Inulin-Type Fructans

Functional Food Ingredients

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Inulin-Type Fructans: Functional Food Ingredients, Marcel Roberfroid

Inulin-Type Fructans

Functional Food Ingredients

Marcel Roberfroid

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To Julie
for all her support.
To George, Paul, Anne, Jan, Glenn
for the exciting adventures
in the exploration of inulin territories.

Foreword

In *Inulin-Type Fructans: Functional Food Ingredients*, Dr. Marcel Roberfroid provides a scholarly and comprehensive review of inulin and inulin-like substances as functional foods, principally in their capacity as "colonic foods." He defines functional foods as naturally occurring substances in food ingested as part of the normal diet that provide health benefits beyond basic nutrition and may reduce the risk of chronic disease. This concept must be distinguished from dietary supplements and nutraceuticals, which are concentrated food components (e.g., lycopene) that are ingested like pharmaceutical products. In an era when the public is better informed about what constitutes a healthy diet and lifestyle, and is also disenchanted with standard forms of medical care, a better understanding of the role of diet in health is needed as an alternative. In addition, the excessive and inappropriate use of antibiotics in animals and humans over the last several decades has resulted in an ever increasing microbial resistance to treatment, resulting in the likelihood of life-threatening diseases, including food-borne infections, particularly in hospitalized young (infants) and elderly patients.

In a recent review in the *New England Journal of Medicine*, Jean-Francois Bach describes the impact of changes in public health worldwide over the last fifty years. He shows that the major infectious causes of death (hepatitis, AIDS, tuberculosis, malaria, etc.) have declined dramatically in developed countries due to public health measures such as improved sanitation, use of vaccines, antibiotics and sterile hospital settings. However, over the same time period, there has been a steady "mirror-image" increase in allergy and autoimmune diseases (e.g., asthma, type 1 diabetes, Crohn's disease). This change in disease prevalence has been explained by the so-called "hygiene hypothesis" which suggests that these public health measures, while reducing life-threatening infection, have actually decreased the microbial burden during development resulting in an increased incidence in immune-mediated disease. The implication is that an appropriate microbial stimulation to the developing immune system with proper initial colonization of the gut is necessary for lifelong health. Inulin-like fructans can function as "prebiotics" to stimulate the increased proliferation of health-promoting colonic bacteria (e.g., bifidobacteria, lactobacilli). Marcel Roberfroid, in conjunction with Glenn Gibson in an article in the *Journal of Nutrition*, coined the term *prebiotic*. Prebiotics are large molecular weight carbohydrates (e.g., oligosaccharides) which are not digested in the small intestine and enter the colon intact as "colonic food" to be fermented by resident bacteria. Because of fermentation producing short-chain fatty acids and an acid milieu, bifidobacteria and other health-promoting organisms (lactobacilli, etc.) proliferate to stimulate active immune function and to prevent infectious diseases. For proper development of mucosal immune responses, this process must occur during infancy. What allows prebiotics to be effective is that these functional foods stimulate the proliferation of

resident endogenous flora rather than an artificial colonization of the gut with non-resident flora, which occurs with the use of probiotics. Early colonization is necessary to stimulate the development of a balanced mucosal immune response.

Dr. Marcel Roberfroid has an encyclopedic knowledge of this field and is well-trained professionally to understand the basic research involved. In this text, he carefully reviews the evidence to support which complex carbohydrates can be considered as prebiotics as well as the experimental evidence to support their function and utility in humans as functional foods. Since the field of prebiotics is relatively new (since 1995), large clinical trials have not been completed to definitively establish their long-term stimulus of endogenous flora. It is known that bifidobacteria and other health-promoting bacteria increase in the colon shortly after ingestion of inulin-type fructans and that their levels decrease with the cessation of their use. However, it would be important to determine whether the prebiotic stimulus can last for extended periods of time (years rather than months) or, instead, whether colonic bacteria can adapt by fermentation occurring with other less desirable bacteria using short-chain fatty acids as food, thus negating the long-term prebiotic effect. In addition, it is presumed that prebiotics, by stimulating proliferation of bifidobacteria and lactobacilli, have the same effect as if those organisms were given as probiotics. However, not knowing their secondary effect is the same as the use of these organisms as probiotics to prevent/treat disease. Additional multicenter trials are needed to confirm this concept.

In addition to the prebiotic effect of inulin-type fructans, these non-digestible oligosaccharides have been shown to have other primary effects (e.g., promote calcium and other mineral absorption, improve elimination of colonic waste, modify lipid metabolism towards a healthier profile, etc.). Again, large additional multicenter trials are needed to support preliminary clinical observations suggesting this effect. Marcel Roberfroid, as Chairman of the ORAFTI scientific group (a nonprofit group of scientists meeting semiannually to review the field of prebiotics) has been committed to filling in the gaps in our knowledge, particularly our clinical knowledge, with regard to the use of inulin-type fructans as functional foods. These activities, along with in-depth knowledge of the subject that appears in this text, provide the basis for what should be considered to be the definitive review of this topic in the field.

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SERIES PREFACE FOR MODERN NUTRITION

The CRC Series in Modern Nutrition is dedicated to providing the widest possible coverage of topics in nutrition. Nutrition is an interdisciplinary, interprofessional field par excellence. It is noted by its broad range and diversity. We trust the titles and authorship in this series will reflect that range and diversity.

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Contributors from any bona fide area of nutrition, including the controversial, are welcome.

We welcome this important and timely contribution to this series. This book will be useful to a broad spectrum of nutritionists and life scientists of all walks.

Ira Wolinsky, Ph.D.
University of Houston
Series Editor

Preface

The current interest in nutritional health and well-being makes this the right time to write a book on inulin-type fructans and their nutritional properties. Even if the concept of "balanced diet" remains the basis for dietary recommendations, new nutrition concepts need to be developed because new challenges have appeared, at least in the affluent societies that characterize most occidental and industrialized countries. These challenges include, among others, the growing costs of medical care and, at the same time, increased life expectancy, better consumer awareness of the relationships between diet and health, impressive progress in scientific knowledge (especially in biology and medicine) and major changes in lifestyles. Moreover, according to the new definition proposed by the World Health Organization (WHO), "health" is no longer limited to the "absence of disease" but also includes physical and psychological well-being. Living longer, while still in good health, is thus a requirement or even an expectation of a large number of consumers in these countries.

Thus, nutrition science must adapt by developing new concepts to be applied to the discovery and validation of new products affecting (beyond what could be expected from traditional nutrition) a variety of body functions relevant to either a state of well-being and health or to the reduction of the risk of disease. Optimum nutrition is such a new concept, aimed at maximizing the physiological as well as psychological functions of each individual through the consumption of food components, with the goal of controlling and modulating body functions to optimize them. In such a context, "functional food" has been proposed as one approach to improve nutrition, and about 15 years of extensive scientific research has demonstrated that inulin-type fructans are among the most interesting and fascinating examples of functional food.

Inulin-type fructans are unique natural food ingredients that are present in a variety of edible plants. Moreover, and because of the diversity of industrial products available and the diversity of their technological attributes, they have already found a wide variety of applications both in human food and animal feed. Inulin-type fructans also have unique physiological effects, the discovery and the understanding of which were at the core of one of the most recent, but already very popular, concepts in nutrition: the concept of "prebiotics." Prebiotics are claimed to provide, by selective modification of the composition of the intestinal microflora, enhanced intestinal and systemic functions, and reduction of risk of diseases.

This book is a review of the research (both experimental and human) data available that demonstrate these physiological effects and contribute to our understanding of their mechanisms. In order to help the reader evaluate these data most accurately, two introductory chapters are included that set the scene by summarizing the state of the art in the science of functional food and in the multiple aspects of

the physiology of the gastrointestinal system. Moreover, at the beginning of each chapter, both a short introduction concerning the basic knowledge in physiology and biochemistry of the particular functions it covers and a survey of the most relevant methodologies that are used to investigate the effects of inulin-type fructans are included. In concluding each chapter and also each part of the book, the results are discussed in detail and critically evaluated, and perspectives for future development are proposed.

After a description of inulin-type fructans, their classification and their terminology, and how inulin-type fructans can be analyzed and quantified, the chapters describe, evaluate, and discuss the nutritional properties of inulin-type fructans. The discussion begins with their effects on the upper gastrointestinal tract, including resistance to digestion, selective intestinal fermentation due to their unique chemical structure, and the beneficial effects on bowel functions, all of which have led to their classification as dietary fiber and low-calorie carbohydrates. This is then followed by a review, evaluation, and discussion of the evidence on the selective and beneficial modification of the composition of the intestinal microflora, a discovery that was at the core of the prebiotic concept proposed in 1995. Finally, three chapters review the data on, and discuss the modulation of, a series of important physiological functions, i.e., mineral absorption, lipid homeostasis, and the body's defenses and their potential to reduce the risk of miscellaneous diseases, especially inflammatory bowel diseases and colon cancer. The last chapter is a general discussion with the objective of confronting the data with the concept of, and the criteria for, the classification of inulin-type fructans as functional food ingredients. For doing so, the data discussed in the different chapters are classified into one of three categories, i.e., the data that justify claims of enhanced function or reduction of disease risk; those that are sound enough to justify human intervention studies (both nutritional and/or clinical) but not yet solid enough to justify claims; and, finally, those that are preliminary but still strong enough to justify further investigations that may, in the future, lead to new claims. In addition, the discussion evaluates the pertinence of classifying inulin-type fructans as a feel-good factor, a new category of food ingredients that fits the new definition of health as well as the demand of consumers for a healthy, long life. The last chapter is also an opportunity to speculate on new ways of looking at the symbiosis that, in the intestine (especially the large bowel), results from the association between the eukaryotic tissue and a large and highly diversified prokaryotic population. Indeed, the microflora that symbiotically colonize the large bowel are key players in keeping the colon (and thus the whole body) healthy as well as in feeling well. This population of unicellular microorganisms is complex and highly diversified, and it is hypothesized that myriad unicellular microorganisms belonging to hundreds of genera, species, and strains that live close together in the large bowel and collaborate to reach a (sometimes precarious) balance of activities are like cells that form a complex tissue-like structure in which the different types of "specialized" (yet to be identified) cells interact to perform a series of physiological functions that are essential for health and well-being. According to that hypothesis, intestinal health and well-being would then result from interactions within and between these two pluricellular worlds. Such an interaction between the two worlds has been referred to as "crosstalk." The multicellular prokaryotic tissue-

like entity would benefit from, but would also provide benefits to, the intestinal mucosa (i.e., the whole and complex pluricellular tissue) and *vice versa*. A major determinant of these interactions would be the composition of these two cell populations, especially that of the prokaryotes. If this speculation turns out to be true, then modulation of the composition of the colonic microbiota by inulin-type fructans thus will become a key determinant of large bowel functions and also, indirectly, systemic functions and finally the host's health and well-being. Conversely, it becomes plausible that systemic dysfunction elsewhere in the body's organs can influence the composition of the colonic flora and, as a consequence, their activities and colonic functions.

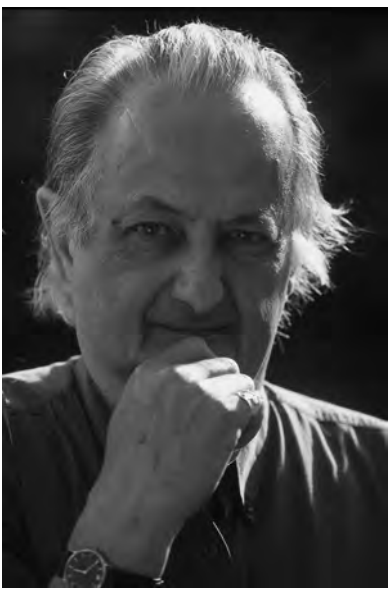
Research on inulin-type fructans has certainly contributed to progress in understanding their nutritional properties, but it has also advanced basic scientific knowledge, especially in gastrointestinal physiology. The recent developments in intestinal endocrinology (e.g., the discovery of incretins), as well as the new data demonstrating active interactions (including up-regulation of gene expression) between some bacteria of the colonic microflora and intestinal cells, appear as the most exciting topics to which such research will contribute in the future.

Before ending this introduction, it must be pointed out that a very large proportion of the scientific data that forms the basis of this book has been generated by studies largely initiated and founded by the European Community, on the one hand, and by the food industry on the other hand. Over the last decade, at least seven multinational research programs approved and funded by the EU Research programs have investigated and substantiated the physiological effects of inulin-type fructans. The acronyms of these programs are ENDO, NUTRIGENE, GUT FLORA, CROWNALIFE, SYNCAN, EU MICROFUNCTION, and PROTECH. More recently, in the U.S., the National Institutes of Health has funded a program on inulin-type fructans and Ca absorption in adolescents (the BONE MAX project), and the National Cancer Institute has included these ingredients in a clinical trial as part of its colon cancer prevention program. During the same period, the food industry has also largely contributed to research. This is the case with a Japanese company (Meiji) that was the first (in the 1980s) to demonstrate that a particular inulin-type fructan, i.e., oligofructose or fructooligosaccharides, selectively stimulated the growth of bifidobacteria in fecal microflora and enhanced mineral absorption in experimental animals. The core of the data available today (especially human data), as well as most of the new developments, including technological developments, results from research projects that have been initiated and largely funded by a Belgian company (ORAFTI) that produces all types of inulin and inulin derivatives extracted from chicory roots at typical farming plants in southern Holland, Belgium, and northern France. It is a fundamental strategy of this company to base its development and business growth on a sound foundation of science, and its competitors largely profit from these investments in research without themselves spending much money.

Writing this book would not have been possible without the kind invitation of Ira Wolinsky of the University of Houston to contribute to the CRC series on *Modern Nutrition*. Also, the review of such a wide variety of data and its scientific evaluation would not have been possible without the many discussions with the best experts

worldwide whom I had the opportunity to visit and to meet many times, and with whom I had so many fruitful and stimulating conversations. This is particularly the case for all my colleagues who joined me in the international BENEO Scientific Committee on inulin that I had the privilege to chair for the last 4 years. These are K. Cashman (Ireland), Ch. Cherbut (France), N. Delzenne (Belgium), A. Franck (Belgium), G. Gibson (U.K.), F. Guarner (Spain), M. Nyman (Switzerland), B. Pool-Zobel (Germany), B. Schneeman (U.S.), J. Van Loo (Belgium), A. Walker (U.S.), and C. Weaver (U.S.). I am also indebted to experts with whom I have had the pleasure to collaborate in different projects, especially St. Abrams (U.S.), J.H. Cummings (U.K.), J. Milner (U.S.), and I. Rowland (U.K.), and many others I cannot list here. To all of them, I extend my sincere gratitude for having joined me in making the inulin story such an exciting scientific adventure, which is still in the early stages of discovery.

Author Biography



Marcel Roberfroid is now a retired professor of the Université Catholique de Louvain in Belgium, the same institution from which he graduated as a pharmacist and completed his Ph.D. in pharmaceutical sciences. He completed his postdoctoral research under B.B. Brodie at the Laboratory for Clinical Pharmacology at the U.S. National Institutes of Health, Bethesda, Maryland. Roberfroid returned to the Université Catholique de Louvain, where he was appointed professor of biochemistry, biochemical toxicology, and experimental nutrition, and where he remained for the rest of his career.

During his academic career, Dr. Roberfroid led the research group that investigated the mechanisms of carcinogenesis, particularly concerning the role of food and nutrition in modulating that process. In Europe, he was also very active in developing the concept of "functional food," and together with his colleague Professor G. Gibson at the University of Reading in the U.K., he conceived of "prebiotics" and "synbiotics," which have become very popular concepts in the science of nutrition. It is because of these concepts that he became involved in the research on inulin-type fructans, and he is now internationally recognized as a leading expert in that field. He has served as the president of the European branch of the International Life Sciences Institute (ILSI Europe) and worked as a scientific consultant for many food industries.

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Part I

Introduction

1 Functional Foods and Claims: Concepts, Strategy of Development, Requirements for the Scientific Substantiation of Claims, and Communication with Consumers

1.1 NUTRITION IN THE 20TH CENTURY: FROM PREVENTION OF DEFICIENCIES TO REDUCTION OF RISK DUE TO EXCESSIVE CONSUMPTION OF NUTRIENTS

Functional food has been introduced as a new concept because the science of nutrition has changed. During the 20th century, nutritionists have discovered the essential nutrients, and they have established nutrient standards (Dietary Reference Intakes), dietary guidelines, and food guides (Figure 1.1). The main objectives of nutrition research was to make recommendations in order to support body growth, maintenance, and development; to prevent nutrient deficiencies[1]; and, more recently, to avoid excessive consumption of some of these nutrients after recognizing their potential role in the etiology of miscellaneous (mostly chronic) diseases.[2–3] In such a context, one of the major contributions of nutrition science during the 20th century has been the concept of the "balanced diet" (Figure 1.1) defined as "*an appropriate mixture of food items that provides, at least, the minimum requirements of nutrients and a few other food components needed to support growth and maintain body weight, to prevent the development of deficiency diseases and to reduce the risk of diseases associated with deleterious excesses.*"[1]

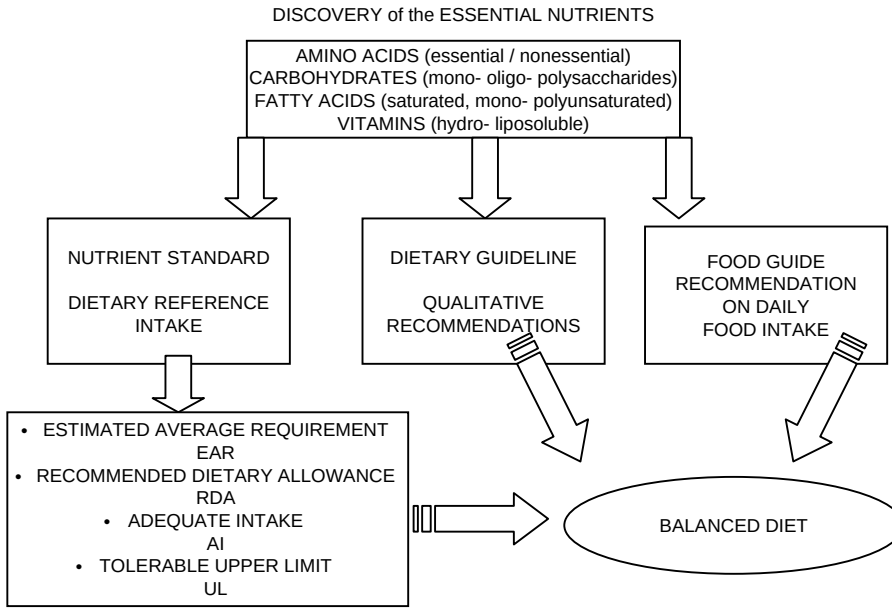

FIGURE 1.1 Summary of the main achievements of the science of nutrition during the 20th century.

1.2 NUTRITION AT THE TURN OF THE 21ST CENTURY: NEW CHALLENGES

At the turn of the 21st century, the "balanced diet" and the recommendations it supports remain key concepts. At least in the society of abundance that characterizes most of the occidental and industrialized countries, new nutrition concepts need to be developed and new dietary recommendations need to be elaborated as new challenges have appeared (Figure 1.2).

These challenges include, among others:

- Growing costs of medical care
- Increase in life expectancy
- Progress in scientific knowledge, especially in the biological and the medical sciences
- Application of new technologies to food development, food production, and food storage
- Major changes in lifestyles

Moreover, according to the new definition recently proposed by the World Health Organization (WHO) "health" is not only absence of disease but includes physical and psychological well-being. Thus, nutrition must adapt by developing new concepts and, consequently, by elaborating additional recommendations.

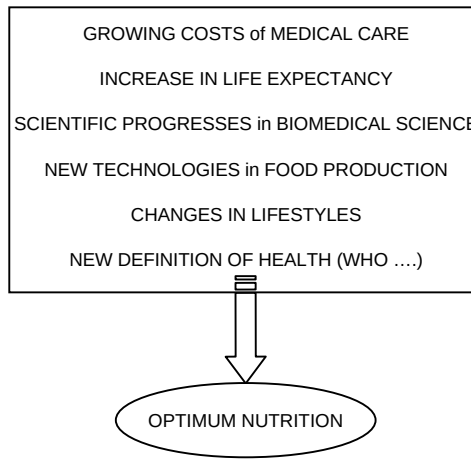

FIGURE 1.2 Summary of the main challenges of the science of nutrition at the turn of the 20th century.

1.3 THE CONCEPT OF OPTIMUM NUTRITION

Optimum nutrition[4] is a very new concept. It aims at maximizing the physiological as well as psychological functions of each individual through nutrition in order to ensure both well-being and health but at the same time, a minimum risk of disease throughout the lifespan. It relies on the hypothesis that the optimized consumption of food components will control and modulate body functions to maximize their efficiency. In such a context, "functional food" has been proposed as one approach to improve nutrition.

1.3.1 Functional Food: A Nutrition Concept

As the science of nutrition progresses, a wide variety of foods are or will, in the future, be characterized as functional food with a variety of components affecting a variety of body functions relevant to either a state of well-being and health and/or to the reduction of the risk of a disease. Consequently, the term *functional food* has as many definitions as the number of authors referring to it.

As discussed previously,[5] these definitions range from simple to elaborate. Simple descriptions may be:

- Foods that may provide health benefits beyond basic nutrition[6]
- Foods or food products marketed with a health benefit message[7]
- Everyday food transformed into a potential life saver by the addition of a "magical" ingredient[8]

Elaborate definitions of functional foods may be:

- Food and drink products derived from naturally occurring substances consumed as part of the daily diet and possessing particular physiological benefits when ingested[9]
- Food derived from naturally occurring substances that can and should be consumed as part of the daily diet and that serve to regulate or otherwise affect a particular body process when ingested[10]
- Food similar in appearance to conventional food, which is consumed as part of a usual diet and has demonstrated physiological benefit and/or reduces the risk of chronic disease beyond nutritional functions[11]
- Food that encompasses potentially helpful products including any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrients it contains[12]

However, the term *functional food* cannot represent a single well defined and well-characterized entity. Instead, functional food has to be understood as a concept that belongs to nutrition and not to pharmacology. It deserves a category of its own, a category different from *nutraceutical, farmafood (pharmafood), medifood, designer food, vitafood*, or any other term that tends to mix up food and drugs. A functional food is, and must remain, a food not a drug and, except in very particular and very exceptional situations, it is not developed to have therapeutic effects. Moreover, its role regarding disease will, in most cases, be in "reducing the risk" rather than "preventing (i.e., suppressing)" it. The regular consumption of a truly functional food as part of the usual recommended diet should be prescribed because it has been scientifically proven to significantly reduce the likelihood of getting a particular disease.

To elaborate the concept of functional food, the following features need to be taken into consideration, and the food or food ingredient must be:

FIGURE 1.3 Functional food: Key features.

- A conventional, everyday food
- Naturally occurring
- Proven to have beneficial effects on target functions beyond nutritive value and basic nutrition
- Proven to enhance well-being and health, reduce the risk of a disease, or improve the quality of life including physical, psychological, and behavioral performances

1.3.2 Functional Food: A Consensus of the European Scientific Community

A European Commission Concerted Action program known as Functional Food Science in Europe (FUFOSE) was initiated in early 1996, coordinated by the European branch of the International Life Science Institute (ILSI Europe). In 1998, it reached a consensus (known as the "European Consensus on Scientific Concepts of Functional Foods"[13]) which proposes the following working definition of a functional food:

> *A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease. A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet: it is not a pill or a capsule, but part of the normal food pattern.*

This definition describes all the main features of functional foods, i.e.:

- Their food nature and their consumption as part of a normal food pattern
- The requirement for a scientific demonstration of the effects
- The beneficial effects on body functions beyond adequate nutritional effects
- The relevance of these effects to improved well-being and health and/or reduction of disease risk

It aims to stimulate research and development in the field of nutrition contributing to set standards for an optimized nutrition. From a practical point of view, a functional food can be:

- A natural food
- A food to which a component has been added
- A food from which a component has been removed
- A food in which the bioavailability of one or more components has been modified
- Any combination of these possibilities

1.3.3 The Strategy for Functional Food Development

As described in the European Consensus Document:[13]

"*The design and development of functional foods is a key issue, as well as a scientific challenge, which should rely on basic scientific knowledge relevant to target functions and their possible modulation by food components.*" Emphasis is then put on the importance of "*the effects of food components on well-identified and well-characterizes target functions in the body that are relevant to well-being and health issue, rather than, solely, on reduction of disease risk.*" In order to achieve such a development, it is necessary to identify potential functional foods or functional food components and, at least partly, to understand the mechanisms by which they modulate target functions (Figure 1.4).

These target functions have to be recognized or proven to be relevant to the state of well-being and health, and/or the reduction of a disease risk. When such a functional effect is demonstrated, it will be used to formulate hypotheses to be tested in human nutrition studies aimed at showing that adequate (in terms of dose, frequency, duration etc.) intake of the specified food or food component will improve one or more target functions, that are, either directly or indirectly in terms of a validated marker, relevant to an improved state of well-being and health and/or to a reduced disease risk.

Human nutrition studies should be hypothesis driven; they should aim at testing the effect of a food as part of the ordinary diet consumed, in most cases, by the general population or large, at-risk target groups; and they should not use a risk vs. benefit approach. Most of these studies will rely on change(s) in validated or relevant markers to demonstrate a positive modulation of target functions after (long-term) consumption of the potential functional foods. A double-blind type of design based

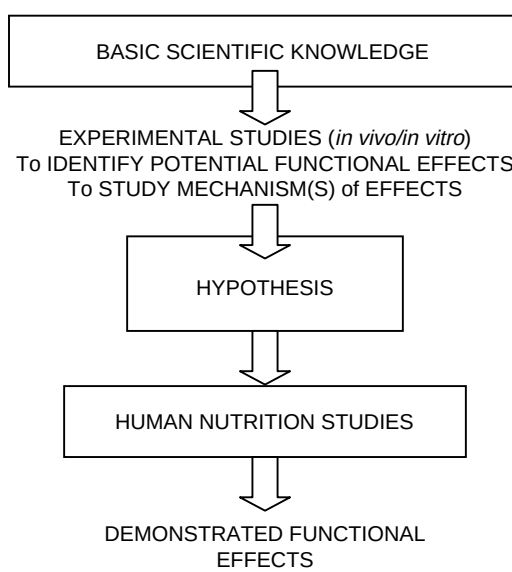

FIGURE 1.4 Functional food: Strategy of development.

on parallel groups rather than crossing-over will generally be appropriate. Data of these studies should be collected and handled according to standards for data management, and data analysis should prove statistical, but perhaps most importantly, biological significance. The markers to be used for the development of functional foods need to be characterized and validated for their predictive value of potential benefits to well-being and health or the risk of a disease.

