richardson and Puri, 2002; Stevens et al., 2003; Richardson and Montgomery, 2005). In addition to improving ADHD symptoms, supplementation with combined n-3 PUFAs reduces parent-rated cognitive problems (i.e., difficulties with attention, concentration, and working memory) (Richardson and Puri, 2002) and improves scores on standardized tests of reading and spelling (Richardson and Montgomery, 2005). Similarly, a number of small, controlled trails have reported that increasing n-3 PUFA consumption improves mood and reduces impulsive and aggressive behaviors (Hamazaki et al., 1999, 2002, 2005; Itomura et al., 2005). For example, EPA supplementation significantly reduced aggression in women with borderline personality disorder (Zanarini and Frankenburg, 2003) and, in incarcerated criminals, supplementation with essential fatty acids and vitamins reduced violent offences by 26% (Gesch et al., 2002).

Additionally, several pediatric behavioral, neurological, and language disorders such as dyslexia (Richardson et al., 2000, 2006), autism (Richardson, 2006), and Asperger's syndrome (Bell et al., 2004) also have been found to be associated with altered levels of phospholipid-PUFAs. Dyslexia is a developmental disorder that is marked by visual dysfunction and difficulties in learning to read and write. It has been noted that dyslexia is associated with increased schizotypal personality features and shares many of the neuropsychological features of schizophrenia including language, attentional, and working memory deficits (Richardson, 2000). Initial studies in a small group of schizophrenic patients have shown impaired electroretinogram (ERG) in patients with reduced levels of RBC-DHA (Peet et al., 2003). Likewise, receptive language difficulties have been observed repeatedly in both schizophrenic patients and in the siblings of individuals with schizophreniform-spectrum disorder (Condray et al., 2002). It is possible that since these disorders are associated with increased risk for adult psychotic disorders (Keshavan et al., 2003), these are related illnesses, even though qualitatively different, and represent a continuum of membrane/phospholipid spectrum disorders.

As discussed previously, PUFA-deficient diets fed to laboratory animals in early development significantly reduces frontal cortex monoaminergic concentrations of dopamine and serotonin as well as their degrading metabolites, 3,4-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindolacetic acid (5-HIAA), respectively (De la Pressa Owens and Innis, 1999). PUFA intake also affects brain development in humans. For example, babies fed formula deficient in long-chain PUFAs have lower brain DHA and poorer cognitive functioning when compared to breast-fed infants (Anderson et al., 1999). Lastly, PUFAs modulate the expression of numerous genes relevant to learning processes (Kitajka et al., 2004).

V. CONCLUSIONS

Evidence to date suggests that the membrane lipid environment has a significant impact on the behavior of neurotransmitter systems. Although, the notion that lipids play a key role in several psychiatric disorders, research has not been exhaustive. Recent investigations have shown that n-6 and/or n-3 membrane PUFAs are significantly lower in schizophrenic and depressive patients than in normal subjects. Such changes in membrane fatty acids not only have been associated with symptom severity but also provide a theoretical basis in predicting psychotropic effect of EFA supplementation. A collaborative 8-nation study of schizophrenia by World Health Organization (WHO) demonstrated a significant correlation between dietary fat intake and the outcome of schizophrenia (Christensen and Christensen, 1988). A good outcome was positively correlated with diets enriched in EFA (e.g., high intake of fish and vegetables). Thus, it is unequivocal that a balanced EFA diet is essential, particularly during the early stages of brain development. On the other hand, to determine whether membrane PUFA deficits are of primary etiological significance in these psychiatric disorders will require further investigation. Since a variety of apparently disparate biological findings have been reported (Lieberman and Koren, 1993; Yao, 2003), very likely there is etiologic heterogeneity that exists a final common pathogenic pathway that mediates the recognizable clinical syndromes. Nonetheless, there is sufficient evidence to support large-scale systematic clinical trials of adjunctive EFA supplementation to modify the course and severity of schizophrenia and depression as well as other clinical group previously characterized by in part n-3 deficiency.

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