1.3.4 Type A and Type B Claims

As stated in the European consensus on scientific concepts of functional foods:[13]

"As the relationship between nutrition and health gains public acceptance and as the market for functional foods grows, the question of how to communicate the specific advantages of such foods becomes increasingly important." Its importance also lies in avoiding problems associated with consumer confusion about health messages. Regarding functional foods, making claims associated with specific food products is the preferable means of communicating to consumers, provided these claims are not misleading but true, as well as scientifically valid, unambiguous, and clear. A general definition of "claim" is widely accepted in the field of nutrition as "any representation which states, suggests, or implies that a food has certain characteristics relating to its origin, nutritional properties … or any other quality."[14] But the fact that distinct types of claims exist makes it difficult to communicate specifically the benefits of functional foods. This is made even more difficult because the term "*health claims*," which is traditionally used to communicate the benefits of foods is understood differently in different parts of the world. Seeking for clarity, Codex Alimentarius has recently classified and defined four different categories of claims, i.e.:

- Claims that relate to dietary guidelines
- Claims that relate to nutrient content
- Claims that are comparative
- Claims that describe nutrient function

Also, they have excluded a category covered by the term "health claim." These claims refer to known nutrients and their role in growth, development, and normal functions as well as to the concept of adequate nutrition. They are based on established, widely accepted knowledge but they do not refer to a particular effect over and above that expected from consuming a balanced diet. These claims are thus not really helpful to communicate the specific benefits of functional foods. Indeed, the claims for functional foods should be based on effects that go beyond what could be expected from the established role of diet. If such effects concern a target function or a biological activity without direct reference to a particular disease or pathological process, the claim will be made for an "enhanced function." But, if the benefit is clearly a reduction of the risk of a disease or pathological process, the claim will be made for a "disease risk reduction." These two types of claims that are specific for functional foods are identified in the European consensus document as "Type A" and "Type B" claims, respectively.[13]

One of the major issues still to be resolved, especially with these two types of claims, concerns the biological level at which evidence can be accepted as "satisfactorily demonstrating" an enhanced function or a reduction of disease risk. This evidence should rely on all data available that can be grouped in three categories[5] (Figure 1.5):

- Biological observations
- Epidemiological data
- Intervention studies, mostly based on markers

All supporting evidence should, however, be:

- Consistent in itself
- Able to meet accepted scientific standards of statistical as well as biological significance, especially dose–effect relationship, if relevant
- Plausible in terms of the relationship between intervention and results, especially in terms of mechanisms of action
- Provided by a number of sources (including obligatorily human studies) that give consistent findings that are able to generate scientific consensus

1.3.5 The Communication Challenge

Regarding the development and marketing of functional foods, truthful, clear, and educational communication to the consumers remains a challenge.[5] New concepts and new approaches to communicate about functional foods and market them are

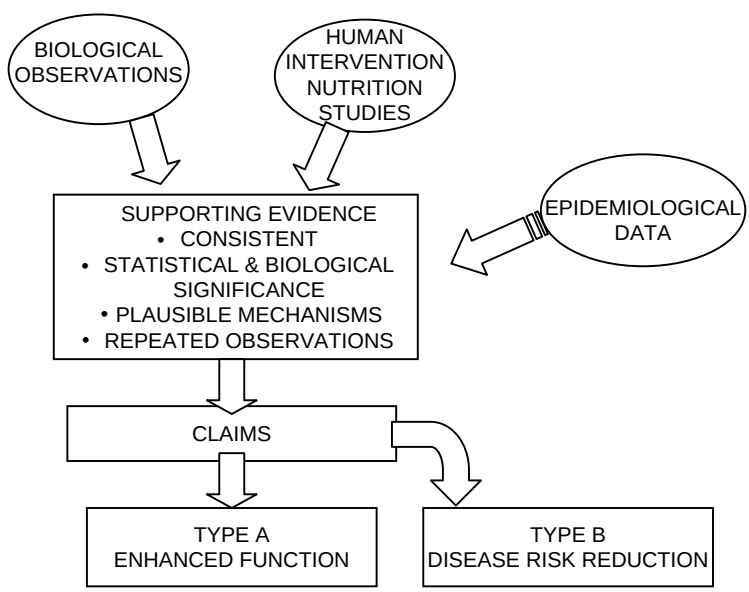

FIGURE 1.5 Functional food: Scientific substantiation of claims.

therefore needed. These should be based on specific consumer research aimed at finding out how consumers think and talk about health and how they feel about functional foods.

In March 2002, Cogent Research of Cambridge, MA, conducted a survey with 1004 randomly selected U.S. adults by the International Food Information Council (IFIC).[15] The major conclusions of that survey are that:

- Almost all (94%) agree that certain foods have health benefits that go beyond basic nutrition and may reduce the risk of disease or other health problems.
- A majority (71%) believe that food and nutrition play "a great role" in maintaining or improving overall health.
- Almost two thirds (63%) say they are eating at least one food to receive a functional health benefit.

According to the IFIC,[15] to develop awareness of the potential health benefits of functional foods, "consumers need a clear understanding of and a strong level of confidence in the scientific criteria that are used to document health effects and claims. When communicating about functional foods, it is important to translate quality science into understandable and usable messages that meet consumer needs." Also, IFIC recommends the following specific communication strategies:

- Cite the need for credible, scientific criteria as the basis for messages about functional foods and the development of new food products.
- Accentuate the "good news" about food.
- Place new research findings into context with the overall body of scientific evidence.
- Discuss the benefits of particular components within the context of familiar foods and overall eating patterns.
- Do not overstate potential benefits.
- Ensure that any claims made on- or off-label are based on reasonable and responsible information.[15]

Combining basic and nutrition research, functional food development, consumer research, and marketing experience are thus major challenges for the functional food industry.

1.3.6 Communication on the Functional Effects of a Prebiotic: An Example

In the particular field of prebiotics and their effects on gastrointestinal functions that are a major target for functional food development (and the main topic of this book), a communication platform has recently been developed with the objective to meet the criteria discussed above. It aims at being a coordinated and balanced way to communicate about gut health to the consumer. It is known as the BENEO® Program, and it is composed of four main elements that are combined to support health messages for everyday foodstuffs:[16]

- Communication based on science
- Clear, simple, and positive messages
- Appearance of a logo (the BENEO® symbol) on all products and carriers of information that participate in the program
- Consistent communication from different partners

The BENEO® logo indicates the presence of an active ingredient (i.e., chicory inulin or any derived product) in sufficient amount to have the benefits claimed on the product pack. It refers to communication supports that are available to the consumer. Such a communication is developed to be simple to understand, clear, and direct, and also positive. Indeed, the consumer is much more interested in positive messages (enhanced function claims) than in disease risk reduction claims. Moreover, functional foods with a positive message have the potential to become real "family products" that anybody may consume at any time.[16] This aspect is probably the most underestimated of functional food marketing today. Indeed, too often functional foods are believed to be medicines disguised as food.

To support the development of the BENEO® communication program, consumer research has been performed in several countries including Belgium, which is often considered as a test market for foods in Europe.

The first part of the research is qualitative. It uses a focus group approach to confront consumers with marketing concepts and techniques. Such a research provides information on how consumers think about food and health (especially intestinal health) and on how they talk about these subjects. It allows formulating proposals and establishing guidelines for communication choices.

The most important conclusion of this part of the research is that consuming functional foods is an easy way to do something beneficial for health. In addition and regarding intestinal health (the major target of prebiotics), the most important information in terms of consumer's perceptions are:

- "Intestinal health" is not a common concept or an easy subject to talk about.
- But "intestinal transit" is seen as a common problem.
- The term *intestinal flora* does exist in the mind of the consumer and it is not a taboo subject.
- It is understandable that a food product can beneficially affect bacteria in the intestines and therefore have benefits in the short term as well as in the long term.
- A food ingredient that improves the intestinal flora is seen as a natural way of protecting the body and making it function better.

In the second, more quantitative, phase of consumer research, actual food products based on the BENEO® concept were tested and evaluated in large groups of women (±500/group), each woman receiving, a different food product packaged in a white box as a powder to be added to the diet. The positioning concepts of the products were explained to the consumers in an interview before the test phase that lasted for 30 d, after which a second interview was done to collect reactions, experiences, and

opinions. The main element of the product positioning was the novelty of the product presented as a "new active plant fiber that nourishes our own good intestinal flora."

The most important general conclusions of this research are:

- The attractiveness of the concept (often translated by the European consumer as "to facilitate the transit" or "good for the intestinal flora)".
- The reports from about two thirds of the consumers of having experienced either an improvement in intestinal function, especially a better transit, or a feeling of well-being.

Based on the results of the consumer research, it was concluded that:

- "Gut health" and "intestinal well-being" are meaningful terms.
- "Gut flora" and "intestinal transit" are familiar European (but probably not US) concepts.

The fact that it is difficult to talk about these terms (especially in the US) does not mean that communication concepts cannot be built around them. Moreover, it is clear that consumers can actually experience a feeling of increased intestinal well-being, especially after consuming chicory inulin or any derived product.

Consumer research can also guide selection of the best channels to communicate the message to consumers. It is clearly not a matter of massive advertising. The types of media used to communicate are also important and, again, this differs from country to country. In Belgium, there is high emphasis on providing correct information to health professionals, dieticians, nutritionists, and physicians.

An essential element of the BENEO® program is the BENEO® Scientific Committee composed of independent scientific experts in health and nutrition (see the Preface). In view of the strong requirement for a scientifically sound communication, it has several tasks. The first task is to put forward the general principles for functional food development (see above) that all partners in the program should respect. Based on these principles, the second task is to review and evaluate the scientific data available concerning the functional effects of inulin or any of its derivatives and to help formulate any communication messages (including the wording to be used) to the health professionals and/or the consumers.

The Scientific Committee gives recommendations and guidance for further research projects. It is a source of valuable scientific input.

1.3.7 Perspectives in Functional Food Development and the Case of the Prebiotics

The concept of functional food, as it has been developed in Europe over the last decade, is an interesting and very stimulating concept. It is one promising opportunity to tackle the new challenges facing the industrialized societies at the beginning of the new century. But today it is still mainly a scientific challenge, and its success in helping to develop and elaborate what might become the "optimized nutrition" will strongly depend on scientific progress in the science of nutrition in the next years to come (Figure 1.6).

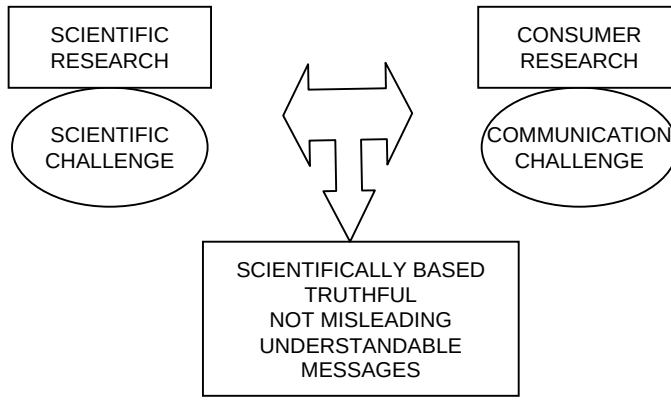

FIGURE 1.6 Functional food development: Perspectives.

The development of functional foods must rely on good science. In particular human data generated by good and well-designed human nutrition studies are an essential requirement for functional food development and for the substantiation of claims. But the communication of scientifically valid "claims" is also a major challenge. If such a communication needs to be clear and understandable, it must also, and more important be truthful and not misleading, as well as adapted to consumers' skills.

The Council of Europe[17] recently issued "Guidelines Concerning the Substantiation of Health-Related Claims for Functional Foods" and ILSI Europe is presently coordinating a new EU-founded concerted action to elaborate further on the Process for the Assessment of Scientific Support for Claims on foods (PASSCLAIM).

As identified in the FUFOSE project,[18] the main targets for functional food development are

1. Growth, development, and differentiation
2. Substrate metabolism and the syndrome X
3. Defense against reactive oxidative species
4. Cardiovascular functions
5. Gastrointestinal physiology and functions
6. Behavioral and psychological functions

Among these, gastrointestinal physiology and functions are key topics that have already attracted a great deal of interest both in the scientific community and the food industry. The physiology of the large bowel and the composition and activities of the microbial ecosystem which colonizes it are major targets which are especially attracting a great deal of interest, as shown by the most recent developments in the fields of probiotic, prebiotic, and synbiotic.[19–21]

Progress has also been made in improving communication to health professionals and consumers. The BENEO® program is one example that demonstrates how

consumer research can complement scientific research to improve the quality and the readability of messages.

This book has two objectives: first, to review extensively the scientific data available on the nutritional properties of chicory inulin and all its derived products. These are model prebiotics that are classified as dietary fiber. The second objective is to discuss their properties by reference to the concept of functional food introduced above. Taking into account the strategy for functional food development that includes basic and experimental research to formulate hypotheses of functional effects to be tested in human nutrition studies, the book is aimed at validating claims to be used to communicate the effects of these food ingredients. As functional food science is a part of the science of nutrition, each chapter is introduced by summarizing the basic scientific knowledge underpinning the relevance of effects on well-being and health, and the reduction of disease risk. The literature reviewed includes all papers published before the end of January 2004, plus a few reports in press or in preparation that the author has had the opportunity to review.

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2 The Gastrointestinal System: A Major Target for Functional Foods

2.1 THE ANATOMY OF THE GASTROINTESTINAL SYSTEM[1]

The human gastrointestinal system is composed of the organs through which the nutrients (i.e., the nutritious substances: carbohydrates, peptides and proteins, lipids, nucleic acids, and vitamins and minerals), other food components (e.g., the phytochemicals), and water, as well as also toxic chemicals and microorganisms (both potentially beneficial or potentially harmful), enter the body.[1] Complex molecules such as proteins, lipids, and some carbohydrates are broken down (digested) into absorbable units (essentially, amino acids and some small peptides, monoglycerides, and fatty acids) and, eventually, into glycerol, simple carbohydrates, or monosaccharides (glucose, fructose, and galactose), respectively. The products of digestion plus vitamins, minerals, and water, as well as other food components, are absorbed, and enter the blood and lymph circulation. Some complex carbohydrates and some peptides or proteins, however, resist the digestive process, but they are fermented essentially in the colon because of the presence of commensal microflora. Most of the exogenous microorganisms are destroyed by various defense mechanisms, but some survive and become part of the microbial ecosystem that permanently colonizes the gastrointestinal system with either beneficial (as is the case for probiotic bacteria) or harmful (as is the case for pathogens) consequences.

The digestive, absorptive, and fermentative functions of the gastrointestinal system depend upon a variety of mechanisms that soften and dissolve the food, propel the chyme (the semifluid mass of partly digested food), and mix it with different exocrine secretions. These mechanisms depend upon intrinsic properties of the smooth muscle, involve the operation of visceral reflexes, or are under the regulation of hormones.

The human gastrointestinal system is composed of the gastrointestinal tract and the attached exocrine glands, i.e., the salivary glands, the liver plus the gall bladder that stores and secretes the bile, and the pancreas (Figure 2.1).

The gastrointestinal tract is the alimentary, tubular, food-carrying passage extending from the mouth to the anus. The different parts of the alimentary canal are: the oral cavity, the esophagus, the stomach, the small intestine composed of the duodenum, the jejunum and the ileum, the cecum plus the colon or large bowel that contains the colonic microflora, and the rectum. The large bowel (or large gut, or

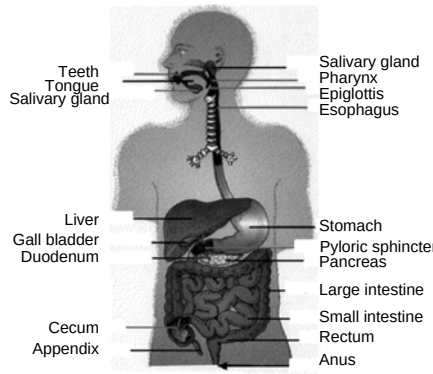

FIGURE 2.1 Schematic representation of the gastrointestinal system.

colon) starts at the ileocecal junction and continues to the anus.[2] It is composed of the cecum, ascending colon, transverse colon, descending colon, and sigmoid rectum.[3] The proximal colon (or right colon) consists of the cecum and ascending colon, whereas the distal colon (or left colon) comprises the descending colon and sigmoid rectum.[4]

The epithelium of the alimentary canal is composed of different types of cells that are specialized in the absorption of nutrients, in the synthesis of mucus, in miscellaneous secretions, or in endocrine activities. Below the epithelium is the connective tissue that contains the immune cells that form the Gastrointestinal-Associated Lymphoid Tissue or GALT, the vessels, and the nerves. The epithelium, along with the subepithelial connective tissue, forms the mucosa. Below the mucosa are two layers of smooth muscles — the longitudinal and circular, including sphincters that control the motility of the tract, and regulate the circulation of the chyme or the delivery of the secretions.

2.2 THE DIGESTIVE FUNCTIONS

The physiological functions of the gastrointestinal system involve digestion and fermentation, absorption and excretion, and motility. Each function is associated with one or a few gastrointestinal organs (Table 2.1).

2.2.1 Digestion and Fermentation

Digestion is a complex process. It includes masticating, mixing, and triturating the food to disrupt the matrix followed by solubilization of the components and micelle formation with the nonsoluble products (especially fats), and, finally, partial or complete hydrolysis of the complex food molecules (peptides and proteins, lipids, some complex carbohydrates, and nucleic acids).

Strictly speaking, digestion comprises only the final hydrolytic step aimed at decomposing (Figure 2.2):

TABLE 2.1
Organs of the Gastrointestinal System and Their Major Physiological Functions

	Mixing	Solubilization and Emulsification	Motility	Digestion and Fermentation	Absorption	Excretion	Defense
Oral cavity	÷	÷	÷	÷	÷		÷
Esophagus			÷				
Stomach	÷		÷	÷	÷		÷
Pancreas		÷		÷ ÷ ÷			÷
Liver bile		÷		÷ ÷			÷
Small intestine	÷	÷	÷	÷ ÷	÷ ÷ ÷		÷ ÷
Colon			÷	÷ ÷	÷ ÷	÷ ÷ ÷	÷ ÷
Colon flora				÷ ÷			÷ ÷

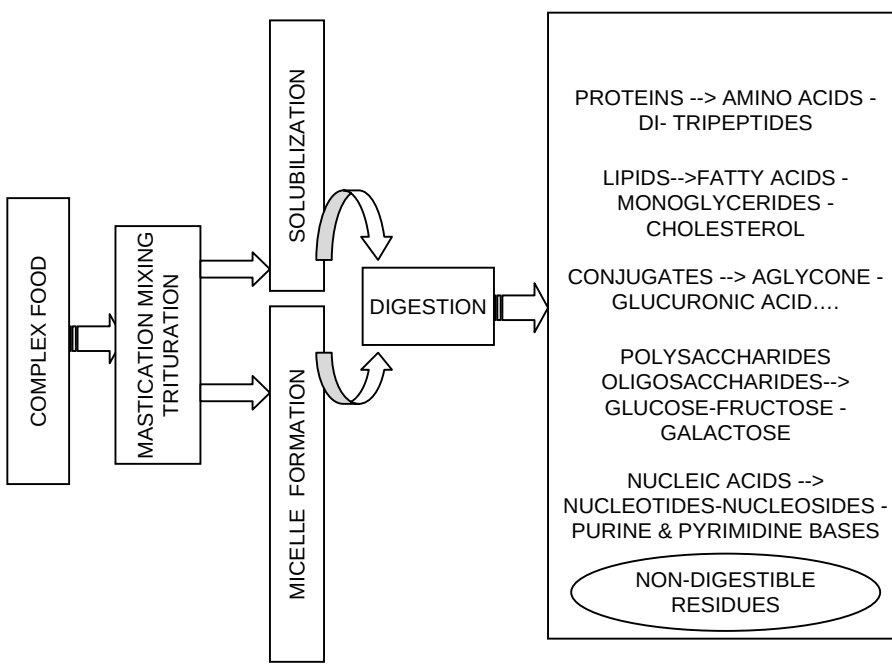

FIGURE 2.2 Schematic representation of the digestion of food.

- Proteins and peptides into amino acids, and di- and tripeptides
- Lipids into monoglycerides, fatty acids and, eventually, glycerol and cholesterol
- Polysaccharides, essentially starches, into free glucose, maltose, maltotriose, and a-limit dextrins (branched polymers containing an average of 8 glucose units)
- Oligosaccharides such as lactose, maltose, maltotriose, a-limit dextrins, and sucrose to glucose, galactose, and fructose
- Nucleic acids into nucleotides, nucleosides or, finally, pyrimidine or purine bases
- Complex conjugated food components into the aglycone plus phenol, alcohol, amino acid, or carboxylic acid

Though the hydrolysis involves chemical processes, it mainly involves enzymatic processes. The most active enzymes are proteases, peptidases, lipases, phospholipases, amylases, glucoamylases or maltase, sucrase, lactase, nucleases, nucleosidases, and phosphatases (Table 2.2). The pH varies in the different organs of the gastrointestinal system to reach the optimum activity of the enzymes they contain.

2.2.1.1 The Oral Cavity

The first step in digestion is mastication in the oral cavity to destroy the matrix, and to mix and triturate the food. But the oral cavity also receives the secretions of the salivary glands (parotid, sublingual, and submaxillary glands), the primary role being moistening of the food. Moreover, because it contains some enzymes (mainly the salivary amylase or ptyalin, plus a low amount of lipase and ribonuclease), the saliva also initiates the hydrolytic process. In addition, the secretions are rich in mucins that act as lubricants, and they are germicidal (via lysozyme and lactoferrin). Saliva also protects the teeth by means of a Ca-binding protein rich in proline, and it contains immunoglobulins A (IgA) that play a role in the defense mechanisms to protect the

TABLE 2.2
Major Hydrolases Secreted by the Human Exocrine Pancreas

Food Substrates	Pancreatic Enzymes
Proteins/peptides	Trypsin
	Chymotrypsin
	Collagenase
	Carboxypeptidase
Lipids	Lipase
	Phospholipase
	Esterase
Carbohydrates	a-Amylase
Nucleic acids	Ribonuclease
	Deoxyribonuclease

body against antigens. Finally, the salivary glands also secrete a large number of physiologically active substances (e.g., growth factors, vasoactive proteases, regulatory peptides, etc.), the exact role of which is largely unknown. The moistened and lubricated food bolus coming out of the oral cavity, which is already partly decomposed and hydrolyzed, is propelled through the esophagus into the stomach.

2.2.1.2 The Stomach

In the gastrointestinal tract, the stomach is the enlarged segment located between the esophagus and the small intestine. It functions temporarily as a storage organ, but its primary role is to continue the processing of food decomposition and food hydrolysis. The role of the stomach in the absorption of water-soluble nutrients or food components is limited, with the exception of ethanol that is, however, absorbed more slowly in the stomach than in the small intestine.

The stomach has a glandular mucosa that secretes:

- High concentration of hydrochloric acid (HCl), which creates a very acidic pH (±1) that denatures proteins, facilitates the hydrolytic processes, and acts as a bactericide
- Pepsinogen, which is hydrolyzed to pepsin at the stomach pH
- Gastric mucus to form a gel that coats and protects the mucosa against the aggressive acidic pH
- HCO_3^- that, in the mucus-protected mucosa, neutralizes the acidity to maintain a neutral pH (±7)
- The intrinsic factor, a glycoprotein that will bind vitamin B_{12} in the ileum so as to permit its absorption
- Miscellaneous hormones
- A gastric lipase that plays a minor role

The stomach releases the processed food known as chyme at a controlled steady state into the duodenum. The acidic pH of the chyme stimulates the flow of bile and pancreatic juice. The rate of stomach emptying into the duodenum depends on the composition of the food, the fastest being for carbohydrate-rich food and the slowest for fatty meals, with protein-rich food being in between. Osmolarity of the chyme also influences gastric emptying. Indeed, hyperosmolarity is sensed by osmoreptors in the duodenum that initiate a decrease in gastric emptying. Such an effect is also neurally mediated when products of protein hydrolysis and protons (H^+) bathe the duodenum mucosa (the enterogastric reflex). Finally, gastric emptying is also controlled by various hormones, especially the gastric inhibitory peptide or GIP.

2.2.1.3 Exocrine Pancreas, the Bile, and the Small Intestine

The small intestine is the organ of the gastrointestinal tract where the final steps in the digestion and absorption of most of the nutrients and food components take place. It is the receptacle for pancreatic juice (the secretions of pancreas) and bile (produced by liver), the two key players in the hydrolysis of proteins, lipids,

oligosaccharides, and polysaccharides, as well as conjugated food components. But it also has its own hydrolases (especially lactase, sucrase, and a-glucoamylase) synthesized by the enterocytes, and it is particularly active in absorbing amino acids, lipids, fatty acids, monosaccharides, vitamins, and most minerals. In a day, the small intestine receives ±2 l of dietary fluid plus ±7 l of gastrointestinal secretions; out of this ±9 l of fluid, only ±2 l will pass into the colon.

The exocrine pancreas is composed of alveolar glands that are similar to salivary glands. The glands are composed of acinar cells that discharge granules containing digestive enzymes (the zymogen granules) by exocytosis into the small pancreatic ducts that coalesce into the duct of Wirsung. The exocrine pancreas produces two secretions: one is electrolytic, and the other is enzymatic. The electrolytic secretion is alkaline (pH = 7.5–9) because it is rich in HCO_3^- (±113 meq/l). It buffers the acidic chyme coming out of the stomach and creates the optimum pH for the activity of the enzymes secreted by the pancreas. The enzymatic secretion of the pancreas contains a large number of different hydrolases (proteases, lipases, and amylases) (Table 2.2). The very powerful protein-splitting enzymes are secreted as zymogens, the inactive proenzymes that become activated in the duodenal lumen through an interconnected cascade of enzymatic reactions (Figure 2.3).

Trypsin also activates a prophospholipase A_2 into phospholipase A_2 that hydrolyses lecithin to produce lysolecithin. The other hydrolases (lipases, amylases, and nucleases) are secreted in their active forms. The exocrine pancreatic secretions are primarily under the control of two hormones: (1) secretin, which stimulates the electrolytic secretion and (2) cholecystokinin (CCK), which acts on the acinar cells to cause release of the zymogen granules. The secretion of CCK is stimulated by the presence of peptides, amino acids, fatty acids, or Ca in the duodenum.

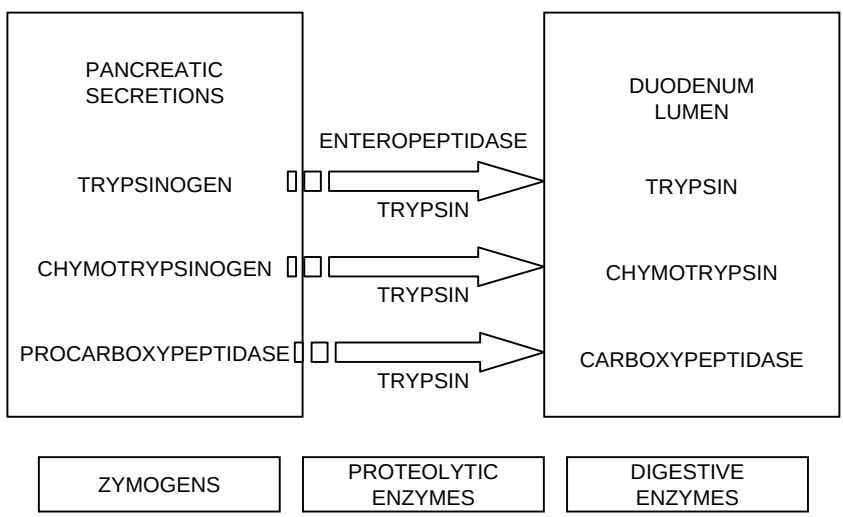

FIGURE 2.3 Schematic representation of the activation of proteolytic zymogens in the duodenal lumen.

The bile is synthesized by the hepatocytes and secreted in the bile canaliculi that are apposed to the hepatic cells. These canaliculi coalesce via the intralobular bile ducts to form the right and left hepatic ducts that join outside the liver to form the common hepatic duct. The common hepatic duct then unites with the cystic duct to form the bile duct. The cystic duct drains the gallbladder where the bile is stored and concentrated (because of water absorption) between meals. The bile duct enters the duodenum via the ampoule of Vater in which the bile mixes with the secretions of the exocrine pancreas. The sphincter of Oddi is usually closed but relaxes when food that enters the oral cavity surrounds the bile duct. Furthermore, the hormone CCK, secreted by the intestinal mucosa when the chyme enters the duodenum, causes the gallbladder to contract, thus increasing the bile flow in the cystic and bile ducts.

Bile (±0.5 l/d in human adults) is a solution of alkaline electrolytes (similar to the pancreatic juice) that contains biliary acids, bile pigments, and traces (0.1%) of cholesterol, phospholipids, fats, fatty acids, and proteins (some of which are with enzymatic activity, e.g., alkaline phosphatase). In a process known as enterohepatic circulation, some of the components of the bile are reabsorbed in the intestine to be transported into the liver via the portal vein, and then, to be excreted again into the small intestine via the bile.

The bile pigments (bilirubin and biliverdin) are the end products of the catabolism of hemoglobin. Bilirubin is present in bile as a water-soluble glucuronide. But in the intestine, the conjugate is hydrolyzed and the bilirubin is reabsorbed (enterohepatic circulation), or it is further metabolized to biliverdin (mostly by the anaerobic bacteria in the large bowel, as discussed shortly) to a series of colorless compounds known as sterco- and urobilinogens.

The bile acids are synthesized in the liver cells from cholesterol and actively secreted via the bile into the small intestine as water-soluble Na^+ and K^+ salts of glycine and taurine conjugates (±20–30 g/24 h). They are effectively reabsorbed (via an active transport in the ileum and a passive transport in the large bowel) via the enterohepatic circulation (±6–7 cycles/24 h). Thus, fecal excretion remains limited (±0.6 g/24 h), but this still represents the major excretion route for cholesterol. The major bile acids synthesized in the liver are the primary bile acids: cholic and chenodeoxycholic acids. In the colon, the anaerobic bacteria metabolize them to produce the secondary bile acids (deoxycholic and lithocholic acids) and, eventually, the tertiary bile acid (ursodeoxycholic acid) (discussed later in Section 2.2.1.4). The bile acids are both hydrophilic (because of the polar peptide bond plus the carboxyl and hydroxyl groups on the surface) and hydrophobic (because of the cycloperhydrophenanthrene nucleus). They are amphipathic, a characteristic that allows them to form micelles. When the critical micelle concentration is reached, lipids collect with cholesterol in the hydrophobic center, and the amphipathic lipids (phospholipids and glycerides) line up with their hydrophobic tails in the center and their hydrophilic heads on the outside. These micelles play an essential role in solubilizing the lipids and lipid-soluble vitamins so as to make them sensitive to enzymatic hydrolysis, as well as to facilitate their intestinal absorption. In addition, the bile acids also solubilize the lipids present in bile (including cholesterol, thus avoiding calculus formation); they stimulate bile secretion as well as colic motility. They are also an essential element of the homeostasis of cholesterol in the body.

The small intestine produces its own digestive enzymes that are synthesized in the enterocytes, the most abundant cells in the intestinal epithelium. These enzymes are either secreted in the intestinal lumen or remain anchored in the brush border of the enterocytes. The most important hydrolases synthesized in the enterocytes are disaccharidases (especially lactase, sucrase, and maltase), amylases, peptidases, nucleases, and alkaline phosphatase. On its luminal side, the brush border is lined by the glycocalix, an amorphous layer rich in neutral and amino sugars, which may serve a protective function.

2.2.1.4 The Large Bowel and Colonic Microflora[7,8]

The large bowel is an organ of the gastrointestinal system in which lives a large population of mainly anaerobic bacteria. The specific symbiotic association of this bacteria largely governs its functions. A major function of the large bowel is to ferment and thus breakdown oligo- and polysaccharides (especially the nondigestible oligosaccharides, dietary fibers, and resistant starches) and proteins that are not completely digested in the upper gut. The products of the fermentation are the linear and branched short-chain carboxylic acids known as short-chain fatty acids (SCFAs), various amines, phenols, and gases (Table 2.3).

The fermentation also produces biomass as a consequence of bacterial proliferation. The SCFAs are readily absorbed and provide energy for the host that, depending on the amount of nondigestible food components present in the daily diet, can provide up to 30% of the basal energy requirement. In occidental populations eating western diets, that percentage usually does not exceed 5%.[4,9,10] Being protonated (because of the acidic pH), the amines, including ammonia, are not absorbed but excreted in the feces, as are the phenols. As stated by Cummings, a gastroenterologist who reviewed the physiological role of the colon:[8] "Anaerobic fermentation dominates large bowel function º It affects every process including salt and water

TABLE 2.3
Major Products of Anaerobic Fermentation of Carbohydrates and Proteins in the Human Large Bowel

Carbohydrates	Proteins/Peptides
SCFAs	**SCFAs**
Acetate	Acetate
Propionate	Propionate
Butyrate	Butyrate
	Branched SCFAs
Gases	**Gases**
H_2	H_2
CO_2	CO_2
CH_4	CH_4
	Amines + ammonia
	Phenols
Bacterial biomass	**Bacterial biomass**

absorption, pH, epithelial cell metabolism, motility and bowel habit and colonization resistance, in addition to providing products that are absorbed and reach the liver and peripheral tissues." In addition to fermenting the nondigestible nutrients, the colonic microflora also contributes to the metabolism of endogenous compounds and xenobiotics: the primary bile acids are transformed, via the 7a-dehydroxylation, into secondary (deoxycholic and lithocholic acids), and, eventually, tertiary (ursodeoxycholic acid) bile acids: biluribin and biliverdin are metabolized to produce the uro- and stercobilinogens: miscellaneous food components like alkaloids, flavonoids, glucosinolates, saponins, terpenes, coumarins, sulfur-containing compound have either beneficial or deleterious effects in the human body are hydrolyzed or reduced.[7] Furthermore, the colonic bacteria produce some of the B vitamins and vitamin K, as well as folic acid, which may contribute to the body's pool of nutrients.

The human adult colon has a length of ±1.5 m and a surface of ±1.3 m^2. Its content has a pH that ranges from 5.8 in the cecum up to 6.5 in the recto–sigmoid (Figure 2.4). Its content represents ±0.25 kg of mass composed mainly of bacteria (±10^{11}/g dry matter) sitting on particles of food residues surrounded by a matrix of glycoprotein and exfoliated cells. In the feces, several hundreds of bacteria have been identified, but some 30–40 species belonging to five or six genera account for 99% of the biomass. Table 2.4 lists the predominant bacteria so far isolated and identified in human feces. Most of these bacteria are nonsporing anaerobes.

The recent development and validation of molecular methodologies (especially the fluorescent *in situ* hybridization technique using specific ribosomal RNA [rRNA] sequences known as the FISH method in Chapter 9, Section 9.3) has initiated very active research on the composition of human fecal flora. The experiments so far reported have already revealed the existence of yet unknown/noncultivable genera and species. The number of bacteria expressed as colony-forming units (cfu) of the different species range from 10^2 up to 10^{10} per gram of fresh feces. Each species has its own nutrition requirements and produces a specific pattern of metabolic end products. The colonic microflora is acquired at birth and during the early days, weeks, and months after birth. It is recognized today that the composition of the first colonic microflora is a key element in the development of the immune system early in life. Furthermore, the maintenance of a well-balanced composition throughout life is considered an important factor for well-being and health. There are differences between the different segments of the colon, especially between the cecum and ascending colon on the one hand, and the descending colon and sigmoid colon on the other. The right part of the colon is relatively rich in carbohydrates that are fermented by saccharolytic species to yield principally linear SCFAs, H_2, and CO_2. The content is well moistened and relatively acidic, and so bacteria proliferate rapidly. The left part of the colon is carbohydrate- and water-depleted. Protein breakdown and amino acid fermentation by proteolytic bacteria become more dominant; this produces more branched-SCFAs, H_2, CO_2, CH_4, phenols, and amines. The pH is closer to neutrality (±7) and proliferation of bacteria is slower. The durations of residence in the different segments of the colon are also different — between 6 and 16 h, and 12 and 36 h in the right and left side, respectively. The average transit time in the large bowel is ±60 h, with a range of 23–168 h.[11]

TABLE 2.4
Major Bacteria Species of Human Feces

Species	Mean Log_{10}/g	Typical Substrates of Fermentation
Bacteroides	±11	Carbohydrates
		Arabinogalactans
		Cellulose
		Galactooligosaccharides
		Guar gum
		Mucins
		Pectins
		Starch
		Xylans
Eubacterium	±10.7	Carbohydrates
		Pectins
		Starch
		Amino acids
Bifidobacterium	±10.2	Carbohydrates
		Arabic gum
		Fructans
		Galactooligosaccharides
		Mucins
		Pectins
		Starch
		Xylans
Clostridium	± 9.8	Carbohydrates
		Cellulose
		Pectins
		Starch
		Amino acids
Lactobacilli	± 9.6	Carbohydrates
		Galactooligosaccharides
		Starch
Fusobacterium	± 8.4	Carbohydrates
		Starch
		Amino acids
Escherichia	± 8.6	Carbohydrates
		Amino acids

The second major function of the colon is absorption of water, Na^+, and other minerals. Indeed, it receives daily 1–2 l of chyme of which it removes ±90% of the fluid to produce 0.2–0.25 L of semisolid feces. Also, the concentrations of Na^+ and Cl^- are, respectively, 5 and 6 times higher in the ileum effluent than in the feces, whereas the concentration of K^+ is 7 times higher in the feces than in the ileum effluent.

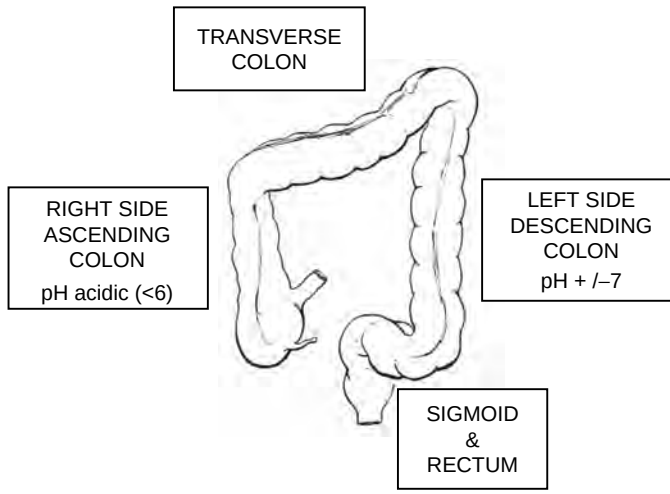

FIGURE 2.4 Anatomy of the human colon.

2.2.2 The Absorption[1,6]

Absorption is the process by which the products of digestion, vitamins, minerals, and fluids selectively cross the gastrointestinal mucosa and enter the blood or lymph. The mechanism of absorption is:

- Primary or secondary (coupled transport) active transport (i.e., involving specific transport protein)
- Passive transport (diffusion, facilitated diffusion, or solvent drag)
- Endocytosis

The transport process can be transcellular (via the enterocytes or M-cells) or paracellular (via the pores of the tight junctions).

The major site of absorption in the gastrointestinal system is the small intestine; this is mostly because it has more surface area (±200–300 m^2), comprising *valvulae conniventes*, villi, and microvilli (brush border). Some absorption also occurs in the other organs of the gastrointestinal system, especially the large bowel.

After the proteins have been hydrolyzed, amino acids, as well as di- and tripeptides, are absorbed, mostly in the small intestine. The absorption of amino acids is an active transport coupled with Na^+ transport. There are different transporters for acidic, neutral, and basic amino acids. The absorbed di- and tripeptides are hydrolyzed in the enterocytes. After absorption, the amino acids enter the portal circulation by simple or facilitated diffusion. The absorption of the amino acids, and di- and tripeptides in the small intestine is very efficient, and only 10% of the *N* of the proteins enters the colon.

In the small intestine, nucleic acids are split into nucleotides by the pancreas nucleases. Mucosal enzymes hydrolyze the nucleotides to phosphates and nucleosides that are further split into ribose or deoxyribose, and purine or pyrimidine bases. The bases are absorbed by an active transport to be either recycled in the synthesis of endogenous nucleic acids or catabolized.

The intestinal absorption of the lipids is a complex process that involves emulsification and micelle formation with the help of the biliary acids. The emulsified lipids are decomposed by lipases that hydrolyze the 1- and 3-bonds in triglycerides and the ester bond in cholesterol esters. The micellar fatty acids, monoglycerides, and cholesterol are then transported in the enterocytes. They enter the cells by passive diffusion. Inside the cells, the fatty acids (10–12 carbon atoms) and part of the cholesterol are reesterified to triglycerides and cholesterol esters. Together with cholesterol, phospholipids, and proteins, these esters form the chylomicrons that leave the cells and enter the lymphatic circulation. Most of the absorption of lipids occurs in the upper parts of the small intestine, but appreciable amounts are still absorbed in the ileum. After a moderately fatty meal, 95% of the lipids are absorbed in the small intestine and very little, if any, fats enter the colon.

The most common dietary carbohydrates are polysaccharides (starches and nonstarch polysaccharides known as dietary fibers), disaccharides (lactose and sucrose), and monosaccharides (glucose and fructose). With the exception of resistant starch (a gelatinized starch formed by heating starch, or that which is found in raw fruits and that escapes digestion), starches are the only polysaccharides that are digested in the small intestine. The absorption of the water-soluble monosaccharides is fast and involves an active transport (coupled with Na^+) for glucose and galactose, and a facilitated passive transport for fructose. The hexoses then diffuse across the enterocytes and enter the portal circulation to reach the liver. Most of the digestion and absorption of the carbohydrates occur in the duodenum and the jejunum (Figure 2.5).

The carbohydrates that resist digestion in the small intestine (nonstarch polysaccharides and nondigestible oligosaccharides) are the dietary fibers. They reach the colon chemically intact, as they have been ingested. Lactase deficiency is common in many ethnic groups. The lactose that escapes digestion enters the colon and causes the syndrome known as lactose intolerance. In the colon, the nondigested carbohydrates are hydrolyzed and fermented by most of the anaerobic bacteria to produce SCFAs, gases, and biomass.[8] The SCFAs are largely absorbed and provide energy for the host. They might also contribute to the regulation of metabolic pathways. Some of the nondigestible oligosaccharides (especially the fructans) are selective substrates for a limited number of bacterial species in the colon (especially the bifidobacteria). When present in food, they selectively stimulate the growth of these bacteria, and they have been called *prebiotics* (see Chapter 9).

The small intestine and the large bowel (but not the stomach) are very active and very efficient (up to 98%) in absorbing water. The absorption is essentially a passive paracellular transport process that goes in both directions across the mucosa in response to osmotic gradients until the osmotic pressure of the intestinal content equals that of the plasma. Na^+ is also actively absorbed throughout the various segments of the small intestine and the colon. In the duodenum and the jejunum it

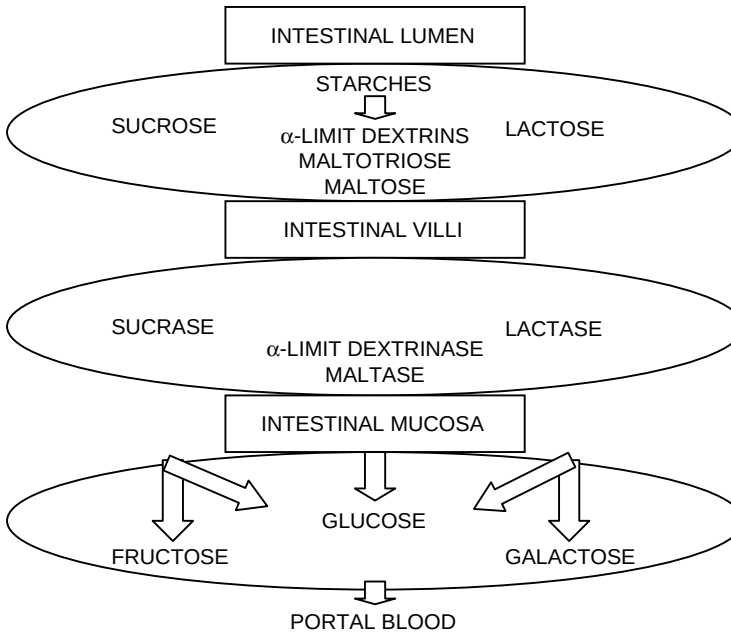

FIGURE 2.5 Schematic representation of the digestion and absorption of dietary carbohydrates.

is coupled with glucose transport. K^+ diffuses across the gastrointestinal mucosa and, to a lesser extent, it is secreted into the intestinal lumen mainly as a component of mucus. In the colon, K^+ is secreted via specific channels, and it moves passively down its electrochemical gradient. In the ileum and the colon where the content tends to be more alkaline, Cl^- is actively reabsorbed in a one-for-one exchange for HCO_3^-. The major site of active absorption of Ca^{2+} (that is facilitated by 1,25-dihydroxycholecalciferol, the active metabolite of vitamin D) is the upper small intestine, but some absorption by passive diffusion may also occur in the ileum as well as in the colon (see Chapter 10). The vitamin D-dependent absorption of Ca^{2+}, but not the passive diffusion, is adjusted to the body's needs. Proteins facilitate Ca^{2+} absorption and the passive diffusion is stimulated by some nondigestible oligosaccharides (especially lactose and fructans; see Chapter 10). The absorption of Mg^{2+} is essentially due to passive diffusion; it is facilitated by proteins, and it occurs mainly in the colon. Iron is actively absorbed, essentially in the duodenum and the jejunum. It is more readily absorbed in the ferrous (Fe^{2+}) state. However, since most of the dietary iron is in the ferric state (Fe^{3+}), it needs to be reduced — a reaction that occurs in the stomach where it solubilizes and forms a complex with ascorbic acid.

Vitamins are absorbed in the duodenum and the jejunum, with the exception of vitamin B_{12}, which is bound to the intrinsic factor that is absorbed in the ileum and has a specific receptor. The absorption of the fat-soluble vitamins (A, D, E, and K) is linked to fat absorption, depending on the presence of biliary acids and micelle formation (see the preceding text).

2.2.3 Excretion[8]

At the end of the gastrointestinal system, the undigested and nonfermented residue (like cellulose) of food and bacterial biomass, plus exfoliated cells, mucus, and endogenous metabolic end products are stored in the rectum and finally excreted as feces. Stool weight varies substantially (from ±70 up to almost 500 g/d) in world populations. For adults eating a Western-type diet, the usual stool weight is ±100 g/d, and most pass one stool per day. However, in each individual, stool weight varies significantly (20–420 g/d) from day to day due to menstrual cycles, dietary patterns, mood changes, travel, and fluid intake.[11] Stool output also varies with age; it is more in early life and becomes less frequent as old age approaches. It also varies with sex, tending to be greater in men than in women. Stool weight correlates inversely with transit time.[12] Indeed, studies with healthy volunteers have shown that reducing colonic transit time from ±70 to ±25 h results in an increase in stool weight from ±150 to ±285 g, whereas increasing transit time results in decreased stool weight.[13] Form and consistency of feces may vary considerably from soft and porridge-like amorphous material to hard, small, and fragmented droppings.[14] The water content of feces varies in parallel with consistency, but in general, it is 70–80%.[15] The solids are composed of bacteria (up to 55%) and dietary fiber (up to 17%).[16]

2.2.4 Motility[5,6]

Gastrointestinal motility allows triturating the chyme but, most important, it controls the time the chyme stays in the different organs. It is under both neural and humoral regulation.

The time it takes material to pass from the oral cavity through the gastrointestinal tract to the rectum is known as the transit time. It is, largely, genetically determined, but it is also controlled by factors such as hormonal status and stress. Moreover, it can vary greatly from day to day in individuals. The transit time is closely related to stool weight; it being ±40 h if stool weight is high, and reaching up to 80 h or more if stool weight is low. The transit time differs in the segments of the colon. It has been estimated at 7–24 h, 9–30 h, and 12–44 h in the right, left, and sigmoid segments of the colon (plus the rectum), respectively.[8]

In the oral cavity, the food is chewed and mixed with saliva. It is then propelled into the pharynx and the esophagus, where it stays only for a few seconds so that it reaches the stomach quickly to avoid reflux of acid or food. The stomach serves as a reservoir to store, mix, and break down food. It also plays a key role in transferring the chyme into the duodenum at a controlled, steady state (especially to ensure a regular caloric flux into the small intestine). Gastric emptying is related to the volume, composition, and caloric content of the meal. It is more rapid for fluids than for solids, and fatty meals are the slowest to be released. Gastric motility is regulated via receptors that respond to distension in the stomach and that sense osmolarity, caloric charge, or pH variations in the duodenum. The regulation involves the nervous system (both sympathic and parasympathic) as well as different hormones, especially gastrin, secretin, and CCK, etc.

The motility of the small intestine depends on the electric activity of the smooth muscular cells that contract the organ. This activity causes two movements:

- The segmentation contractions that mix the chyme with bile and pancreatic and mucosa secretions, and facilitate its contact with the mucosa
- The peristaltic waves that propel the chyme toward the colon

The motility of the small intestine is under both neural (via both the intrinsic nervous system, extrinsic vagus, and splanchnic nerves) and humoral regulation (especially motilin). Each time a peristaltic wave reaches the end of the ileum, the ileocecal valve opens briefly and squirts some of the ileal chyme into the cecum. The cecum relaxes and the flux through the ileocecal valve increases when the chyme leaves the stomach (gastroileal reflex).

As in the small intestine, the motility of the large bowel involves two movements: (1) the segmentation contractions and (2) the peristaltic waves that have essentially the same functions. Also, an additional specific contraction occurs in the colon, i.e., the mass-action contraction, in which the smooth muscle contracts simultaneously in large confluent areas to move the chyme from one segment of the colon to another, and, finally, to the rectum. The movements of the large bowel are coordinated by a slow wave that increases from the cecum (±2/min) to the sigmoid (±6/min). The distension of the rectum initiates defecation. The motility of the large bowel varies with the nychtemer, being weak during sleep and increasing on waking up and after meals (gastrocolic reflex).

2.3 ENDOCRINOLOGY: PEPTIDE HORMONES[5]

The mucosa of the gastrointestinal system produces many different hormonally active peptides known as the gastrointestinal hormones. These hormones play essential roles in the regulation of gastrointestinal secretions and motility, as well as in the regulation of more systemic functions like insulin secretion and carbohydrate metabolism (Figure 2.6). These belong to one of the following four categories:

- Gastrin family (gastrin, CCK, etc.)
- Secretin family (secretin and gastrointestinal peptide [GIP])
- Incretin family (glucose-dependent insulinotropic polypeptide, and glucagon like peptide 1 or GLP-1)
- Ghrelin

The main site of gastrin secretion is the G-cell in the glandular stomach. Its main activity is to stimulate motility and growth of the mucosa, as well as secretion of acid and pepsin in the stomach. It also stimulates the growth of small intestinal mucosa (trophic effect). After a protein-rich meal (but not a carbohydrate-rich meal), circulating endogenous gastrin stimulates insulin and glucagon secretion. Gastrin secretion is controlled by the stomach content, the vagus nerves, the presence of amino acids, and a series of blood-borne factors like Ca^{2+} and epinephrine.

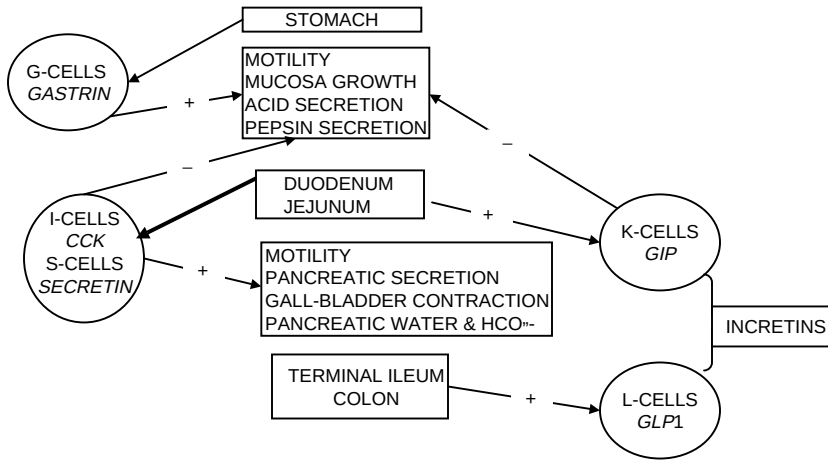

FIGURE 2.6 Schematic representation of the integrated actions of the major gastrointestinal hormones. (CCK= cholescystokinin; GIP = gastrointestinal peptide; GLP1 = glucagon-like peptides.)

CCK is produced by the I-cells in the mucosa of the duodenum and the jejunum. In the gastrointestinal system, it has two main actions:

- Stimulation of the pancreatic secretions
- Stimulation of the contraction of the gall bladder

In addition, it inhibits gastric emptying, it enhances the action of secretin, and it may play a role in stimulating the motility of the small intestine and the large bowel. Its production is regulated by contact of the duodenum and jejunum mucosa with digestion products, especially peptides, amino acids, and fatty acids (≥10 carbon atoms).

The S-cells in the mucosa of the duodenum and the jejunum produce secretin. This secretion is stimulated by the products of protein digestion and by the acidity of the chyme coming from the stomach. It is structurally very similar to glucagon, GIP, and VIP. Its main intestinal activity is to cause the secretion of a watery and alkaline pancreatic juice by stimulating the production of HCO_3^-. It also inhibits gastric acid secretion and augments the stimulatory action of CCK on pancreatic secretion of digestive enzymes.

The K-cells in the mucosa in the duodenum and the jejunum, and the L-cells in the jejunum, ileum, and large bowel secrete GIP and GLP-1 (one of the products of the posttranslational processing of proglucagon), respectively. These secretions are stimulated by the presence of glucose and fats. GIP and GLP-1 inhibit gastric secretion and motility. However, these polypeptides have also a systemic effect; they act as pancreatic b-cell-stimulating hormones that induce insulin secretion. For that reason GIP is also called the *glucose-dependent insulinotropic hormone*, and GIP and GLP-1 are known as the *incretins*.

The incretins are the secretagogue hormones that are produced by intestinal enteroendocrine cells and constitute one arm of the enteroinsular axis.[17] The concept of *incretin* originates from the observation that in nondiabetic subjects oral ingestion of glucose triggers a greater insulin response than intravenous administration for equivalent serum glucose concentration. GIP and GLP-1 account for about 20 and 80% respectively of the intestinal incretin effect.[18]

The enteroendocrine K- and L-cells secrete GIP and GLP-1, respectively, in response to fatty meals and dietary carbohydrates. They have been shown to enhance glucose-mediated insulin release (most likely via a stimulation of the proliferation of the pancreatic b-cells) and glucose disposal in the peripheral tissues (muscle and adipose tissue), to inhibit glucagon secretion (via an inhibition of pancreatic b-cells), and to promote satiety (most probably via an action in the hypothalamus). Moreover, after intravenous injection, GLP-1 or its long-acting analog exendin-4 has been shown to have antidiabetic effects.[19]

Ghrelin contributes to the regulation of feeding behavior by modulating the expression levels of orexigenic peptides in the hypothalamus. It has been implicated in the coordination of energy balance and weight control.[20]

2.4 DEFENSE MECHANISMS[21,22]

The gastrointestinal system is an essential barrier to protect the body against pathogenic microorganisms, antigens, and potentially toxic chemicals that might be contaminating food (see Chapter 12). It has three defense mechanisms:

- Innate, relatively nonspecific mechanisms
- Specific and acquired immune mechanisms
- Xenobiotic-metabolizing enzymes

The innate, relatively nonspecific defense mechanisms in the gastrointestinal system include:

- Motility that, when it is reduced, causes stagnation of the chyme with, as a consequence, a pullulation of the microorganisms, especially in the small intestine, which, in humans, is normally close to sterility
- Secretions, especially acid in the stomach, bile acids, and pancreatic secretions (lysozyme and lactoferrin), and antimicrobial peptides secreted by the Paneth-cells located in the basal part of the crypts in the small intestine
- Mucus composed of a glycoprotein-protective gel between the lumen and the epithelium that retains secretory IgA, lactoferrin, and lysozyme, and to which microorganisms attach

- Endogenous flora, especially the colonic microflora, that, when it has a well-balanced composition, protects the gastrointestinal system against colonization by exogenous potential pathogens (see Chapter 12)
- Unstirred mucosal layer and the epithelium that form a physical barrier to control gastrointestinal permeability and to prevent bacterial translocation

The specific and acquired immune mechanisms aim at protecting the host against pathogenic microorganisms and at controlling the reactions against antigens. The small intestine and the large bowel represent the largest mass (producing ±60% of total daily immunoglobulin) of lymphoid tissue in the body. The GALT contains over 10^6 lymphocytes/g of tissue and it represents ±25% of the intestinal mucosa. These immune cells are present in the epithelium, are distributed in the mucosa as diffuse lymphocytes, or are organized in lymphoid structures, especially the Peyer's patches and the mesenteric ganglions. The intraepithelial lymphocytes are a heterogeneous population of cells that mediates both non-major-histocompatibility-complex-restricted and major-histocompatibility-complex-restricted cytotoxicity, and regulates neighboring immune and epithelial cells via the secretion of cytokines. The diffuse lymphocytes in the mucosa are T-helper cells, granulocytes, and mast cells. The Peyer's patches and a few isolated follicles are lymphoid follicle aggregates that contain the M-cells to capture microbes and antigens, and transfer them to the antigen-presenting cells that then migrate in germinating B follicles and interfollicular T-zones. The Peyer's patches are easily accessible to microorganisms because, compared to the gut epithelial cells, they have less mucosa-secreting goblet cells. The first line in the intestinal defense mechanisms against offending antigens is immune exclusion involving secretory IgA antibodies, a process that results from T-cell regulation in the Peyer's patches. Secretory IgAs resist intraluminal proteolysis, do not activate the complement, and do not initiate inflammatory responses. The main function of secretory IgAs is to mediate immune exclusion of foreign antigens by preventing epithelial adherence and translocation of invasive pathogenic microorganisms, by neutralizing toxins, and by inhibiting viral multiplication. If the antigens have penetrated the mucosa, a second line of defense mechanisms starts to play that involves mainly IgG and a large number of mediators such as cytokines; this initiates an inflammatory response. There are differences between the small intestine and the large bowel in isotype distribution of immunoglobulin-producing cells. In particular, two subclasses of IgA have been identified: IgA_1 immunocytes predominate in the small intestinal mucosa whereas IgA_2 immunocytes are most frequent in the colonic mucosa. Moreover, IgA_2 antibodies resist proteolysis by bacterial proteases. The immunity of the gastrointestinal system has, however, a special nature that has evolved under the constant exposure to miscellaneous environmental antigens. Indeed, it initiates an effective and continuous response to invading antigens while showing selective hypo responsiveness by prior oral administration of dietary antigens that induce oral tolerance. At birth, the gastrointestinal system is sterile; it becomes colonized after birth by bacteria that act as a source of antigens and nonspecific immunomodulators that influence the number and distribution of GALT cell populations and that play major roles in regulating the immune

responses. The intestinal microflora is the major antigenic stimulus responsible for the migration and maturation of precursor lymphoid cells (especially IgA plasmocytes) in the Peyer's patches. The intestinal flora also modulates the specific immune response and allows the persistence of the systemic unresponsiveness to an antigen induced by previous feeding with the same antigen (oral tolerance).

The enzymes of the xenobiotic-metabolizing systems, especially the phase II enzymes, inactivate and eliminate toxic chemicals and their metabolites.[22] Many organs, including the small intestine and the large bowel, do have such enzymatic activities, especially with acetyl-, glucuronyl-, glutathione-, methyl-, and sulfotransferases. Their role is to increase the hydrophilicity of the xenobiotics and their metabolites to facilitate urinary and fecal excretion. If xenobiotics and their metabolites are highly reactive compounds that can initiate a toxic response, such metabolism will serve as an inactivation mechanism that protects the host from potentially deleterious effects. The activity of the phase II xenobiotic-metabolizing enzymes is often sensitive to induction or inhibition by miscellaneous chemicals (especially drugs) as well as phytochemicals present in food like indols, flavonoids, sulfur-containing molecules, and coumarins.

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Part II

Inulin: Origin, Chemistry, Biochemistry, and Technological Properties

3 Inulin: A Fructan

3.1 FRUCTANS

3.1.1 Definition

A fructan is any compound where one or more fructosyl-fructose linkages constitutes a majority of linkages.[1] Even though according to classical rule, names of molecules ending with "an" should be used to designate polymers with DP > 10, no longer is there today a distinction between polymers and oligomers. Consequently, fructan is used to name molecules that have a majority of fructose residues whatever the number is. It even includes the disaccharide composed exclusively of two fructose residues, specifically the fructosyl-fructose or inulobiose but not sucrose, isomaltulose, and galactosucrose, etc.[1,2] In addition, fructan is also sometimes either a cyclic or a branched molecule (Figure 3.1). Fructan is also known as *polyfructosylfructose*.[3] All natural (plant and microbial) fructans are a mixture of oligomers or polymers or both, which is best described by the mean (or average) and the maximum number of fructose units, residues, or moieties,* known as the average and the maximum degree of polymerization (DP_{av} and DP_{max}), respectively. More than 50 generic names of fructans have appeared in old literature including, to cite only a few, inulin, levan, and phlein (see definition below) but also fructoholoside, fructosan, graminin, inulenin, lävulan, levulosan, levosin, and pseudo-inulin, etc.,[4] but their usage should be avoided.[1]

A FRUCTAN is:

A CARBOHYDRATE THAT CONSISTS MOSTLY OF FRUCTOSE
(+ GLUCOSE)

A MOLECULE THAT HAS A MAJORITY OF FRUCTOSE RESIDUES
(+ GLUCOSE)

A FRUCTAN can be:

–LINEAR:
INULIN (2, 1 fructosyl-fructose)
LEVAN (2, 6 fructosyl-fructose)

–BRANCHED (2, 1 or 2, 6 and 2, 6)

–CYCLIC

FIGURE 3.1 Definition of Fructan.

* These terms will be used interchangeably throughout the book to designate the fructose monomers.

3.1.2 Chemistry of Linear, Branched, and Cyclic Fructans

From a chemical point of view, the linear chain of fructans is either a β-D-glucopyranosyl-[-β-D-fructofuranosyl]$_{n1}$-β-D-fructofuranoside ($G_{py}F_n$) or a β-D-fructopyranosyl-[-β-D-fructofuranosyl]$_{n1}$-β-D-fructofuranoside ($F_{py}F_n$). The fructosyl-glucose linkage is always β-(2<–>1) as in sucrose,* but the fructosyl-fructose linkages are either β-(1➡2) or β-(6➡2). In branched fructans the branching linkages are usually β-(2➡6). Fructans are mainly of plant origin, but they are also found in fungi and bacteria. In plant fructans the number of fructose monomers does not exceed 200, whereas in bacterial fructans it can be as high as 100,000, and it is highly branched.

The general terms to describe fructans are: inulin, levan, graminan, phlein, and kestoses (Table 3.1).[1]

- **Inulin** is a material that has mostly, or exclusively, the β-(1➡2) fructosyl-fructose linkage, and glucose is allowed at the terminal position in the chain but is not necessary. Until recently, inulin was considered to be a linear molecule with β-(1➡2) linkages exclusively. However, using optimized permethylation analysis, it has been possible to demonstrate that even native inulin has a very small degree (1–2%) of branching.[5] All fructans in dicotyledons, but only part of the fructans in monocotyledons, are inulin-type fructans.[4] Inulin exists also in a cyclic form that contains 6,7, or 8 fructofuranose rings.[6]

TABLE 3.1
Chemistry of Fructans

General Structure

α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$- β-D-fructofuranoside ($G_{py}F_n$)

β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$- β-D-fructofuranoside ($F_{py}F_n$)

Name	Linkage (fructosyl-fructose)	Chemical Structure	Natural Origin
Inulin	β (2,1)	Linear, branched, cyclic	Plant, bacteria, fungi
Levan	β (2,6)	Linear, branched	Plant, bacteria, fungi
Phlein	β (2,6)	Linear, branched	Plant
Graminan	β (2,1) and β (2,6)	Linear, branched	Plant
Kestoses	β (2,1) and β (2,6)	Linear, branched	Plant

* In such a representation, the numbers indicate the linkage's position on the C atoms of the fructose or glucose rings and the arrow points away from the reducing C atom (C_2 in fructose or C_1 in glucose).

- **Levan** is a material that has mostly, or exclusively, the b-(6➟2) fructosyl-fructose linkage. Glucose is allowed at the terminal position in the chain but is not necessary. Levans are found mostly in bacteria (high molecular weight) but to some extent in higher plants also (short polymers). The levans of higher plants are heavily branched molecules through the formation of b-(2➟1) linkages.[2]
- **Phlein** has substantially the same meaning as levan, but the name has commonly been used to describe plant- (and not bacteria-) based material which contains, most exclusively, the b-(6➟2) fructosyl-fructose linkage; a glucose is allowed at position 1 in the chain but is not necessary. In general, plant-based fructans are of lower molecular weight (DP < 100) than those derived from bacteria, and thus this distinction has been useful. Phlein-type fructans occur mainly in monocotyledons, which represent the most frequently identified fructans.[4]
- **Graminan** is a material that has both b-(1➟2) and b-(6➟2) fructosyl-fructose linkages in significant proportions; glucose is allowed at position 1 in the chain but is not necessary.
- **Kestoses or kesto-*n*-oses** are trimeric or oligomeric fructans containing one glucose and two or more fructose units linked by b-(1➟2) and/or b-(6➟2) fructosyl-fructose linkages.

More specific terms have been still used:

- **Bifurcose:** a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(6➟2)-b-D-fructo-furanosyl-(1➟2)-b-D-fructofuranoside.
- **Inulo-n-ose:** Oligomeric fructofuranosyl-only fructans that have all-(1➟2) linkages like inulobiose and inulotriose.
- **Fructooligosaccharides, oligofructan, and oligofructose:** Oligomeric linear fructans with b-(1➟2) linkages. They can be of both ($G_{py}F_n$) and ($F_{py}F_n$) types. Among others, these terms include 1-kestose, neokestose, and nystose.
    - **1-Kestose:** a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(1➟2)-b-D-fructo-furanoside
    - **6-Kestose:** a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(6➟2)-b-D-fructo-furanoside
    - **Levan-n-ose:** oligomeric fructofuranosyl-only fructans that have all b-(6➟2) linkages like levanbiose, levantriose, etc.
    - **Neokestose:** b-D-fructofuranosyl-(2➟6)-b-D-glucopyranosyl-(1<–>2)-b-D-fructofuranoside
    - **Nystose:** a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(1➟2)-b-D-fructofuranosyl-(1➟2)-b-D-fructofuranoside

3.1.3 Biochemistry: The Biosynthetic Pathways of Fructans

Intermediate in the synthesis of plant fructans is a trisaccharide formed by the transfer of a fructosyl residue from 1 sucrose (a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranose) to another at either the O_1 (in inulin) or O_6 (in levan) position of the fructofuranose moiety, producing:[7]

1-kestose [a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(1➟2)-b-D-fructofuranoside] or 6-kestose [a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(6➟2)-b-D-fructofuranoside].

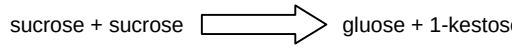
Higher homologues are synthesized by chain elongation that involves the sequential addition of fructosyl residues to the O_1 (in inulin) or O_6 (in levan) position of the terminal fructofuranose unit (Figure 3.2).

Enzymes that catalyze the synthesis of linear fructans are:

- *Sucrose-sucrose fructosyl transferase* (EC 2.4.1.99) which catalyzes the transfer of a fructosyl residue from one sucrose molecule to another, producing glucose, and 1-kestose (a-D-glucopyranosyl-(1<–>2)-b-D-fructofuranosyl-(1➟2)-b-D-fructo-furanoside), which is considered to be the universal fructosyl donor in plant
- *Two fructan-fructan fructosyl transferases* (EC 2.4.1.100) (O_1-fructose-fructosyl transferase for inulin and O_6-fructose-fructosyl transferase for levan synthesis, respectively), which catalyzes the transfer of fructosyl residues from 1-kestose to sucrose, kestose, and fructosylkestose, etc.

In fungi and bacteria,[8] fructans are assumed to be synthesized by a repeated transfer of fructosyl moieties from sucrose, as unique fructosyl donor, to the growing fructan chain. The enzymes are, respectively:

1. *Sucrose-sucrose fructosyltransferase* (*EC* 2.4.1.99)



2. *Fructan-fructan fructosyltransferase* (*EC* 2.4.1.100)

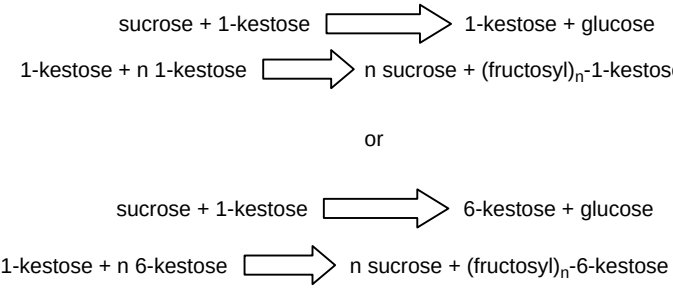


FIGURE 3.2 Major steps in the plant biosynthesis of linear fructans

- Inulo-sucrose-O_1-fructose-fructosyl transferase (EC 2.4.1.9) or inulin sucrase
- Levan-sucrose-O_6-fructose-fructosyl transferase (EC.2.4.1.10)
- Levan sucrase

In a strain of *Bacillus circulans* isolated from soil, a cycloinulooligosaccharide fructanotransferase catalyzes the formation of a mixture of cycloinulo-hexaose (60%), cycloinuloheptaose (20%), and cycloinulooctaose (trace) from inulin.[6]

3.1.4 Natural Occurrence of Fructans

Fructans are reserve carbohydrates in at least 10 families of higher plants that store them in a soluble form in vacuoles in crowns, leaves (short time storage), roots, stems, tubers, or kernels. Fructan metabolism may be a way by which plants control sucrose content within or between cellular and organ compartments.[7] The fructans and their linkage type and length differ greatly, depending on the plant and the plant organ. Moreover, the chain length of plant fructans can be modulated through changes in DP as a means to modulate osmotic potential. This observation has led to suggest that fructans may also play a role in protecting plants against cold-induced desiccation.[9] They are also present in fungi and bacteria.[10]

3.1.4.1 Occurrence of Fructans in Plants[10,11]

Fructan-containing plants are mainly angiosperms. The fructan-containing species belong to both mono- and dicotyledonous families. Some of these plants are eaten as vegetables such as artichoke, asparagus, chicory, garlic, Jerusalem artichoke, leek, onion, and salsify, etc.

In monocotyledons, fructans are widely present in the aerial parts of young seedings of Gramineae but significant concentration is found only in northern grasses (Pooideae), oat (*Avena sativa*), barley (*Hordeum vulgare*), rye (*Secale sativa*), and wheat (*Triticum aestivum and Triticum durum*). It is also present in the order of the Liliaceaes. Indeed, the bulbs, tuber, and tuberous roots of Amaryllidaceae, Agavaceae, Haemodoraceae, Iridaceae, Liliaceae, and Xanthorrhoeaceae produce and store fructans. Especially, fructans have been found in the family of Liliaceae, in the leaf and bulb of leek (*Allium ampeloprasum*), the bulb of onion and shallot (*Allium cepa*), garlic (*Allium sativum*), and the tuber of asparagus (*Asparagus officinalis and Asparagus racemosus*) and in the family of *Agavaceae* in the tuber of palm lily (*Cordyline terminalis*) and *Dracaena australis*.

In dicotyledons, the fructans-containing orders are the Asterales, the Campanulales, the Dipsacales, the Polemoniaceae and the Ericales. As far as is known, all members of the major family Compositae (Asterales order) store significant amounts of fructans in their underground storage organs such as tap roots and tubers but not in their leaves. This is the case for chicory (*Cichorium intybus*), elecampane (*Inula hellenum*), dandelion (*Taraxacum officinale*), Jerusalem artichoke (*Helianthus tuberosus*), murnong (*Microseris lanceolata*), salsify (*Tragopogon porrifolius*), and yacon (*Polymnia sonchifolia*). In the other orders, the fructan-containing families

usually have morphological affinities to the Compositae, namely Campanulaceae, Goodeniaceae, Lobeliaceae, Stylidiaceae (within the order Campanulales), and Calyceraceae (within the order Dipsacales). Within the order Polemoniaceae, three families with distant affinities to Asterales and Campanulales also contain fructans, namely Boraginaceae, Menyanthaceae, and Polemoniaceae.

In bryophytes, fructans have been reported to occur only in six orders of liverworts (Hepaticopsida) within land plants and in some species of the genus sphagnum within the mosses (Bryopsida). Fructans have not been found in Pterodophyta (ferns, club mosses, and horsetails) or in Gymnospermae (conifers and cycads).

Only brief summaries of the occurrence of fructans in algae have been provided. Still, inulin has been identified in members of both the Dasycladales order (especially *Acetabularia mediterranea*) and the Cladophorales order (four species of *Cladophora* and two species of *Rhizoclonium*).

3.1.4.2 Occurrence of Fructans in Fungi[10]

Fructans accumulate in various species of aspergillus, but some species also synthesize it extracellularly from sucrose. Especially, it has been reported that *Aspergillis sydowi* synthesizes an inulin that has a molecular weight greater than that of plant inulin. However, fructan has not been demonstrated in penicillium, pestalotiopsis, myrothecium, or trichoderma. This observation correlates well with the fact that sucrose has not been confirmed as a fungal carbohydrate. Indeed, the most characteristic endogenous disaccharide of all fungal groups is trehalose (1-1-di-glucose).

3.1.4.3 Occurrence of Fructans in Bacteria[10]

With the exception of certain strains of *Streptococcus mutans* (a major component of dental plaque) that produce inulin-type fructans, the bacterial fructans are essentially of the levan type. Fructans or the genes for their synthesis appear essentially in five orders or families of bacteria, namely the Gram-negative aerobic (Pseudomonadaceae) and facultative, anaerobic (Enterobacteraceae) rods and cocci, the Gram-positive cocci (Streptococcaceae), endospore-forming rods and cocci (Bacillaceae), and Actinomycetaceae.

Within these groups not all genera synthesize or process fructans. For example, only ±10% of tested *Pseudomonas species*[12] and ±40% of tested *Bacillus species*[13] have the capacity to synthesize levans. Indeed, the biosynthetic capacity may be limited to a few strains of particular bacterial species that otherwise appear to use a very similar mechanism of synthesis.

3.2 INULIN

3.2.1 History of Inulin[4]

The use of miscellaneous fructan- (but mostly inulin-) containing plants as food seems to be quite old, dating back to at least 5000 years, and one of the most commonly consumed vegetables in ancient times was onion (*Allium cepa*). Reference to the consumption of chicory by humans was made almost 2000 years ago by Pedanios

Dioscoride,[14] a physician in the Roman army who praised the plant for its beneficial effects on stomach, liver, and kidneys. More recently, around the mid-19th century, Jerusalem artichoke (*Helianthus tuberosus*) pulp, prepared by cooking and drying the tubers, was added in a 50:50 ratio to flour in order to bake cheap bread.[15] It was in the same century that a German scientist discovered inulin after he had isolated a "peculiar substance of plant origin" from the boiling-water extract of *Inula helenium*.[16] That substance was called inulin.[17] One of the pioneers in fructan research is the German plant physiologist Julius Sachs, who also discovered starch in chloroplasts.[18] After ethanol precipitation, Sachs, indeed, identified with a microscope the spherocrystals of inulin in the tubers of *Dahlia variabilis*, *Helianthus tuberosus*, and *Inula helenium*. Later on, the inulin nature of the precipitate was confirmed by staining.

The first scientific report on the health benefits of inulin for humans also dates back to the last quarter of 19th century. Indeed, referring specifically to inulin, Külz reported as early as 1874 that no sugar appears in the urine of diabetics who eat 50 to 120 g of inulin per day.[19] At the end of the 19th century, feeding diabetic patients with pure inulin (40 to 100 g/d) was reported "with much benefit" (Von Mehring, 1876 cited in De Roover et al.[20]). In 1912, the first studies on the effects of inulin on healthy humans appeared,[20] and some years later (1935), Shannon and Smith dramatically demonstrated the absence of toxicity when one of the authors injected himself intravenously with 160 g inulin.[21] Over the last 10–15 years, a spectacular increase in the number of scientific publications dealing with the functional and nutritional effects of inulin has been observed. More recently, inulin and its derivatives have attracted the interest of the food industry which has already developed a series of food products with wide applications, thus stimulating research and publications in all the domains relevant to its production and nutritional use.

3.2.2 Chemistry and Biochemistry of Inulin

From a structural or polymeric point of view, (linear) inulin can be considered as a polyoxyethylene backbone to which fructose moieties are attached, as are steps to a winding stair. It is a polydisperse carbohydrate material consisting mainly, if not exclusively, of b-(2➠1) fructosyl-fructose linkages.[1] A starting a-D-glucose moiety can be present but is not necessary. $G_{py}F_n$ and $F_{py}F_n$ compounds are included under the same nomenclature, and they are both a mixture of oligomers and polymers that are best characterized by the average and the maximum DP. The general molecular structure of inulin compounds is shown in Figure 3.3.

The degree of polymerization (DP) of inulin and the presence of branches are important properties that influence its functionality strikingly. Therefore, a strict distinction must be made between inulin of plant and bacterial origin. The DP_{max} of plant inulin is rather low (maximal DP < 200), but DP_{max} and DP_{av} vary according to plant species, weather conditions, and the physiological age of the plant (see Section 3.3.3). Until recently, plant inulin was considered to be a linear molecule, but, by applying optimized permethylation analysis, it has been possible to demonstrate that even native chicory inulin (DP_{av} = 12) has a very small degree of branching (1–2%).[4] The DP_{max} of bacterial inulin varies from 10,000 up to 100,000, and it is highly branched (15%).

3.2.3 Distribution of Inulin in Plants

Inulin is present in significant amounts in several fruits and vegetables that have been analyzed by Van Loo et al.[22] To avoid both chemical and enzymatic hydrolysis of the b-(2,1) fructosyl - fructose linkages, a fresh sample of miscellaneous plants was extracted at neutral pH (5.5–8.5) by boiling in water. The aqueous supernatant was then treated with mixed resins to remove interfering ions. The concentration of fructose, glucose, sucrose, and inulin oligomers with DP ≥ 5 was determined by high performance liquid chromatography (HPLC), using Aminex HPX87K columns. The oligomers with DP in the range of 2 to 10 were also quantified by capillary (OV1: 6 m length ¥ 0.53 mm diameter) gas chromatography (CGC) after oximation and silylation of the carbohydrates, using a flame-ionization detector. The quantitative determination of all types of inulin was performed before (to quantify native fructose, glucose, and sucrose), and after (to quantify inulin), enzymatic (Novozym 230) hydrolysis of all oligomers and polymers into their component monosaccharides, i.e., fructose and glucose. Together with sucrose the monosaccharides were then quantified by CGC as described above. To compute the results, response factors were determined using standard solutions of the pure carbohydrates. Inulin was characterized by the description of its DP distribution, using a DIONEX chromatography and a pulsed electrochemical detector.

Table 3.2 summarizes the data of such inulin analysis for miscellaneous plants, most of which are edible plants. It illustrates the diversity of inulin types in different plant species. Inulin content ranges from less than 1 up to some 20% of fresh weight. Moreover, not considering the structure of inulin (linear or linear and branched), the length of the chain also varies:

- In banana, 100% of oligomers have a DP < 5, but in salsify, 75% have a DP ≥ 5.
- DP ranges from 2 to 12 and from 2 to 65 in onion and chicory, respectively.
- In wheat, 50% of oligomers have a DP £ 5, but in globe artichoke, 96% have a DP > 5.
- Chicory and Jerusalem artichoke produce an inulin with 83 and 94% of the chains having a DP < 40, respectively.
- In globe artichoke, 87% of polymers have a DP ≥ 40.

Based on these data, the average daily consumption of the various types of inulin has been estimated to be between 3 and 11 g in Europe[22] and between 1 and 4 g in the U.S.,[23] the most common sources being, in both the studies, wheat, onion, banana, garlic, and leek.

With the exception of agaves (*Agave azul tequilana*) that are grown commercially in Mexico for the production of the alcoholic drink tequila, the only plants that have so far been used industrially for the extraction of fructans belong to the Compositae family, i.e., chicory, Jerusalem artichoke, and dahlia.

Chicory is a biennial plant. During the first season, the plants remain in the vegetative phase and put forth only leaves, taproots, and fibrous roots. The roots look like small oblong sugar beets. The inulin content is high (16–18%) and fairly constant from year to year for a given region. Yields are around 45 ton roots per

TABLE 3.2
Inulin Content and Chain Length of Miscellaneous Plants

Plant	Inulin g/100g	Chain Length Degree of Polymerization (DP)
Globe Artichoke	2–7	DP ≥ 5 = 95%
(*Cynara scolymus*)		DP ≥ 40 = 87%
Banana	±1	
(Musa cavendishii)		DP < 5 = 100%
Barley	0.5–1	
(*Hordeum vulgare*)	±22	
very young kernels		
Chicory	15–20	DP < 40 = 83%
(*Cichorium intybus*)	Mean 16.2	DP 2–65
		DP ≥ 40 = 917%
Dandelion (leaves)	12–15	
(*Taraxacum officinale*)		
Garlic	16	DP ≥ 5 = 75%
(*Allium sativum*)	Mean 13	
Jerusalem Artichoke	17–20.5	DP < 40 = 94%
(*Helianthus tuberosus*)		DP 2–50
		DP ≥ 40 = 6%
Leek	3–10	DP 12 is most frequent
(*Allium ampeloprasum*)		
Onion	1–7.5 Mean 3.6	DP 2–12
(*Allium cepa*)		
Salsify	Mean ±20	DP ≥ 5 = 75%
(*Scorzonera hispanica*)		
Wheat	1–4	DP £ 5 = 50%
(*Triticum aestivum*)		

Source: Adapted from Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., Smits, G., On the presence of inulin and oligofructose as natural ingredients in the Western diet, *Critic. Rev. Food Sci. Nutr.*, 35, 525–552, 1995.

hectare. A strict crop rotation is necessary (once every 5 years). Commercial inulin production is essentially from chicory (*Cichorium intybus*). Jerusalem artichoke shows a rather high inulin content (17–20.5%). The tubers are small and irregular, hence a high amount of soil is attached to them. Jerusalem artichoke inulin has only 20% of chains with a degree of polymerization longer than 10. Many dahlia cultivars are available, but they have all been selected for their flowers, rather than for inulin production. The tuberous roots can be propagated only if they are attached to stem tissue. When propagated from seed, sowing has to be delayed until late spring, given dahlia's extreme sensitivity to frost. Mechanical harvesting of the tubers is feasible only in sandy soil. Although the degree of polymerization of dahlia inulin is higher than that for chicory, the yield is only half that of chicory. For all these reasons, dahlia does not appear to be an interesting crop for inulin production, and practically only chicory (*Cichorium intybus*) is used today as an industrial crop; its fructan is known as chicory inulin.[24,25]

3.2.4 Biological Functions of Inulin in Plants

Despite major advances in understanding the metabolism of fructans, the exact physiological functions of inulin is still a subject of debate. Most documented is its role as a long-term reserve carbohydrate stored in underground overwintering organs. Two other functions are often quoted: cryoprotection and osmotic regulation, allowing not only just survival but also growth under conditions of water shortage, whether induced by drought or by low temperatures. During drought, increased amounts of glucose, fructose, sucrose, and inulin are found in the roots and in the leaves.[26] In winter (e.g., 3 weeks at 4˚C), chicory inulin becomes hydrolyzed, resulting in lower-DP fractions and increased amounts of free fructose, which are osmotically more active than native inulin. The role of fructans as true cryoprotectant is still under discussion because the increase in free fructose and sucrose upon depolymerization of fructans would only account for a decrease in the freezing point of 0.2–0.5˚C.[27] On the other hand, inulin was shown to interact directly with membrane lipids upon freeze-drying, preserving the membranes in a liquid-crystalline phase at room temperature, and preventing a phase transition and solute leakage during rehydration.[28]

3.3 CHICORY INULIN

3.3.1 Description of Chicory Inulin

Native chicory inulin is a nonfractionated inulin, extracted from fresh roots, taking precautions to inhibit the plant's own inulinase activity as well as acid hydrolysis. It always contains glucose, fructose, sucrose, and small oligosaccharides.[25] It crystallizes along a pseudo-hexagonal sixfold symmetry with an advance of 0.24 nm per monomer. Moreover, two inulin crystalline allomorphs exist: a semihydrated one and a hydrated one. The difference between the unit cells seems not to correlate with any change in the conformation of the sixfold helix, but rather to a variation in water content. Oligomers up to DP 5 can adopt structures resembling the conformation of cyclo-inulohexaose. Oligomers between DP 7 and 8 adopt a conformational change probably because these oligomers form helical structures that become more rigid as the DP increases.[29] This hypothesis of change of conformation gives also a very reasonable explanation for the observation that between DP 6 and 9, the elution sequence of the oligomers on a reversed phase C18 nucleosil column is completely reversed.[5]

Because of the beta configuration of the anomeric C_2 in its fructose monomers, inulin is resistant to hydrolysis by human small intestinal digestive enzymes, which are specific for a-glycosidic bonds (see Chapter 2 and Chapter 4). It has thus been classified as "nondigestible" oligosaccharide (NDO).[30,31]

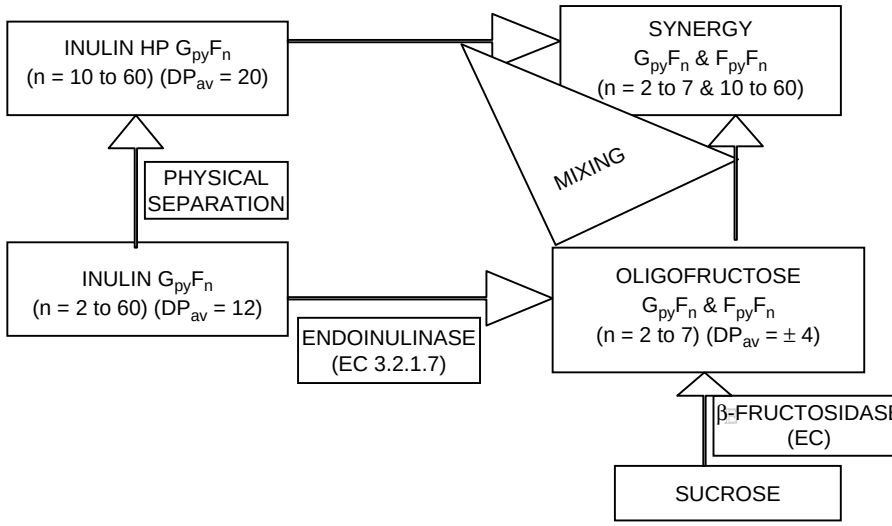
Chicory inulin is a linear - (2➟1) fructan composed of a mixture of oligomers and polymers in which the DP varies from 2 to, more or less, 65 units with a DP_{av} = 12. About 10% of the fructan chains in native chicory inulin have a DP ranging between 2 (F_2) and 5 (GF_4). The partial enzymatic hydrolysis of inulin using an endoinulinase (EC 3.2.1.7) produces oligofructose that is a mixture of both $G_{py}F_n$ and $F_{py}F_n$ molecules, in which the DP varies from 2–7 with a DP_{av} = 4. It is composed

primarily of lower DP oligosaccharides, namely, 1-kestotriose, 1,1-kestotetraose and 1,1,1-kestopentaose, as well as inulobiose, inulotriose, and inulotetraose. Oligofructose can otherwise be obtained by enzymatic synthesis (transfructosylation) using the fungal enzyme b-fructosidase (EC 3.2.1.7) from *Aspergillus niger*. In this reaction, in a process similar to the plant biosynthetic pathway, sucrose serves as a substrate to which 1, 2, or 3 additional fructose units are added by forming new b-(2,1) linkages. In such a synthetic compound, DP varies from 2–4 with DP_{av} = 3.6 and all oligomers being the $G_{py}F_n$-type.

By applying specific separation technologies, the food industry also produces a long-chain inulin known as inulin HP (DP 10–60) with a DP_{av} = 25. Finally, a specific product known as Synergy 1®* is produced commercially by combining chicory oligofructose and long-chain inulin. The different industrial products vary in $DP_{av,}$ DP_{max}, and DP distribution (Figure 3.3), and they have different properties.[32]

3.3.2 Nomenclature of Inulin

In the following chapters of this book that review the nutritional properties and health benefits, the term inulin shall be applied as a generic term to cover all b-(1➟2) linear fructans when the properties reviewed concern all types of molecules. In any other circumstances that justify the identification of oligomers vs. polymers, the terms *oligofructose* and *inulin* will be used, respectively. Even though the inulin hydrolysate and the synthetic compound have a slightly different DP_{av} (4 and 3.6,



$G_{py}F_n$ = α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside

$F_{py}F_n$ = α-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside

FIGURE 3.3 Chemical description of chicory inulin, oligofructose, and their derivatives.

* In the rest of the book this specific product will be identified as oligofructose-enriched inulin Synergy 1.

respectively), the term *oligofructose* shall be used to identify both. Indeed, *oligofructose* and (short-chain) *fructooligosaccharides* are considered to be synonyms to name the mixture of small inulin oligomers with $DP_{max} < 10$.[33–37] Moreover, as outlined by Farnworth, "although the initial findings (on the effects of inulin) were based on Neosugar (the synthetic or so-called short-chain fructooligosaccharide), it has become evident that many of the conclusions extend to other sources of dietary fructans (and especially inulin and inulin derivatives)."[38] The term *inulin* will be used to identify both native chicory inulin and any other derived industrial products except oligofructose. When appropriate, the mixture oligofructose and inulin HP will be identified as oligofructose-enriched inulin Synergy 1.

3.3.3 Industrial Production of Inulin and Oligofructose and Related Products

During the early 1990s, several attempts were made to isolate and purify inulin and oligofructose for use as dietary supplements. Nowadays, they are used in a pure form as ingredients in many food products. The roots of chicory, which are also used in different countries for the production of a coffee substitute (after roasting), look like small oblong-shaped sugar beets. Their inulin content is high (more than 70% of dry matter) and fairly constant from year to year. The production process involves extracting naturally occurring inulin from chicory roots by diffusion in hot water, in a manner very similar to the extraction of sucrose from sugar beets. The raw extract is then refined by using technologies from the sugar and starch industries (e.g., ion exchangers), and then evaporated and spray dried. Chicory oligofructose is obtained by partial enzymatic hydrolysis of inulin, eventually followed by spray drying (Figure 3.4).[25]

Hydrolysis is catalyzed either by exo-inulinase (EC 3.2.1.80), by the combined action of exo- and endo-inulinases, or solely by endoinulinase (EC 3.2.1.7). Although the best source of these enzymes is *Kluyveromyces fragilis* that produces only an exo-inulinase, most inulin-hydrolyzing enzymes of yeast origin have both exo- and endoinulinase activity.[8] The enzymes used for the commercial production of fructose and oligofructose come from *Aspergillus niger* or *Aspergillus ficuum.*

The long-chain inulin or inulin HP is produced by using physical separation techniques to eliminate all oligomers with a DP < 10. The product known as Synergy 1 is obtained by mixing 30:70 w/w oligofructose and inulin HP.

Other products are also made from inulin by intermolecular (depolymerizing) fructosyl-transferases (from *Arthobacter globiformis, Arthobacter urefaciens,* and pseudomonas) like DFA's (difructose dianhydrides) and cyclic forms of difructose.[39] Cyclofructans are also produced using an extracellular enzyme of *Bacillus circulans*. This enzyme forms mainly cycloinulohexaose (CFR-6), but also small amounts of cycloinuloheptaose and -octaose by an intramolecular trans-fructosylation reaction.[40]

3.3.4 Technological Properties of Chicory Inulin and Oligofructose

Chicory inulin, inulin HP, and the oligofructose-enriched inulin Synergy 1 mixture are available as white, odorless powders, and oligofructose as powders and colorless

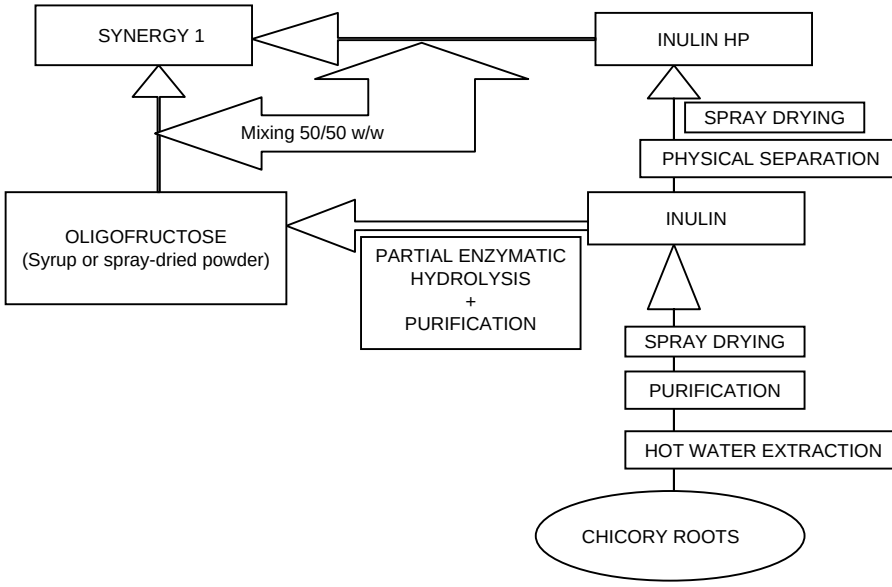


FIGURE 3.4 Industrial processes to produce chicory inulin, oligofructose, and their derivatives.

viscous syrups (75% of dry matter), all with a high purity and a well-known chemical composition. Their physicochemical and technological properties are summarized in Table 3.3.

Inulin has a bland, neutral taste, without any off-flavor or aftertaste. Because it contains fructose, glucose, and sucrose, native inulin is slightly sweet (10% sweetness in comparison with sugar), whereas inulin HP is not. It combines easily with other ingredients without modifying delicate flavors. Solubility in water is moderate (maximum 10% at room temperature) and brings a rather low viscosity (less than 2 mPa for a 5% w/w solution in water).

When thoroughly mixed with water or another aqueous liquid, submicron crystalline inulin (especially inulin HP) particles form a tri-dimensional gel network resulting in a white creamy structure with a short spreadable texture, which can easily be incorporated into foods to replace up to 100% fat.[41] Large amounts of water are immobilized in this network, which assures its physical stability. Special instant qualities of inulin, that do not require shearing to give stable homogeneous gels, also have been developed using a specific spray-drying process. Inulin works in synergy with most gelling agents like gelatin, alginate, k- and i-carrageenans, gellan gum, and maltodextrins. It also improves the stability of foams and emulsions such as aerated desserts, ice creams, table spreads, and sauces.[42]

Oligofructose is much more soluble than inulin (about 80% in water at room temperature). In the pure form, it has a sweetness of about 35% in comparison with sucrose. Its sweetening profile closely approaches that of sugar, the taste is very clean without any lingering effect, and it also enhances fruit flavors. It combines with intense sweeteners such as aspartame and acesulfame K, providing mixtures

TABLE 3.3
Physicochemical and Technological Properties of Chicory Inulin, Oligofructose, and Their Derivatives in Powder Form

	Inulin	Inulin HP	Oligofructose	Synergy 1
Chemistry	$G_{py}F_n$ DP 2–60	$G_{py}F_n$ DP 10–60	$G_{py}F_n$ and $F_{py}F_n$ DP 2–7	$G_{py}F_n$ and $F_{py}F_n$ DP 2–7 DP 10–60
DP_{av}	12	25	4	
Content (%dry matter)	92	99.5	95	95
Dry matter (%)	95	95	95	95
Sugars (% dry matter)	8	<0.5	5	
pH (10% in H_2O)	5–7	5–7	5–7	5–7
Ash (% dry matter)	<0.2	<0.2	<0.2	<0.2
Heavy metals (% dry matter)	<0.2	<0.2	<0.2	<0.2
Color	White	White	White	White
Taste	Neutral	Neutral	Moderately sweet	Moderately sweet
Sweetness vs sucrose (%)	10%	None	35%	
Water solubility (% at 25˚C)	12	2.5	>75	
Water viscosity (5% at 10˚C)	1.6 mPa	2.4 mPa	<1 mPa	
Food application (specific)	Fat replacers	Fat replacers	Sugar replacers	
Food application (synergism)	+Gelling agent	+Gelling agent	+Intense sweetener	

Source: Adapted from Franck, A., Technological functionality of inulin and oligofructose, *Br. J. Nutr.*, 87 (suppl. 2), S287–S291, 2002.

with a rounder mouth feel and a better sustained flavor with reduced aftertaste, as well as improved stability. Combinations of acesulfame K–aspartame blends with oligofructose also exhibit a significant quantitative sweet taste synergy.[43] Oligofructose is a stabling sty during usual food processes (e.g., during heat treatments) even if the b-links between the fructose units are (partially) hydrolyzed in very acidic conditions, at high temperatures, and under low dry-substance conditions. Oligofructose also contributes toward improved mouth feel, shows humectant properties, reduces water activity ensuring high microbiological stability, and affects boiling and freezing points. In summary, oligofructose is, technologically speaking, similar to sucrose and glucose syrup.[44]

At a high concentration (>25% in water for standard chicory inulin and >15% for long-chain inulin), inulin has gelling properties and forms a particle gel network after shearing. When it is thoroughly mixed with water or another aqueous liquid, using a shearing device such as a rotor-stator mixer or a homogenizer, it forms a white creamy structure that can easily be incorporated into food products to replace fat up to 100%.[45] Such a gel provides a short spreadable texture, a smooth fatty mouth feel, as well as a glossy aspect and a well-balanced flavor release. As far as fat replacement is concerned, long-chain (high performance) inulin shows about twice the functionality compared to standard chicory inulin, thus allowing for lower dosage levels.

The gel strength obtained depends on the concentration of inulin and total dry substance content; on the shearing parameters (such as temperature, time, speed, or pressure); and also on the type of shearing device used, but is not influenced by pH (between 4 and 9). Electron cryomicroscopy analysis has shown that such an inulin gel is composed of a tridimensional network of insoluble submicron inulin particles in water. These particles (±100 nm in size) aggregate to form larger clusters with a diameter of 1–5 microns. Large amounts of water are immobilized in this network, as determined by NMR. X-ray diffraction analysis confirmed the crystalline nature of the gel particles, whereas the starting inulin powder is essentially amorphous. An inulin gel exhibits a viscous-elastic rheological behavior and shows shear-thinning and thixotropic properties. It is characterized by a relatively low yield stress (e.g., 1540 Pa for a gel of 30% standard inulin in water at 25˚C). Inulin also displays synergy with most gelling agents (e.g., gelatine, alginate, kappa and iota carrageenan, gellan gum, and maltodextrin).[46]

Furthermore, inulin improves the stability of foams and emulsions, e.g., in aerated dairy desserts, ice creams, table spreads, and sauces. It can therefore replace other stabilizers in different food products.

Inulin and oligofructose can be used for either their nutritional advantages or technological properties, but often their applications offer a dual benefit: an improved organoleptic quality and a better-balanced nutritional composition. Table 3.4 gives an overview of their applications in foods and drinks.

The use of inulin or oligofructose as a dietary fiber ingredient in bakery products and breakfast cereals often leads to an improved taste and texture, and gives more crispiness.[42] These ingredients also keep breads and cakes moist and fresh longer. Their solubility allows fiber incorporation in watery systems such as drinks, dairy products, and table spreads.[47,48] Oligofructose is also often formulated in other (low-calorie) dairy products, frozen desserts, and meal replacers.[49]

Because of its specific gelling characteristics, inulin is used to develop low-fat foods without compromising on taste and texture. In table spreads, both fat and water-continuous, inulin allows the replacement of significant amounts of fat and the stabilization of the emulsion, while providing a short spreadable texture. It can also be applied in fat-reduced spreads containing dairy proteins, as well as in butter-like recipes and other dairy-based spreadable products. In low-fat dairy products, such as fresh cheese, cream cheese, or processed cheese, the addition of a few percents of inulin gives a creamier mouth feel and imparts a better-balanced round flavor. Inulin can also be used as a fat replacer in frozen desserts, providing easy

TABLE 3.4
Typical Examples of Food Technology Applications of Chicory Inulin, Oligofructose, and Their Derivatives

Food Products	Applications
Dairy products	Body and mouth feel
	Foam stability
	Sugar and fat replacement
	Synergy with sweeteners
Frozen desserts	Sugar and fat replacement
	Synergy with sweeteners
	Texture and melting
Table spreads	Fat replacement
	Texture and spreadability
	Emulsion stability
Baked goods and breads	Sugar replacement
	Moisture retention
Breakfast cereals	Crispness and expansion
Fruit preparations	Sugar replacement
	Synergy with sweeteners
	Body and mouth feel
Meat products	Fat replacement
	Texture and stability
Chocolate	Sugar replacement
	Heat resistance

Source: Adapted from Franck, A., Coussement, P., Multi-functional inulin, *Food Ingred. Anal. Int.* October, 8–10, 1997.

processing, a fatty mouth feel, and excellent melting properties, as well as freeze–thaw stability, without any unwanted off-flavor. Fat replacement can further be applied in meal replacer, meat products, sauces, and soups, e.g., to produce sausages and pâtés with a creamier and juicier mouth feel, and improved stability due to better water immobilization. The incorporation of inulin (1–3%) in fruit yogurts, possibly through fruit preparations, improves the mouth feel and offers a synergistic taste effect in combination with aspartame or acesulfam K or both. Furthermore, it increases the stability of foams and mousses, and improves the processability of dairy-based aerated desserts. The resulting products retain their typical structure longer and show a fat-like feeling.

Inulin also has found an interesting application as a low-calorie bulk ingredient in chocolate without added sugar, often in combination with a polyol. It is also used as a dietary fiber or sugar replacer in tablets.

Inulin has thus become a key ingredient in the food industry, offering new opportunities for the development of well-balanced and yet better-tasting products.[32]

3.3.5 Analytical Methodologies

Presently, appropriate legal authorities in most countries have confirmed that inulin and oligofructose can be labeled as "dietary fiber" for food labeling (see Chapter 6). However, because of its solubility in ethanol/water (4/1), the classical methods for the analysis of dietary fiber do not analyze oligofructose and only partly inulin that are, moreover, partly degraded in the acid hydrolysis steps.[33,50] Recently, based on the results of a collaborative ring test, AOAC International has adopted, as method number 997.08, the *fructan method* that allows specifically the accurate quantitative determination of inulin and oligofructose in foods.[51] The method, which is shown in Figure 3.5, involves treatment of the sample with amyloglucosidase and inulinase enzymes, followed by determination of the released sugars by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

The method can be combined with the standard AOAC total dietary fiber method to quantify the total amount of fiber used for food labeling.[33] Another variant of a similar methodology is applicable to quantify all nondigestible oligosaccharides (or resistant short chain carbohydrates). It involves enzymatic hydrolysis of a-glucans to glucose and fructans to fructose, precipitation of nonstarch polysaccharides in ethanol, or sulfuric acid hydrolysis of all nondigestible oligosaccharides (or resistant short chain carbohydrates) to their monosaccharide constituents followed by their chemical ($NaBH_4$) reduction to acid-stable alditols that are quantified by gas–liquid chromatography as alditol acetate derivatives.[52] To quantify the individual oligomers

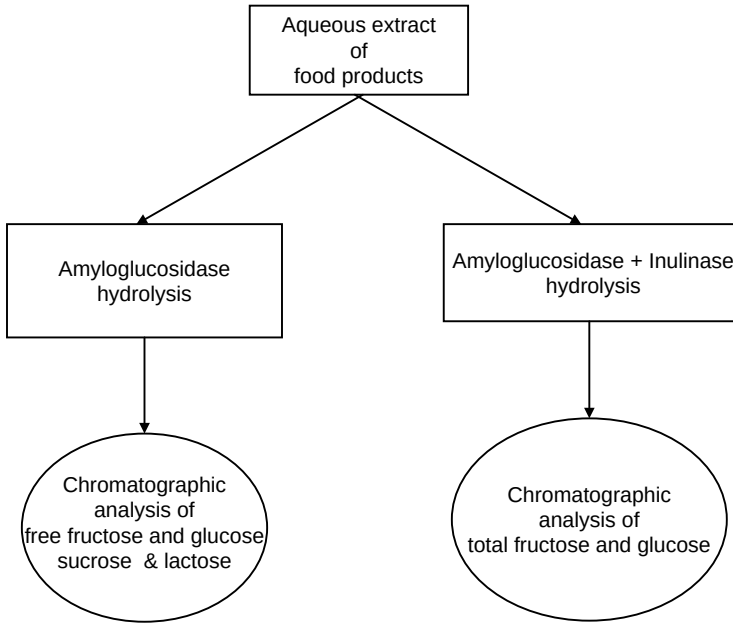


FIGURE 3.5 Schematic representation of the steps in the analysis of inulin and oligofructose in food products.

(up to DP = 10), a capillary gas chromatographic method that includes the derivatization (oximation and silylation) of extracted sugars (isooctane extracts), a cool on-column injection on an apolar Al-clad column, and flame ionization detection has been developed.[53] This method has been used to quantify oligofructose in plants (see Section 3.2.3) and is routinely used for analysis of food products. A typical chromatogram identifying all $G_{py}F_n$ and $F_{py}F_n$ oligomers in oligofructose is shown in Figure 3.6. DIONEX chromatography and pulsed electrochemical detection are used to analyze the composition of different industrial preparations of inulin by identifying all oligomers and polymers up to a DP 60 or even higher (Figure 3.7).

To quantify total oligofructose and/or inulin in plants or food products, various enzymatic methods have been developed and eventually validated. These methods involve the following hydrolysis steps and detection methodologies:

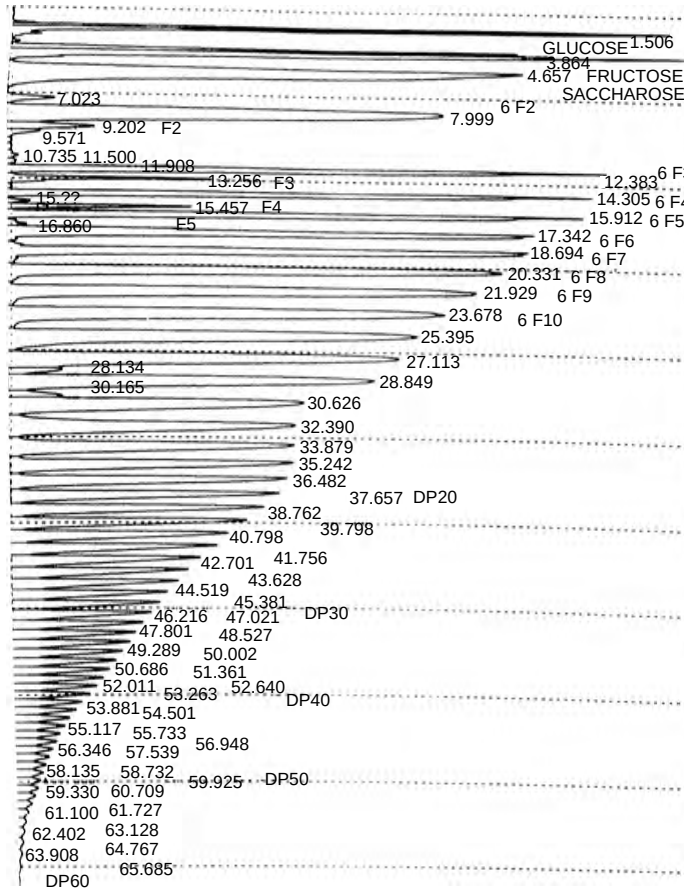


FIGURE 3.6 Distribution of the fructan chains in chicory inulin as analyzed by High Performance Anion Exchange Chromatography and Pulse Amperometric Detection (HPAEC-PAD).

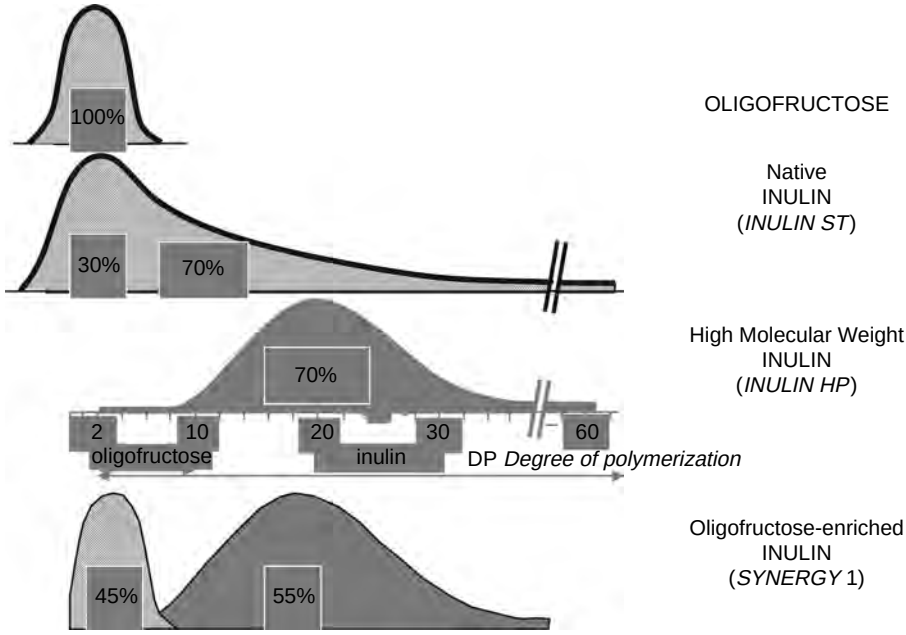


FIGURE 3.7 Schematic representation of the DP distribution of the fructan chains in oligofructose, native inulin, high molecular weight inulin, and oligofructose-enriched inulin as analyzed by HPAEC-PAD.

- CGC quantification (see above) of sucrose glucose and fructose before (to quantify native mono- and disaccharides) and after (to quantify inulin) enzymatic (Novozym 230) hydrolysis of all oligomers and polymers[53]
- Oxidation of native glucose by glucose oxidase (EC 1.1.3.4) enzymatic determination of native fructose, followed by hydrolysis of inulin by inulinase (EC 3.2.1.7), and quantification of inulin-derived fructose by using a series of biochemical reactions that include its phosphorylation to fructose-6-phosphate by hexokinase (EC 2.7.1.1) and ATP, followed by isomerization to glucose-6-phosphate by phosphoglucose isomerase (EC 5.3.1.9), oxidation to gluconate-6-phosphate by glucose-6-phosphate–$NADP^+$ oxidoreductase (EC 1.1.1.49), and finally the spectrophotometric (340 nm) determination of NADPH[53]
- Fractionation of the hot water extract of the plant or food product into three extracts used, respectively, to measure free glucose, fructose, and sucrose (after yeast a-glucosidase hydrolysis), glucose from starches and other a-gluco-oligo/polysaccharrides (after *Aspergillus niger* a-amyloglucosidase hydrolysis), and total fructose and glucose from inulin (after fructanase hydrolysis) and other oligo/polysaccharides (after a-amyloglucosidase hydrolysis), respectively, followed by spectrophotometric measurement of NADPH, as described above, and calculation of glucose and fructose released from fructans by the differences[54]

- Hydrolysis of sucrose (by sucrase) to fructose and glucose, and starch to glucose (by a mixture of a–amylase, pullulanase and maltase), and reduction of the hexoses (using alkaline borohydride solution) to sugar alcohols, followed by hydrolysis of inulin (by a mixture of purified *exo*- and *endo*-inulinases) to fructose and glucose that are then measured with a spectrophotometer after reaction with *para*-hydroxybenzoic acid hydrazide.[55, 56]

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4 The Digestive Functions: Inulin-Type Fructans as Nondigestible Oligosaccharides

Digestion is a complex process that includes:

- Masticating, mixing, and triturating the food to disrupt the matrix
- Solubilization and micelle formation
- Partial or complete hydrolysis of the complex food

Strictly speaking, digestion comprises only the final hydrolytic step (see Figure 2.2).

4.1 DIGESTION OF CARBOHYDRATES IN THE GASTROINTESTINAL TRACT

In the human diet, the most common carbohydrates are starch, sucrose, lactose, fructose, glucose, and dietary fibers. Most (±50–60% of daily intake) carbohydrates are starch, which is a mixture of linear (amylose) and branched (amylopectin) polymers of glucose with a-1, 4 and a-1, 4 + a-1, 6 linkages, respectively. Starch, as well as the disaccharides lactose and sucrose, is hydrolyzed in the upper part of the gastrointestinal system (Figure 4.1), essentially the oral cavity and the small intestine, whereas the dietary fibers are not. The monosaccharides that preexist in the diet (fructose and glucose) and that are produced by the hydrolysis of starch and disaccharides (lactose and sucrose) are absorbed and reach the systemic circulation via the portal vein. But the oligo- and monosaccharides that reach or are produced in the large bowel, essentially by bacterial hydrolysis of dietary fibers and, in some populations, lactose, are not absorbed but fermented. Strictly speaking, the digestion process concerns only starch, lactose, and sucrose, and the absorption process in the small intestine concerns fructose and galactose but mainly glucose.

4.1.1 Carbohydrate Hydrolysis in the Oral Cavity and the Stomach

Digestion of amylose but not amylopectin starts in the oral cavity because of the presence of salivary ptyalin, a a–amylase that specifically splits the a1, 4 glucose-

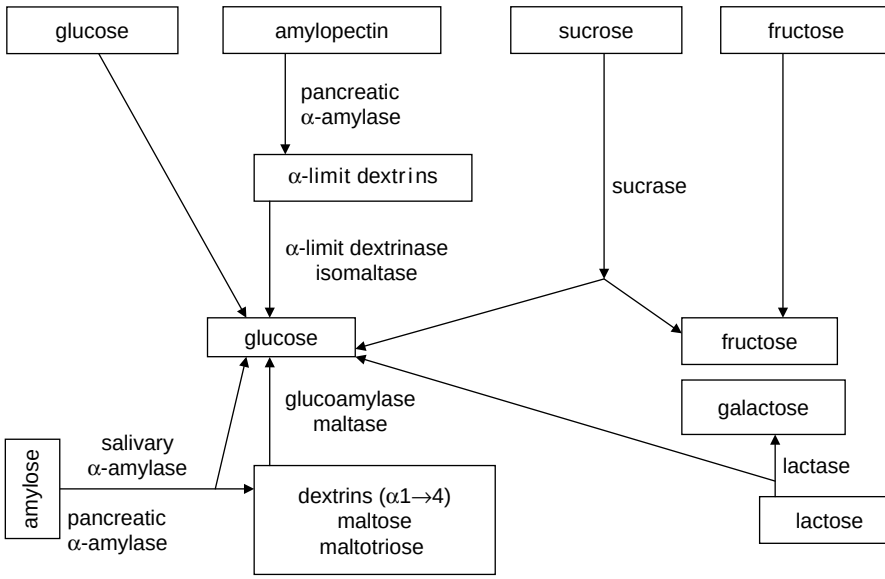

FIGURE 4.1 Fate of dietary carbohydrates in upper organs of the gastrointestinal system.

glucose linkages in linear but not in branched chains. The products of amylose hydrolysis in the oral cavity are maltose (a-1, 4-D-glucopyranosyl-a-D-glucopyranose) and maltotriose (a-1, 4-D-glucopyranosyl-a-D-glucopyranosyl-a-D-glucopyranose). In the stomach, due to the acidic pH, free ptyalin is rapidly inactivated. However, in the presence of oligosaccharides (DP 3–8), the enzyme is protected from acidic inactivation, and it remains the only carbohydrate-hydrolase that is active in the stomach, an organ in which no such enzyme is produced. But in addition and because of the low pH, acid hydrolysis does occur more or less extensively depending on the residence time, the concentration of the carbohydrates in the stomach, and on the granulometry of starch (after chewing and mastication). In the chyme that leaves the stomach and enters the duodenum, dietary lactose, sucrose, and glucose-oligosaccharides are thus not digested, whereas starch is only partly hydrolyzed (amylose) or practically not hydrolyzed (amylopectin).

4.1.2 Carbohydrate Hydrolysis in the Small Intestine

The only carbohydrate hydrolase in the lumen of the small intestine is a pancreatic a–amylase that hydrolyses both amylose and amylopectin but cannot hydrolyze a-1,4 glucose–glucose linkages close to a-1,6 branching points. It does not hydrolyze all types of starches because the extent of starch digestion in the small intestine is variable depending on physical form of starch-containing food and of starch itself.[1] Moreover, it does not produce free glucose from starch. Indeed, the end products of a–amylase hydrolysis of starch in the small intestinal lumen are maltose, maltotriose, and a-limit dextrins (glucose oligomers with DP 5–10 and a-1, 4 and a-1, 6 glucose–glucose linkages). These short chain glucose oligomers as well as the dietary

disaccharides (lactose and sucrose) are rapidly split up by hydrolases that are located in the outer portion of the brush border, the membrane of the microvilli of the small intestine. The human brush border hydrolases are glucoamylase or maltase, α-limit dextrinase, lactase, and sucrase that hydrolyze α-limit dextrins, maltose and maltotriose, lactose, and sucrose, respectively, to produce glucose, fructose, and galactose. The hydrolytic activity is relatively low in the duodenum; it reaches its maximum activity in the jejunum and then falls off to a relatively low level in the ileum. Consequently, the hydrolysis of carbohydrates is nearly completed in the mid-jejunum in normal physiological conditions. In humans, high dietary intake of fructose and sucrose, but not of glucose, stimulates the biosynthesis of the sucrase–isomaltase complex, but it has no effect on lactase activity that is also not stimulated by lactose intake.

4.1.3 Methods to Study the Digestibility of Oligo- and Polysaccharides[2]

The methodologies to demonstrate that oligo/polysaccharides resist digestion in the upper part of the gastrointestinal system are:[2]

- Chemical analysis to confirm the configuration (β) of the osidic linkages
- *In vitro* incubation in fresh saliva or gastric juice or in the presence of pancreatic or small intestinal tissue homogenate
- *In vivo* disappearance of ingested carbohydrate from the intubated rat small intestine
- *In vivo* recovery in feces of germ-free or antibiotic treated rats to suppress the intestinal microflora
- *In vivo* aspiration of residual digesta from the ileum using intubation
- Recovery in the pouch of ileostomy
- Ingestion of the carbohydrate resulting in change in glycemia and insulinemia

4.2 ABSORPTION OF HEXOSES IN THE SMALL INTESTINE

The free hexoses originating from the diet and from the hydrolysis of starch and disaccharides are rapidly absorbed all along the small intestine by crossing its wall. The process is very efficient and all of the monosaccharides (up to 120 g/h) are removed from the intestinal lumen before the remains of the meal reach the terminal part of the ileum. After absorption the sugar molecules cross the mucosal cells and enter the blood stream in the capillaries draining into the portal vein and finally, via the portal circulation, into the liver. The transport of glucose and galactose through the brush border of the intestinal mucosa is an active symport with Na^+ but their transfer out of the cell into the interstitium and from there into the portal capillaries is by a simple and facilitated transport mechanism. The absorption of fructose is independent of Na^+ and involves a facilitated diffusion.

4.3 INULIN-TYPE FRUCTANS AS NONDIGESTIBLE OLIGOSACCHARIDES (NDOS)

The enzymes that decompose starch, lactose, or sucrose cannot hydrolyze the osidic linkages in chicory inulin and oligofructose. Indeed, the specific hydrolytic activity of these enzymes is on a–(1➟4) or eventually a–(1➟6) linkages whereas inulin and oligofructose have almost exclusively b-(1➟2) [and a very few b-(6➟2)] linkages. Demonstrating that inulin and oligofructose are indeed nondigestible *in vivo* in humans is not an easy task because measuring *in vivo* digestion of any substance in the stomach and small bowel is difficult. The methodologies and their results (Table 4.1) are reviewed in the next section.

4.3.1 Methodologies and Results

TABLE 4.1
Inulin and Oligofructose as Nondigestible Oligosaccharides: Methodologies and Results

Methodologies	Results
Linkage analysis	
GLC-MS & ^{13}C-NMRS	b-(2➟1) (b-(2➟6)
In vitro incubation with 0.05 *M* HCl	
Human saliva	No significant hydrolysis
Human gastric juice	
Rat pancreatic homogenate	
Rat small intestinal mucosa	
Human small intestinal mucosa	
Sucrase–maltase	
In vivo	
Glycemia, insulinemia, and C-peptide in blood after oral consumption in humans	No change
Intubation of rat gastrointestinal tract and recovery in various segments	Complete recovery up to the end of ileum
Intubation of rat gastrointestinal tract and recovery in various segments	Complete recovery up to the end of ileum
Intubation of human gastrointestinal tract and collect of ileal fluid	89% recovery in ileal fluid
Recovery in ileostomy volunteers	86–88% recovery

4.3.1.1 Linkage Analysis of Inulin-Type Fructans[3]

Essentially, two analytical tools are used to identify the nature and configuration of fructosyl-fructose linkages in inulin, namely gas–liquid chromatography coupled to electron-impact mass spectrometry (GLC-MS) and Carbon-13 Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMRS). In the first methodology, the (1➟2) linear, plus eventually the (6➟2) branching fructosyl-fructose linkages, are identified by mass spectrometry after permethylation of the hydroxyl groups. If the molecule has

a DP > 4, it must be partly hydrolyzed (enzymatic hydrolysis) prior to the analytical processing. In the second methodology, distinctive chemical shifts of each of the carbons of D-glucosyl and D-fructosyl residues can be characterized. Based on the resonance of the anomeric carbons (i.e., C_2), the nature and the configuration of the fructosyl-fructose linkages as well as the furanose form of all the fructosyl units has been demonstrated.[4]

4.3.1.2 *In Vitro* Models To Demonstrate Resistance of Inulin-Type Fructans to Digestion

To demonstrate *in vitro* resistance to acid (stomach) and enzymatic (saliva, pancreatic, and small intestinal) hydrolysis, inulin and/or oligofructose are dissolved in appropriate medium (saline or buffer solution) and incubated at 37°C in the presence of 0.05 *M* HCl (to mimic acidic pH in the stomach), human saliva, human gastric juice collected after fasting overnight (eventually after administration of pentagastrin to stimulate secretion), homogenates of rat pancreas or rat and human small intestinal mucosa, or sucrase-maltase enzymatic preparations purified from duodenal, jejunal, or ileal mucosa.[5–9]

Pancreas and the various segments of the small intestine are taken from rats after sacrifice, the former being homogenized in physiological saline and the later being split open and clean before mucosa is scraped and homogenized. The sucrase–maltase complex is then extracted and purified.[10] Human jejunal or duodenal mucosa is scraped out of intestinal tissues either taken from patients who had died because of nonintestinal disease[10] or sampled during gastroduodenal endoscopy in patients referred with dyspeptic syndromes. After appropriate incubation, the products of hydrolysis (D-glucose and eventually D-fructose) are assayed chemically or enzymically using the standard methods.[10–12] Any other method involves the incubation of isolated everted jejunum segments of rat small intestines containing an inulin solution and measuring the transmural potential difference evoked by Na^+-dependent transport of glucose which is supposed to be produced by the hydrolysis of inulin.[13]

4.3.1.3 Rat Models to Demonstrate, *In Vivo,* the Resistance of Inulin-Type Fructans to Digestion

In rats the resistance of inulin to any endogenous digestive process can be shown by measuring the recovery in feces of an oral dose given to a germ-free or an antibiotic (Neobacitin) pretreated rat to suppress the intestinal flora and comparing this to untreated rats.[5,7,14]

Other more invasive methods used by Nilsson et al. involve the intubation of an inulin solution into the gastrointestinal system of living anaesthetized rats.[8] In such models, inulin is:

- Either intubated into the stomach, followed 2–4 h later by opening the abdomen, clamping at five to six locations of the gastrointestinal tract to isolate the stomach, the upper, middle, and lower third of the small

intestine, the large intestine and finally transfer and homogenization of the different clamped segments.

- Or intubated directly into the small intestine after abdomen opening, insertion of a tube, and clamping of the intestinal segment (±30 cm in length) filled with the solution, followed by removal of samples at regular intervals for 90 min. Both the homogenates and the intestinal samples are then analyzed to quantify their content in free glucose[12] and free fructose.[15]

4.3.1.4 Human Models To Demonstrate, *In Vivo,* the Resistance of Inulin-Type Fructans to Digestion

The models applicable to humans to demonstrate, *in vivo*, the resistance to digestion involve either the direct recovery of nondigested molecules or an indirect assessment demonstrating that neither glycemia nor insulinemia are significantly increased after oral administration of inulin and oligofructose. For the first approach, a dose of inulin is given orally to:

- Normal healthy volunteers who are then intubated through the oral cavity to allow distal ileum fluid sampling[9]
- Patients who have been subjected to proctocolectomy for ulcerative colitis or familial polyposis without resection of the small intestine or the stomach, who have a stable ileostomy function, who do not have current antibiotic treatment, and whose ileostomy bags are sampled at regular intervals[16,17]

The intubation technique, with a nonabsorbable marker, allows a quantitative assessment of ileal flow.[18–21] In this technique, a triple lumen tube led by a mercury bag, which can be inflated with air to accelerate progression of the tube, is introduced through the oral cavity into the gastrointestinal tract of a volunteer in a semirecumbent position. X-ray confirms the location of the tip in the tube. The major drawbacks of the technique are that it is time consuming, relatively invasive, and expensive. Moreover, it has been shown that the presence of the tube in the intestine affects the gastrointestinal functions,[22] and it is difficult to assess the ileal flow rate of solids with precision.[23]

The ileostomy model is widely accepted as a valuable alternative to study the small intestinal excretion of nutrients.[24–27] The main advantages of the ileostomy model are[24,28,29]

- Short transit time allowing the one day's intake to be completely excreted within 24 h, making short-time balance studies feasible[24, 28]
- Small within-patient, within-diet, and day-to-day variation[30]
- Transit time through the stomach and the small intestine of ileostomy subjects which is similar to that observed in healthy subjects[22]
- Easy collection of ileostomy contents by the subjects who are accustomed to routinely handling the excreta

In the case of inulin, after ingestion of a known amount, the chyme coming out of the small intestine in the pouch is analyzed for inulin content and eventually for oligo and polymer composition. Additional measurements such as bacterial contamination or presence, amount and composition of fermentation products (e.g., SCTAs) are also possible and explain the eventual low percentage of disappearance (apparent digestion).[16]

In the indirect model, the increment in blood glucose, insulin, and C-peptide (the linking region between the A and B chains of pro-insulin that is cleaved when it is converted to insulin and that serves as a marker of pancreatic b-islet cell function), as well as the area under the curves, are measured in healthy volunteers after an oral dose of inulin compared to an equivalent dose of fructose.[31]

4.3.1.5 Experimental and Human Data Demonstrating That Inulin-Type Fructans Resist Digestion

That inulin is a linear b-(1➡2) fructan is well demonstrated.[3,4] Its nondigestibility has been demonstrated both in *the in vitro* and in the *in vivo models* described above.

Incubated *in vitro* with saliva (as in the oral cavity), acid (as in the stomach), gastric juice, pancreatic homogenate, small intestinal mucosa homogenate, or mucosal carbohydrate hydrolases both from rat or human origin (both oligofructose and inulin) remain largely unchanged.[5–9,32] Especially oligofructose (synthetic oligofructose, essentially GF_2 and GF_3) has been incubated *in vitro* with either rat pancreatic homogenate and data show that it is "hardly digested."[5,32] In an early study, McCance and Lawrence had shown inulin to be labile in gastric juice.[33] But Nilsson et al incubated various cereal fructans and inulin in fresh human gastric juice for 1 h and showed that at pH 1.05, 10–15% was hydrolyzed, but above pH 1.8 less than 1% was degraded. These authors concluded that "the stomach hydrolysis of (inulin-type) fructans is likely to be of limited physiological significance."[8] When incubated with homogenized rat jejunal mucosa, the rate of hydrolysis of inulin was ±1/200th and 1/900th compared to sucrose and maltose, respectively.[5] Inulin disappearance from the intubated rat small intestine *in vivo* was virtually nil.[8] By measuring the transmural potential difference (DPDs) evoked by Na^+-dependent transport of glucose after incubation of isolated everted jejunum segments of rat small intestines containing miscellaneous carbohydrate solutions, Tsuji et al. reported that, as compared to glucose, maltose, and sucrose DPDs evoked by oligofructose was negligible.[13] No change in blood glucose, insulin, or C-peptide was seen after 25 g of inulin had been given to healthy subjects, nor when inulin extracted from Jerusalem artichokes (30% GF_7 or greater) at doses of 5, 10, or 20 g were taken either alone or with other carbohydrates.[31,32] Inulin fed to germ-free or antibiotic pretreated rats to clear the colon of its microflora was almost completely recovered (> 80%) in feces, showing its resistance to any digestive process all along the gastrointestinal tract.[5,7,14]

The most convincing data regarding the nondigestibility of inulin came from the *in vivo* human studies.[34] Using the ileostomy model, Bach-Knudsen and Hessov, and Ellegärd et al. have demonstrated that 86–88% of the ingested dose (10, 17, or 30 g) is recovered in the ileostomy effluent.[16,17] Using an intubation technique, Molis

et al. have similarly reported that oligofructose is unabsorbed in the small intestine and that ±89% of the ingested dose (20 g) reach the terminal part of the ileum.[9] These figures (±85–90%) are comparable to the recovery of obtained with pectin or wheat bran but slightly lower than that for complex cereal foods or potatoes.[24,26,28,35,36]

4.4. INULIN-TYPE FRUCTANS AS NONDIGESTIBLE OLIGOSACCHARIDES: DISCUSSION AND CONCLUSION

All the data reported above convincingly demonstrate that inulin-type fructans indeed resist digestion and absorption in the upper parts of the gastrointestinal system. Even though they may be hydrolyzed in very acidic conditions and/or if the gastric retention time becomes abnormally long, in normal physiologic condition they pass unchanged through the oral cavity and the stomach. In the chyme, inulin is thus still chemically intact. Moreover, neither the pancreas nor the mucosa of the small intestine secrete or contain enzymes that are able to hydrolyze β-(1→2) fructosyl-fructose linkages. Even the β-(1→2) glucosyl-fructose linkage that may be present at the beginning of the inulin chains largely resists even the hydrolytic activity of the sucrase-maltase complex. The *in vivo* human studies largely confirm that essential property, even though they indicate a small but significant loss (±12%) of inulin during the passage through the small intestine.[34,37]

This can be explained in three ways as:

1. Acid and/or enzymatic hydrolysis especially of the β-(2→1) glucosyl-fructose linkage in GF_1 (or sucrose) and GF_2 and eventually the very first β-(1→2) fructosyl-fructose linkages. However, such a hydrolysis is very limited. Indeed, as stated by Oku et al., the more fructose units the inulin contains, the more nondigestible it became.[5]
2. Absorption of intact short-chain oligosaccharides in the small intestine. Indeed, a very slow rate of absorption (0.15 to 0.25%/5 h) of di- (especially sucrose and lactose) and trisaccharides has been reported. Moreover, it has been shown that these sugars pass across the normal small intestinal wall probably by a process of passive diffusion.[5,38] If such an absorption occurs after inulin or oligofructose ingestion, it will concern only the $F_{py}F$, $F_{py}F_2$, $G_{py}F_2$, and eventually $G_{py}F_3$ oligomers that represent only a small percentage of the oligofructose, the native inulin and the oligofructose-enriched inulin Synergy 1, respectively. Moreover, these absorbed oligomers will be excreted unchanged in the urine because no carbohydrate–hydrolase activity has been detected in the blood or the internal organs.[5,38] By injecting [U-^{14}C] oligofructose intravenously, Oku et al. have shown, especially, that more than 95% of the intravenously injected dose was recovered in the urine within 24 h. In human volunteers fed a

dose of 20 g/d of low molecular weight oligofructose, Molis et al. reported 0.12%/24 h recovery of intact molecules in the urine.[9]

3. Fermentation of oligofructose and inulin by the microbial population that colonizes the ileum, especially in ileostomy patients who have100 times more bacteria in the terminal small intestine (i.e., 10^7–10^8/g) than normal people (10^5–10^6/g).[39,40] In support of that argument, Bach-Knudsen and Hessov have measured lactic acid and the short chain fatty acids, the end products of anaerobic fermentation of carbohydrates, and they have reported that preferential fermentation of inulin is a plausible explanation for the 12–14% loss that occurs during the passage through the small intestine in ileostomy patients.[16]

In conclusion, oligofructose and inulin are nondigestible oligosaccharides; they pass through the upper gastrointestinal system without significant hydrolysis, and they reach the colon as they have been ingested. They have, thus, the basic characteristic of dietary fiber* and should be classified and labeled as such.[5,7,37,41]

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* For a more in-depth discussion of the dietary fiber properties of oligofructose and inulin, see Chapter 6, Section 6.2.

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5 The Digestive Functions: Inulin-Type Fructans as Fermentable Carbohydrates

5.1 THE COLON AS A FERMENTER

As a physiologically important organ that contributes greatly toward host health and well-being, as well as nutrition, the colon contains some 0.25 kg of materials (15–20% water and 80–85% solids), including a large and mixed population of bacteria.[1] The major cations are K^+, Ca^{++}, Mg^{++}, and NH_4^+ while the dominant anions are the organic short chain fatty acid anions, mainly acetate, propionate, and butyrate (Table 5.1).

Through fermentation it produces a wide range of compounds with either beneficial or potentially harmful effects on gut physiology, intestinal as well as systemic immunity, metabolic activities, health, and well-being (Table 5.2).

In monogastric mammals, and especially in humans, the intestinal tract is colonized by a wide variety of bacteria — mostly anaerobes and facultative anaerobes. In the small intestine, the number as well as the diversity of the microorganisms is rather low and not really known. Mostly because of rapid transit, the small intestinal lumen does not contain many bacteria, but it is entirely possible that a mucosal microbiota exists there. Indeed, individual species and groups of microorganisms may exist in different microhabitats and metabolic niches associated with the mucosa or the mucus layer lining the epithelium. Still, very little is known about bacterial communities in human intestinal biofilms.[2]

In contrast, the large bowel contains a large population of microorganisms that represents a high proportion of its content as well as of its mucosa: the colonic microflora. The total number of microorganisms in the colon is close to 10^{13} cells (equivalent to $\pm 10^{12}$ cells/g of content) or ±90% of the total number (10^{14}) of cells (both eucaryotic and prokaryotic) in the human body. The human colonic microflora is composed of ±50 genera and several hundred individual species and strains of bacteria,[3–6] the vast majority of which are anaerobes,[7,8] even though they vary from relatively oxygen-tolerant, like bifidobacteria[9] or bacteroides,[10] to strict anaerobes. In humans, the actual composition of the colonic microflora is still not known because many bacteria cannot be cultured,[8] but the most important species (in number) are likely to be bacteroides, bifidobacteria, eubacteria, and anaerobic gram-positive cocci. Each genus of bacterium may have a specialized ecological niche to

TABLE 5.1
Adult Human Colon: Anatomical Characteristics, Major Components of Luminal Content and Major Physiological Functions

Anatomical characteristics	
Average length	1.5 m
Mean surface (nondissected)	1,300 cm^2
Major components of luminal content	
Average composition	
Water content	15–20%
Dry matter content	80–85%
Bacteria	10^{11} cfu/g dry matter
Major ions	K, Ca, Mg, NH
	SCFA's anions: acetate, propionate, butyrate, etc.
Major physiological functions	Fermentation of nondigestible food components
	Transport of Na^+/Cl via Na^+/H^+ and Cl/HCO_3 exchanges
	Water absorption
	Stool formation

fulfill. There is, however, a high inter-individual variation in the relative percentage of species (e.g., 10^9 to 10^{13} cfu/g for bacteroides and 10^5 to 10^{13} cfu/g for bifidobacteria[4]). The nondigested part of the chyme that leaves the small intestine reaches the large bowel and affects ecological, physiological, and metabolic events. Inside the colon, the transit is slow and the time of residence is long (±54 h).[11] Consequently, bacteria (mostly if their number is 10^7/g) have time to breakdown and to ferment a large proportion of the nondigested components of the chyme as well as endogenous compounds (mainly proteins and carbohydrates). Fermentation allows bacteria to grow and proliferate, but it also salvages part of the energy of the nondigestible nutrients and produces gases (H_2, CH_4, CO_2, H_2S), short chain carboxylic acids (mainly acetic, propionic, butyric acids), better known as the short chain fatty acids (SCFAs), and other miscellaneous compounds (ammonia, amines, phenols).[1,11,12] The colon behaves, thus, like a fermenter that feeds the bacteria and provides the host with physiologically active, but sometimes also potentially toxic, metabolites. A total amount of 50–70 g/d of nondigestible dietary substrates (Table 5.3) is needed to maintain the colonic microflora[1].

The activities of the colonic bacteria are affected by the physiology and the anatomical architecture of their environment, and they fluctuate in response to substrate availability and distribution in the different segments of the large bowel, to luminal pH, to redox potential (Eh), and to oxygen concentration (pO_2).[1,5,13] In the right/proximal colon (cecum and ascending segment), dietary nutrients are abundant; bacteria grow fast and produce large quantities of SCFAs that acidify the

TABLE 5.2
Health-Related Effects Associated with Colonic Microflora

Targets	Effects
Colonic mucosa	Structure of villi
	Epithelial cell growth
	Epithelial cell differentiation
	Cell cycle
	Mitotic index
	Apoptotic index
Colonic functions	Motility
	Transit of bolus
	pH of bolus
	Stool bulking
	Stool frequency
Nutrition	Hydrolysis and fermentation of nondigestible carbohydrates (dietary fibers, oligosaccharides)
	Hydrolysis and fermentation of nondigested proteins and peptides
	Hydrolysis of nondigested lipids and production of free fatty acids
	Partial salvage of energy of nondigested dietary components
Metabolic activities	Production of
	• Short-chain fatty acids (SCFAs)
	• Branched short-chain fatty acids (BSCFAs)
	• NH_3/NH_4^+
	• Amines
	• Phenols
	• Indols
	Deconjugation and dehydroxylation of bile acids
	Degradation of cholesterol
	Catabolism of steroid hormones
	Synthesis of vitamins
	Disposal of hydrogen
	Production of mutagens (e.g., nitrosamines)
	Metabolism of xenobiotics
Defence mechanisms	Stimulation of immune system
	Enhanced resistance to infections
	Barrier effect
	Colonization resistance
Endocrine activity	Modulation of activity(ies) of endocrine-active cells in colonic mucosa (e.g., L-cells)

content (low pH). In the left/distal (descending segment and sigmoid-rectum) colon, the availability of substrates (especially carbohydrates) is usually low, bacteria grow slowly, and pH is close to neutrality (7).[1] Moreover, the breakdown and fermentation of organic materials reaching the large bowel is a complex process that involves different groups of bacteria with miscellaneous and complementary activities. Bacteria are generally not specific for particular metabolic activities; they all metabolize

TABLE 5.3
Main Substrates of Bacterial Fermentation in the Large Bowel

Nondigestible dietary carbohydrates	Dietary fibers: Nonstarch polysaccharides (NSPs) Nondigestible oligosaccharides (NDOs) Sugars (e.g., lactose, raffinose, stachyose, etc.,) Polyols
Nondigestible dietary proteins and peptides	
Endogens compounds	Carbohydrates Proteins and peptides (e.g., pancreatic enzymes) Mucins

many different substrates as well as intermediate or even end metabolic products, and they interact with each other to form trophic chains.[14]

5.2 THE ANAEROBIC FERMENTATION OF PROTEINS

Nitrogen in the chyme that reaches the large bowel is almost exclusively in the form of proteins and peptides, both form exogenous (dietary) and endogenous (pancreatic enzymes, mucins, etc.) origin. Daily, in an average European-type diet, a total amount of 7–15 g of proteins reach the colon and are likely to be fermented, these include 3–9 g of dietary and 4–6 g of endogenous (mainly pancreatic enzymes) peptides and proteins.[15] For the carbon, nitrogen, and energy of these compounds to benefit the bacteria and, eventually, the host, proteins and peptides need to be hydrolyzed. The colon, which contains both pancreatic and bacterial endopeptidases, is a very effective proteolytic environment[16] that makes available short peptides and free amino acids. Indeed, bacterial proteases (serine, thiol, and metallo-preoteases) complement pancreatic enzymes to hydrolyze peptides and proteins, and especially the globular proteins that resist largely the activity of the sole pancreatic serine proteases. The most active proteolytic bacteria strains belong to clostridia and bacteroides. The main bacterial peptidase activity in human colon is a dipeptidyl-peptidase activity, especially in bacteroides that probably play a key role in initiating peptide hydrolysis.[8]

After protein and peptide hydrolysis, the short peptides and free amino acids become available for fermentation to serve as sources of C, N, and energy, mainly for the microorganisms and probably not really for the host.[8] Indeed, some colonic bacteria depend obligatorily on amino acids while others depend on both amino acids and carbohydrates for their energy requirements. During bacterial fermentation the amino acids are catabolized (Figure 5.1) in a wide variety of reactions that include:[8]

- Oxidative deamination to form a-keto acid
- Reductive deamination to produce saturated fatty acid
- Hydrolytic deamination with production ammonia and a-hydroxy acid

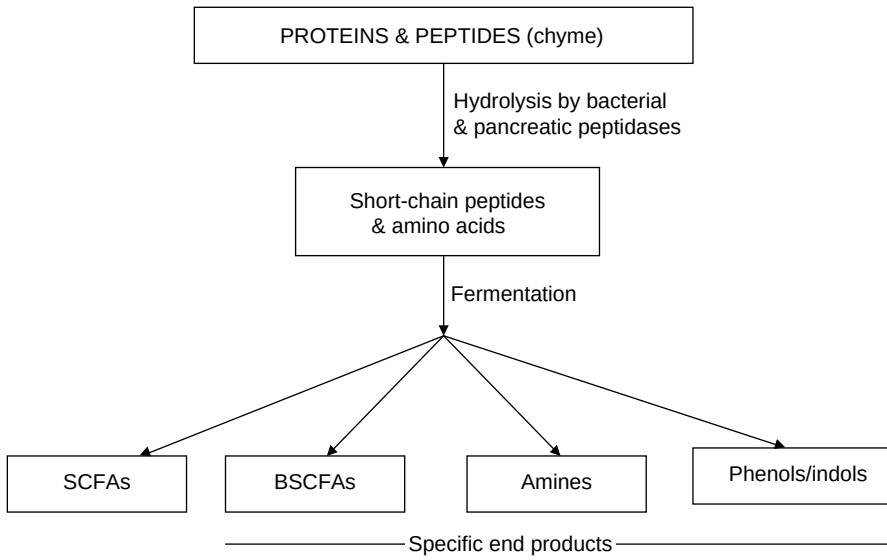

FIGURE 5.1 Schematic representation of the catabolism of proteins, peptides, and amino acids in the large bowel.

- Coupled oxido-reduction between a suitable pair of amino acids (Stickland reaction) that leaves one substrate oxidatively deaminated and decarboxylated and the other reductively deaminated
- Hydroxylation
- Dehydroxylation
- Decarboxylation

The products of bacterial amino acids fermentation are:[8,17]

1. Linear SCFAs, more specifically acetate and propionate from aspartate; acetate and butyrate from arginine, glutamate, histidine, and lysine; propionate and butyrate from methionine; acetate and propionate and butyrate from alanine, cysteine, and glycine
2. Branched short-chain fatty acids (BSCFAs), more specifically isobutyric and isovaleric acids that are one C atom shorter than the parent amino acids, i.e., valine and isoleucine
3. Ammonia
4. Amines including aliphatic amines (methylamine, dimethylamine, propylamine, butylamine, and 2-methylamine), aromatic amines (tyramine, tryptamine, and phenylethylamine), polyamines (putrescine and cadaverine), and N-containing heterocylic compounds (pyrrolidine and piperidine). But these amines are produced mostly at neutral pH and they are usually further metabolized, especially in the presence of carbohydrates
5. Phenols and indols

BSCFAs and phenolic compounds are thus the most specific end products of amino acids fermentation. Measuring these compounds in the different segments of the large bowel of sudden death victims, Macfarlane et al. have shown that the fermentation of amino acids is quantitatively more important in the distal than in the proximal colon.[18] Using an *in vitro* three-stage continuous culture model of the colon, the same authors have also demonstrated that carbohydrate availability regulates amino acid fermentation. It is inhibited in acid pH condition characteristic of the right colon where most of the carbohydrate fermentation takes place. Moreover, increasing retention time in the system (from 27 to 67 h) resulted in a threefold increase in the production of amino acid metabolites, illustrating how prolonged colon transit time predisposes towards the production of putrefactive substances.[19] Many products of the fermentation of amino acids in the colon are considered harmful to the host if availability of organic-N containing compounds is high and/or if the supply of carbohydrates that hinder the putrefactive process is too low. These include high concentrations of ammonia,[20] phenolic/indolic compounds (as co-carcinogens),[21] and amines (as precursors of nitrosamines),[22] that might play a role in increasing the risk of cancer. Moreover, ammonia might also contribute towards the onset of portal-systemic encephalopathy in patients with liver disease.[23]

5.3 ANAEROBIC FERMENTATION OF CARBOHYDRATES

5.3.1 Introduction

In human colonic microflora, the majority of bacteria use nondigestible carbohydrates as the main if not exclusive feeding substrates. The metabolism of these compounds is quantitatively more important than amino acid fermentation, especially in the cecum and ascending segment where substrate availability is greatest. Carbohydrate fermentation is thus an important force driving the micro ecology and the physiology of the large intestine.[8] Even though a wide variety of anaerobic bacteria are capable of fermenting many different types of carbohydrate, they use a small number of metabolic pathways to produce a limited range of end products.

5.3.2 Substrates of Colonic Carbohydrate Fermentation

The most important and most frequent nondigestible carbohydrates in human diet that serve as substrates for colonic fermentation are (Table 5.4):

- Nonstarch or plant cell wall polysaccharides including cellulose or β-D-(1➟4) polymers of glucose (the major structural component in the cell wall of all plants); hemicelluloses or (β-D-(1➟4) polymers of xylose, mannose and glucose, particularly arabinoxylans in cereals (rye and wheat); β-D-(1➟3,4)-glucans in cereals (barley and oat) and xyloglucans

TABLE 5.4
Main Substrates of Colonic Carbohydrate Fermentation

Major substrates	Nonstarch (plant cell wall) polysaccharides
	•Cellulose or b-D-(1➡4) glucans
	•Hemicelluloses or b-D-(1➡4) polymers of glucose, galactose, mannose, xylose, and branching (arabinose, galactose, glucuronic acid)
	•Pectins a-D-(1➡4) polygalacturonic acids
	Oligosaccharides especially inulin b-D-(1➡2) fructans
	Resistant starch or a-D-(1➡4) glucans
Minor substrates	Chitin or poly-N-acetylglucosamines
	Gums like karaya, arabic, guar, xanthan gums
	Mucillages (heteropolymers of arabinose, galactose, galacturonic acid, glucose, mannose, and xylose). Synthetic compounds like lactulose, lactitol, polydextrose
	Synthetic cellulose derivatives like carboxymethyl-, methyl-, hydroxypropyl-, hydroxymethylpropyl-, ethylmethyl-celluloses

in vegetables; and pectins or a-D-(1➡4) polygalacturonic acids in fruits and vegetables.

- Oligosaccharides, especially inulin-type fructans (see below), but also lactose that is poorly digested in the small intestine of the so-called lactose intolerant individuals.
- Resistant starches or starches that, for various reasons, are not hydrolyzed by pancreatic amylases.[24] Resistant starch (RS) is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals."[25] RS is classified into three major types,[26] i.e., RS1 (starch entrapped within the plant cells), RS2 (native crystalline starch stored in cell plant granules), and RS3 (starch that has been retrograded after cooling of gelatinized starch).

Other nondigestible carbohydrates are also found but usually in smaller amounts or less frequently. These include:

- Chitin or poly-N-acetylglucosamine (the organic matrix of insects and crustaceans also present in small amounts in mushrooms).
- Gums, the viscous mucilaginous compounds present in some edible beans and mostly found in human diet because of their use as emulsion stabilizers or thickening agents. They are extracted from tree exudates like karaya gum (from *Sterculia arens*), plant seeds like arabic or acacia gum (from *Acacia senegal*), guar gum (from *Cyamopsis tetragonoloba*), and plant roots like konjac gum (from *Amorphophallus konjac*), or produced by microorganisms like dextran gums (from *Leuconostoc mesenteroides*) or xanthan gums (from *Xanthomonas campestris*).

- Synthetic compounds like lactulose (b-fructosyl-(1➟4)-a-D-galactose), lactitol (a sugar alcohol derived from lactulose, polydextrose (a glucose oligomer prepared by heating glucose, and sorbitol with citric acid).
- Synthetic cellulose derivatives used as food additives such as carboxymethylcellulose (an inert filler in "slimming aids" and a whipping agents in combination with stabilizers), methylcellulose (a gel-forming emulsifier and thickener), hydroxypropyl cellulose, hydroxypropylmethyl cellulose, and ethylmethyl cellulose (all three used as emulsifiers and stabilizers).

The amounts of dietary nondigestible carbohydrates reaching the human colon vary markedly from diet to diet, but are not less than 20 g/d reaching 60 to 80 g/d when carbohydrate foods form the major staple.[8] Nonstarch polysaccharides, resistant starches, and nondigestible oligosaccharides contribute 10–25 g/d, 5–35 g/d, and 2–8 g/d, respectively.[11] With respect to the efficiency of fermentation, 100 g of nondigestible carbohydrates yield 25–36 g of bacterial mass.[27] The rates of fermentation of carbohydrates differ in the proximal or carbohydrate-rich vs. the distal or carbohydrate-depleted segments of the colon.[12]

5.3.3 Anaerobic Degradation of Carbohydrates during Colonic Fermentation

The anaerobic degradation of carbohydrates in the colon is a complex process that involves different groups of bacteria with different and complementary enzymatic and activities and metabolic pathways. These bacteria interact between each other to form complex networks of reactions that transform most of the nondigestible carbohydrates in a three-stage process.

5.3.3.1 Hydrolysis of Oligo- and Polysaccharides

The first step (Figure 5.2) in the degradation of the oligo- and polysaccharides is hydrolysis to liberate either disaccharides like cellobiose (from cellulose), maltose (from starch) and xylobiose (from hemicelluloses), and the constitutive sugar moieties i.e., glucose (±50% of all sugars), fructose, arabinose, galactose, mannose, rhamnose, and xylose but also sugar derivatives like uronic acids.

With the exception of cellulose that resists partly, the dietary oligo- and polysaccharides reaching the colon are more or less quantitatively hydrolyzed by bacterial glycosidases. These enzymes are either associated with the bacterial wall or located intracellularly.[28]

5.3.3.2 Catabolic Pathways of Carbohydrates in Colonic Microorganisms

The second stage in the degradation of the oligo- and polysaccharides involves metabolic pathways in the microorganisms that oxidize hexoses and pentoses via

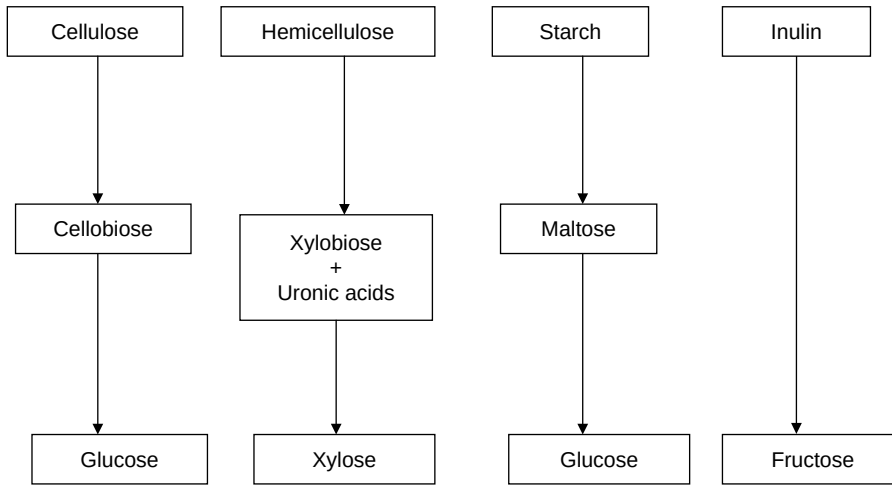

FIGURE 5.2 Major hydrolytic steps in the colonic degradation of the most common nondigestible carbohydrates.

the Embden-Meyerhof pathway (Figure 5.3) or the pathway specific for bifidobacteria (Figure 5.4).

Before entering these pathways, fructose and glucose (via glucose-6-phosphate) are transformed into fructose-6-phosphate; galacturonic acid is transformed into xylose, and xylose is transformed into fructose-6-phosphate via the pentose phosphate pathway. In most bacteria, the Embden-Meyerhof pathway oxidizes fructose-6-phosphate into pyruvate but in bifidobacteria, in the absence of fructose-1, 6-diphosphate lyase (aldolase), fructose-6-phosphate phosphoketolase (EC 4.2.1.22) opens a specific pathway that transforms fructose-6-phosphate into acetic and lactic acids via the bifid shunt.[29]

5.3.3.3 Metabolic Pathways Transforming Pyruvate in Colonic Microorganisms

The third stage in the degradation of the oligo- and polysaccharides transforms pyruvate into these fermentation end products (Figure 5.5):

- SCFAs (mainly acetate, propionate, and butyrate)
- Ethanol
- Gases (CO_2, H_2, CH_4)
- Intermediate metabolites such as formate, lactate, and succinate which, however, do not normally accumulate because they mainly serve to oxidize the reduced coenzymes NADH and NADPH[14,30].

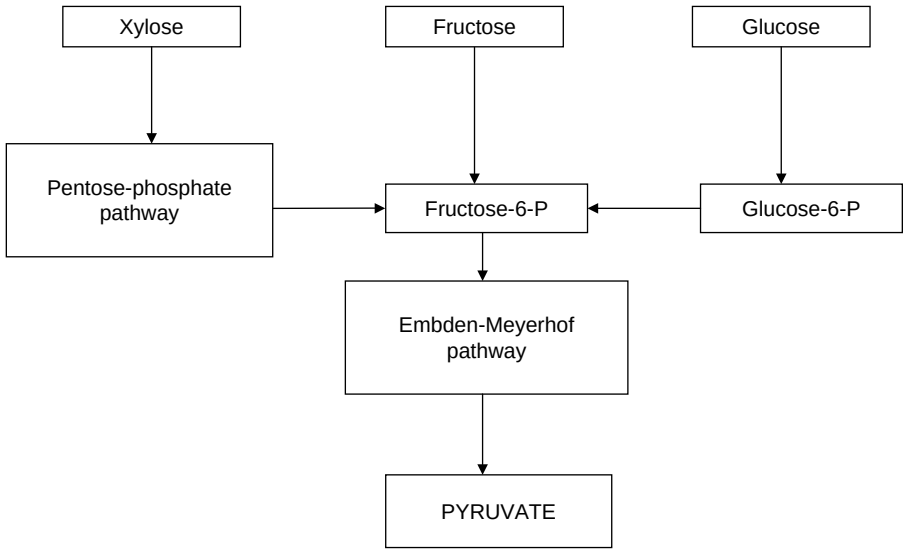

FIGURE 5.3 Major metabolic pathways in the anaerobic oxidation of carbohydrates to pyruvate.

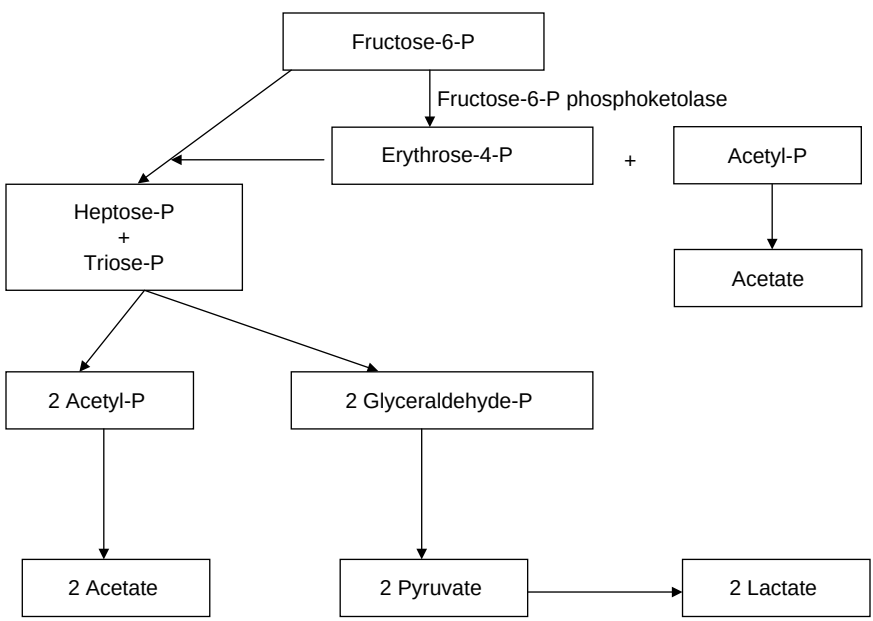

FIGURE 5.4 Schematic representation of the bifid-shunt pathway in bifidobacteria

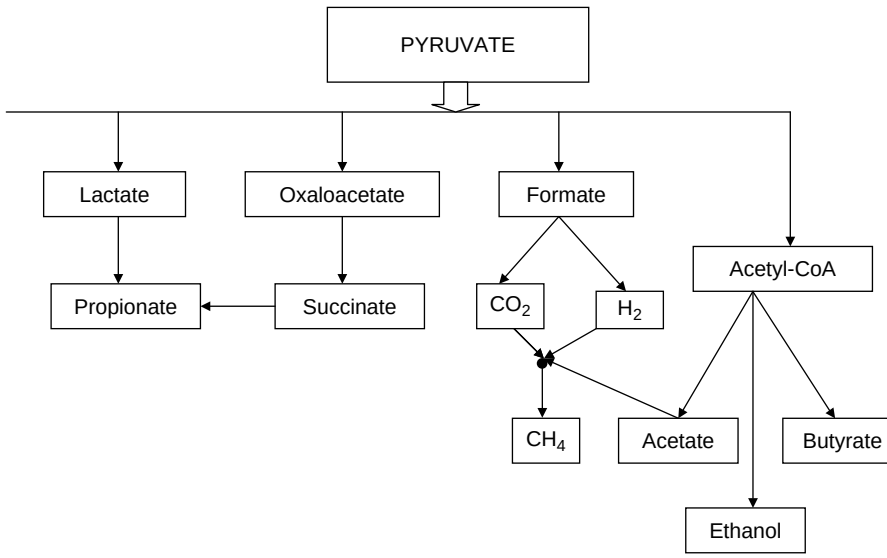

FIGURE 5.5 Main metabolic pathways that utilize pyruvate in colonic microflora.

5.3.4 Overview of the Biochemistry of Production of Fermentation End Products by Human Colonic Microflora

5.3.4.1 The Concept of Healthy Colonic Microflora

In colonic microflora, no single bacterium is capable of producing all the metabolites listed above. As a matter of fact, most bacteria contribute to complex metabolic interactions making the colonic microflora a true interactive ecosystem in which many different microorganisms play distinct complementary roles. As discussed below, this conclusion supports strongly the concept of "healthy flora" in which adequate populations composed of adequate number of individuals cooperate to support colonic functions.[31]

5.3.4.2 Production of SCFAs

In the normal human colonic microflora, acetate resulting from the oxidative decarboxylation of pyruvate or directly from acetyl-phosphate is produced by the major species of bacteria (bacteroides, bifidobacterium, eubacterium, clostridium, fusobacterium, lactobacillus, etc.); it is thus, quantitatively, the major metabolite. The major producers of propionate (from lactate or succinate) belong to the species bacteroides, propionibacterium, and veillonella. Even though not yet fully understood, the metabolic pathway to produce butyrate is likely to involve the condensation of 2 acetylSCoA. Such a pathway is expressed, just to cite the most frequent, in bacteria belonging to the species clostridium, eubacterium, and fusobacterium. The total amount of SCFAs in the human right colon is ±140 mmol/kg of content but it is

only ±95 mmol/kg in the left colon, and consequently the pH is lower in the former than in the latter. The molar ratios of acetate/propionate/butyrate show little difference.[32]

5.3.4.3 Production of Lactate

The bacteria species that ferment carbohydrates to produce lactate are named the lactic acid bacteria. In the human colon these belong to the species *Bifidobacterium* and *Lactobacillus* but also *Streptococcus* and *Enterococcus*. In these bacteria, lactate is the reduction product of pyruvate. In the complex colonic ecosystem, lactate does not really accumulate. Indeed, it can further be transformed to propionate (via succinate) and eventually to butyrate.

5.3.4.4 Production of Gases

Even though the microflora producing them has still not be completely identified, relatively large quantities of gases are released by fermentation in the human colon (from 0.5 up to 4 l/d). The composition of the gas flatus varies considerably from one individual to the other.[11,33,34] Healthy subjects pass flatus gas up to 14 times a day with a total volume of between 0.35 and 1.4 l,[11,33] and they absorb and excrete, in-breath, a variable proportion of all gas (mainly H_2 and CO_2 but also CH_4 in some individuals) produced in the colon. The excretion of semi-in-breath is used as a quantitative measurement of carbohydrate fermentation[35,36] (see Section 5.4.1.2).

5.3.4.5 Metabolism of H_2

While CO_2 equilibrates with the body bicarbonate pool, H_2 and CH_4 are unique to fermentation; the latter is a product of the transformation of the former. Indeed, H_2 is utilized by miscellaneous bacteria species to produce CH_4 (methanogenic bacteria), H_2S (sulphate-reducing bacteria), acetate (homoacetogenic bacteria), and eventually NO_2 (nitrate-reducing bacteria like *Escherichia coli* and *Veillonella*) (Figure 5.6).

AU: Figure not cited in text.

- Methanogenesis is an important mechanism that *Methanobrevibacter smithii* utilizes to reduce CO_2 by H_2. CH_4 production does not seem to be much influenced by variations in diet.[37,39] It occurs predominantly in the distal colon.[18]
- Sulphate-reduction is a process by which *Desulfovibrio* and *Desulfobulbus* species utilize CH_4 to reduce sulphate (SO_4^{--}) to produce SO_4^{--} that is toxic to colonic cells, impairing cellular metabolism and mucosal barriers.[40] The reductive process is coupled with ATP production. It is highly dependent on the amount of SO_4^{--} available in the colon either from dietary or endogenous (mucins) sources. Sulphate-reduction also occurs predominantly in the distal colon [18] and an inverse relationship exists between reduction and methanogenesis.[41,42]
- Reductive acetogenesis is an acetate biosynthetic pathway in which 2 mol of CO_2 are reduced by 4 mol of H_2 to produce acetyl-SCoA that is further

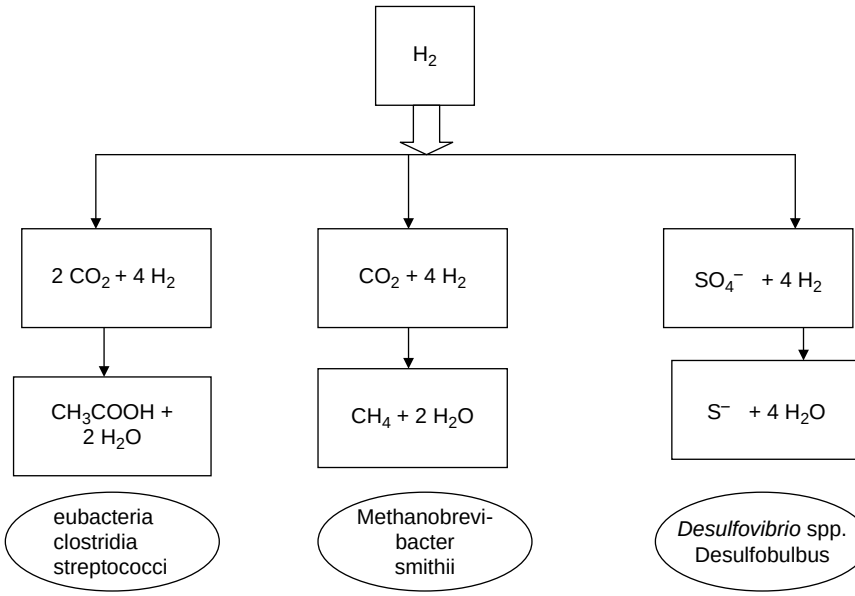

FIGURE 5.6 Main metabolic pathways utilizing H_2 in the colonic microflora.

transformed in acetate in a reaction coupled with ATP production.[43] The acetogenic reductive microflora is mostly active in nonmethanogenic subjects. It comprises species belonging to genera Eubacterium, Clostridium, and Streptococcus.[44,45]

5.3.5 Methodologies for the Study of the Colonic Fermentation of Carbohydrate

5.3.5.1 Introduction

As discussed above (see Section 5.3.2), some carbohydrates are not (or only partly) absorbed or digested in the upper part of the gastrointestinal system. These are the nondigestible carbohydrates that reach the large bowel. Provided they become fermented, these carbohydrates serve as feeding substrates and consequently they support the growth and the activity (or activities) of the large population of mixed bacteria living in the colon. Anaerobic fermentation of the dietary nondigestible carbohydrates in the colonic environment is thus an essential step in supporting the functions of the colonic microflora. It needs to be studied, and different models, both *in vitro* and *in vivo* (including human), exist to do so. Still, acquiring knowledge and an understanding of the colonic functions is extremely difficult, especially because of its relative inaccessibility. Indeed, with the exception of accidental (sudden death victims) or surgical situations (patients waiting for proctocolectomy), the content of the human large bowel is not accessible for experiments or even for analysis. It has thus become a current practice to use feces as a surrogate in an intraluminal situation, examining their activities and their composition (especially

regarding the bacteria population). Such an approach is far from perfect, and it has often been criticized. But for some aspects of colonic functions, especially the composition of the gut microflora in the lumen, feces are probably an acceptable substitute for ceco-colonic contents.[31,46] To obtain meaningful values of pH or short-chain fatty acids concentrations and pool composition, fecal analysis is not an acceptable approach and cecal or colonic contents must be collected.[46]

5.3.5.2 *In Vitro* Models to Study the Fermentation of Carbohydrates by the Colonic Microflora

The most commonly used *in vitro* models to study anaerobic fermentation of carbohydrates by mixed bacteria population, particularly fecal bacteria, are:[47]

- Batch culture fermenters (50–350 ml working volume) that are inoculated either with pure culture of selected genera or species of bacteria or with fecal slurry (5–10% w/v fresh feces homogenized in anaerobic buffer pH 7.0 and subsequently sieved) and the carbohydrate (±1% w/v) to be studied. Feces are collected anaerobically from volunteers with no preceding history of gastrointestinal disorder and who have not been prescribed antibiotics for at least 3 months. Before incubation, the slurry is gazed out with argon leaving a slight positive pressure of gas. The fermenters are then incubated for up to 48 h and samples of gas and liquid are taken for analyses at regular time intervals. The gas samples are analyzed for H_2, CH_4, and CO_2 concentrations by gas liquid chromatography. In the liquid samples, the concentration of SCFAs is determined by gas liquid (GLC) or high-pressure liquid (HPLC) chromatography. Lactate and eventually succinate are also quantified by GLC after methylation. The concentration of the residual carbohydrates is measured by using the adequate specific methodology, being either enzymatic and/or physical (GLC or HPLC).
- Multichamber continuous culture systems that have been developed in an attempt to reproduce some of the different physical and nutritional characteristics of the different segments (ascending, transverse, and descending) in the colon.[15,48] Such a system is composed of three vessels aligned in series so that adequate culture medium pumped in vessel 1 (320 ml, pH 5.5) sequentially feeds vessel 2 (320 ml, pH 6.2) and then vessel 3 (500 ml, pH 6.8) at dilution rates of 0.15/h, 0.15/h, and 0.08/h, respectively. Vessel 1, rich in nutrients with a fast transit and acidic pH, is similar to proximal colon, whereas vessel 3, poor in fermentable substrate and with a neutral pH, resembles the distal colon where bacteria grow slowly. All of the system is maintained in oxygen-free conditions at 37˚C, and the pH is strictly controlled in each vessel.[24] After inoculation with a fresh fecal sample (16% w/v), the system is allowed to stabilize for 2 weeks after which time additional carbohydrate (15 g/d) is pumped in for 2 weeks. The concentration of SCFAs and carbohydrate substrate is deter-

mined (as indicated above for batch fermenters) in samples periodically removed from the vessels.

5.3.5.3 *In Vivo* Models to Study the Fermentation of Carbohydrates by the Colonic Microflora

In vivo fermentation of nondigestible carbohydrates can be studied both in experimental and domestic animals as well as in pets or in human volunteers.

In experimental animals, especially rats, the carbohydrate under investigation is added to food (5, 10, or even 15% w/w) or drinking water (5 to 10% w/v, depending on water solubility), but it can also be administered by gastric intubation. Animals are then anaesthetized and sacrificed at predetermined time intervals. Fecal samples and the content of the gastrointestinal tract (including the cecum and the various segments of the colon) are collected for pH measurement and analysis of SCFAs, lactate, eventually succinate, and residual carbohydrate. A particularly interesting model to study carbohydrate fermentation in experimental animals is the heteroxenic rat bearing human fecal flora. In that model, axenic or germ-free rats born in a sterile environment in an anaerobic chamber, known as the Freter's chamber, are subsequently inoculated *per os* twice consecutively with 1 ml of a suspension (1% w/v) of a homogenized fresh sample of human feces collected anaerobically from an healthy volunteer. That model allows studying the effects of the flora on the host as well as the effects of the composition of the diet on the flora. The human flora conserves its major properties: specified bacterial population, enzymatic activities, and fermentative profile.[13]

To study fermentation of dietary carbohydrates in humans, two major approaches are used:

- One is indirect and collects breath air at regular time intervals to measure the concentration of gases, essentially H_2, in volunteers previously given a single oral dose of the carbohydrate. The assumption is that the production of H_2 is constant for all fermentable substrates and that H_2 excretion in breath and flatus production are linearly related. However, Christl et al. have reported that this not necessarily the case.[49] Indeed, on a per g of fermentable substrate basis and as compared to pectin and resistant starch, volunteers fed lactulose excrete, in breath, four times more H_2. Moreover, the proportion of H_2 gas produced in breath air is very variable (25 to 65%), depending on whether low (<200 ml/d) or high (>500 ml/d) H_2 and CH_4 production rates occurred. This is because at higher production rates, H_2 and CH_4 are more likely to accumulate in significant amounts in the colon as flatus as a result of stimulating motor activity than at low rates, a situation that favors rapid diffusion into blood rather than accumulation.[11] But, as discussed by Cummings, the most important finding from Christl's study is the very small amounts of H_2 collected compared to the theoretical amounts expected from the stoichiometry of fermentation equations. (In the case of lactulose test, only about 10% of total possible H_2 was accounted for.[11]) This is because there are a number of

alternative pathways for H_2 disposal and utilization other than excretion as gas (see Section 5.3.4.5). Breath is thus a qualitative, eventually semi-quantitative, test of carbohydrate fermentation in the large bowel. It can by no means give reliable information about the proportion of the carbohydrate that resists digestion, nor the amount that is fermented in the colon.

- The other approach to study fermentation of dietary carbohydrates in humans is rather straightforward. It consists in collecting feces after oral feeding and quantitatively measuring the concentration of the residual carbohydrate. If all fecal samples are collected over, e.g., 24 or 48 h, the test allows the determination of the proportion of the carbohydrate that has been digested and/or fermented. But if adequate tests have demonstrated that the product is nondigestible (see Chapter 4, Table 4.1), then it is a measure of fermentation and, if the feces contain no residual carbohydrate, then the carbohydrate tested is completely fermentable.

In the *in vivo* tests just described, both in animals and in humans, change in pH and an increase in the fecal concentration of SCFAs are also currently tracked. When assessed after feeding a particular carbohydrate, these parameters are tentatively used to demonstrate colonic fermentation. However this is of low, if any, value.[50] Indeed, the SCFAs produced in the colon are largely absorbed (up to 95% in human intestine) and, at least in humans, do not really appear in the feces[51] (or only a small, unrepresentative amount[52]). Thus, change in fecal pH or increase in the fecal concentration of SCFAs hardly correlate with colonic carbohydrate fermentation and, as stated by Cummings, fecal measurement of SCFAs prove an insensitive guide to events going on more proximally in the large bowel.[11] Similarly, and for the same reasons, attempts to demonstrate changes in the composition of the SCFAs colonic pool (especially the ratios acetate/propionate/butyrate) by recording changes in the SCFAs fecal pool after feeding a particular fermentable carbohydrate either to experimental animals or to volunteers have not really been successful. Still, the individual acids have differing metabolic significance,[53] and the pattern of SCFAs formed upon fermentation of easily fermentable carbohydrates is a factor of nutritional importance, especially because butyric acid appears to be essential in the maintenance of a healthy colonic mucosa, whereas propionic acid might be connected with beneficial effects on systemic carbohydrate- and lipid metabolism. SCFAs may also influence the colonic motility. The pattern of formation of SCFAs is not that easy to measure, and different methodologies have been used such as *in vitro* incubations with human or animal feces, or animal studies that focus on SCFAs pattern in the cecum.

5.4 ANAEROBIC FERMENTATION OF INULIN-TYPE FRUCTANS

5.4.1 The Process of Fermentation: Results and Discussion

5.4.1.1 *In Vitro* Data

That inulin and oligofructose are completely and rapidly fermented has been demonstrated in anaerobic batch culture fermenters inoculated with either the pure culture of selected species of bacteria or mixed human fecal microflora (10% w/v).[54,55] In experiments in which the fructans (0.5 or 0.7% w/v) were incubated (96 or 24 h) at 37°C with pure culture of selected species of bacteria, changes in the pH of the culture medium were used as an indirect marker of fermentation resulting from the production of SCFAs or other acids (Table 5.5). Data show that bifidobacteria ferment equally well both glucose and the fructans.

Even though they are both fermented by all the other tested species, oligofructose and inulin are comparatively less well fermented than glucose ($p < 0.05$). Except for bacteroides, staphylococcus and bifidobacteria, change in culture pH induced by inulin incubation are always lower than that induced by oligofructose fermentation, thus indicating that for these bacteria, the latter is a more efficient substrate than the former.[56] Additional experiments have also shown that, among the different bifidobacteria strains tested, *B. angulatum, B. breve, B. catenulatum, B. infantis, B. longum, and B. pseudolongum* all fermented oligofructose and inulin equally well, but that *B. animalis* was more efficient in fermenting oligofructose than inulin and that *B. bifidum* metabolized neither oligofructose nor inulin.[47,57] Other studies have similarly shown that a range of bifidobacteria (different species but also different strains of the same species) can utilize oligofructose as well as inulin for growth.[58–64] Although other enteric bacteria were also able to grow, especially *Bacteroides* species, the utilization of oligofructose by lactobacilli *E. coli* and *Clostridium perfringens* was poor. However, results are sometimes contradictory, depending on experimental conditions.

After incubation in the presence of mixed human fecal microflora, the change in pH was used to follow carbohydrate fermentation. As shown by the drop in pH within 24 h, oligofructose is more rapidly fermented than inulin.

When gas liquid chromatographic analysis of the culture supernatant was used to follow the subsequent disappearance of the fructans (initial concentration: 0.7% w/v), data showed that both oligofructose and inulin (both $G_{py}F_n$ and $F_{py}F_n$) disappeared completely within 4 to 5 h at a similar relative rate of fermentation. A more detailed analysis, however, revealed that the rate of fermentation of oligomers with DP < 10 is more or less twice as fast as that of molecules with DP > 10.[57] These data have been confirmed by Karpinen et al.,[65] Durieux et al.,[63] and Perrin et al.[66]

When incubated *in vitro* with human fecal slurries, inulin and oligofructose also produced gases and SCFAs.[47,55] As compared to other oligosaccharides like cellobiose, lactulose, or sucrose that produced between 0.04 and 0.05 µmol of H_2/g of fresh feces/24 h, oligofructose and inulin produced only 0.02 and 0.025 µmol of H_2/g of fresh feces/24 h, respectively, amounts that were lower than that produced by polydextrose and equivalent to that produced by pectin or hemicelluloses fermentation.

TABLE 5.5
Drop in Culture pH due to Fermentation[1] of Oligofructose, Inulin, and Glucose by Different Genera of Bacteria

Bacteria (n)	Oligofructose DpH[2]	Inulin pH	Glucose pH
Bacteroides spp. (16)[3]	0.9[1, 4]	0.6[1]	1.1[2]
Clostridium spp. (26)	0.4[2]	0.2[1]	1.4[3]
Enterococcus faecalis (6)	0.6[2]	0.4[1]	1.9[3]
	1.0[2]	0.5[1]	2.2[3]
Lactobacillus spp. (9)	1.2[2]	0.3[1]	2.0[3]
Proteus spp. (2)	0.7[1]	0.3[1]	1.9[2]
Staphylococcus spp. (3)	2.8[1]	2.8[1]	3.1[1]
Bifidobacterium spp. (8)			

Note: [1]Fermentation conditions were: A. Inoculation of pure bacteria strains on modified GAM broth medium followed by incubation at 37°C for 24 h in anaerobic conditions. B. Inoculation of 0.03 ml of the culture in a basal culture medium adjusted at pH 7.6 and containing 0.5% of the tested carbohydrate followed by incubation at 37°C for 96 h in anaerobic conditions.

[2] DpH is the change in pH due to fermentation of the carbohydrate; it is equal to pH at the start (i.e., 7.6) – pH at the end of the incubation.

[3]The number in () is the number of tested strains for the different genera.

[4]Drop of pH values with different superscript letters are statistically different (one factor ANOVA test, $p < 0.05$).

Source: Adapted from Gibson, G. R., Wang, X., Regulatory effects of bifidobacteria on the growth of other colonic bacteria, *J. Appl. Bacteriol.*, 77, 412–420, 1994; Wang, X., Gibson, G. R., Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine, *J. Appl. Bacteriol.*, 75, 373–380, 1993; Wada, K., *In vitro* fermentation of oligofructose and inulin by some species of human intestinal flora. Technical report of Calpis intestinal flora laboratory, available from ORAFTI, Aandorenstraat 3, B-3300 Tienen, Belgium, 1990.

In these experiments, the fermentation of oligofructose, inulin, hemicelluloses, lactulose, pectin, and polydextrose produced nondetectable or very low amounts of CH_4, whereas the fermentation of cellobiose and sucrose produced between 2 and 6 μmol of CH_4/g of fresh feces/24 h. In an experiment comparing inulin, oligofructose, lactulose, xylooligosaccharides, transgalactooligosaccharides, soybean oligosaccharides, and isomaltooligosaccharides, Rycroft et al. reported, however, that in static batch culture inoculated with human fecal slurries, in terms of total gas production, inulin fermentation produced the most, followed by oligofructose, lactulose, and xylooligosaccharides, whereas transgalactooligosaccharides, soybean oligosaccharides, and isomaltooligosaccharides produced the least.[67] Similarly, in comparing the gas production after 24 h *in vitro* batch fermentation of inulin and predigested oat, rye, and wheat brans by human fecal flora, Karppinent et al. concluded that inulin produced the highest volume of gas.[65]

The total amount of SCFAs produced by oligofructose and inulin fermentation reached 56 and 61 μmol/24 h, respectively. That was equivalent to glucose or fructose

(57 and 59 μmol/24 h, respectively) but higher than hemicelluloses, pectin, polydextrose, or starch (40, 53, 48, and 43 mmol/24 h, respectively) fermentation.

In vitro fermentation of oligofructose and inulin was used to study the pattern of SCFAs production. These experiments resulted in variable and sometimes contradictory results. In her study, using batch fermenters inoculated with human fecal slurries, Wang did not find any significant differences in SCFAs patterns (acetate 72–78%; propionate 14–19%; butyrate 8–10%) when fermenting oligofructose, inulin, or pectin.[47] In these experiments, only starch fermentation produced a specific SCFAs pattern in which butyrate was proportionally increased (25%), whereas acetate was reduced (58%). Luo et al. reported higher molar ratios of propionate and butyrate (13 and 25%, respectively) with oligofructose than with lactulose (7 and 19%, respectively)[68] but lower than many types of starches.[69] However, Clausen et al. found very similar proportions of acetate, propionate, and butyrate with both lactulose and oligofructose.[70] More recently, Karppinen et al.[65] and Rycroft et al.[67] have reported data that show clearly that the *in vitro* batch fermentation of inulin and oligofructose for up to 24 h, in the presence of human fecal slurries, was the only process (as compared to fermentation of oat, rye, and wheat brans, lactulose, xylooligosaccharides, transgalactooligosaccharides, soybean oligosaccharides, and isomaltooligosaccharides) that produced higher proportion of butyrate. The data also show that the increase in butyrate was most probably due to further metabolism of acetate and eventually propionate.

5.4.1.2 *In Vivo* Data

Having reached the cecum, inulin and oligofructose are likely to be fermented since they are water soluble and relatively simple molecules. A series of experimental studies have analyzed the effect of inulin or oligofructose feeding on different short chain fatty acids production in the cecum of rats harboring either a human or a conventional microflora (see Table 6.3, Chapter 6, Section 6.2.4.4). In germ-free rats inoculated with a human fecal flora, inulin (10% w/w in diet) and oligofructose (4% w/w in diet) induced an increase (on average, 1.7 fold) in total SCFAs production. In these rats, the relative proportion of butyrate increased from 8 to 15% in control animals, up to 20% or even 34% of all SCFAs, respectively.[13,71–74] In the same experimental model, the relative proportion of butyrate was higher (30%), whereas that of propionate was lower (7%) in the cecum after inulin feeding than after bran (10 and 20%, respectively), beet fiber (6 and 23%, respectively), or carrot fiber (12 and 20%, respectively) feeding.[13]

In other studies with conventional rats, inulin or oligofructose (6% or 10% w/w in diet) also induced a higher cecal concentration of SCFAs (1.4 to 1.8 times), as well as a higher relative proportion of butyrate (from 12–14% up to 20–22%).[46,75–77] Le Blay et al. confirmed the butyrogenic effect of oligofructose.[78] All studies except one[75] reported no significant difference in the cecal concentration and/or molar ratio of acetate or propionate. In addition to butyrate, lactate production was also enhanced by oligofructose fermentation as shown by its increased concentration in cecal content.[74,75,78] Even though of less significance because of their very efficient colonic absorption, total fecal SCFAs concentration showed an increase (up to 3.7 fold).[79]

Heteroxenic rats harboring a human fecal flora fed inulin also excreted an increased amount of breath hydrogen as a result of large bowel fermentation. That effect was more pronounced in rats harboring fecal flora from nonmethane than from methane producers. In no case did inulin intake induce an increase in breath methane, an excretion that was reduced or even completely abolished in rats harboring a methane-producing human flora.[13,71–74]

After feeding inulin and oligofructose to humans, studies have consistently failed to identify any of these materials in feces, indicating that they are completely fermented in the colon and, consequently, they markedly enhance colonic fermentation.[80–86] This is also true in infants (9–18 months old) fed 2 g/d of oligofructose (N. Jonkers, ORAFTI, Tienen, Belgium, personal communication). However, the oral consumption of oligofructose or inulin increases breath hydrogen excretion.[85,87]

In humans, the increase in butyrate proportion reported in rats has not been confirmed. Indeed, generally no effect on fecal concentration or of molar proportions of acetate, propionate, and butyrate has been seen with inulin or oligofructose in humans.[70,85,88–90] However, an increased concentration of lactic acid at higher doses (80 g/d) has been reported.[70] Further, in the study by Kleessen et al., the proportion of butyrate tended to be higher at the higher doses (40 g vs. 20 g) with inulin.[89]

5.4.2 Side Effects of Fermentation of Inulin-Type Fructans

The gases (mainly CO_2, CH_4, and H_2) are inevitable products of fermentation but also provide the major clinical disincentive to consumption of high doses of fermentable carbohydrates that might create unwanted symptoms relating to their production in the gut of flatulence, bloating, borborygmi, and cramps. But intestinal acceptability of nondigestible carbohydrates that are fermented in the large bowel is also dependent on the osmotic effect that brings more water into the colon.

A number of human feeding studies have investigated changes in intestinal symptoms eventually associated with ingestion of inulin-type fructans in human nutrition intervention studies.

1. In Stone-Dorshow and Levitt's study, 12 subjects consuming 15 g of oligofructose daily for 12 d reported mild symptoms of abdominal pain, eructation, flatulence, and bloating when compared with a group of five subjects taking an equivalent dose of additional sucrose. There was no adaptation over the test period.[91]
2. In Briet's study aimed at establishing a threshold for oligofructose-induced intestinal side effects, some 15% of the 14 volunteers consuming 10 g/d reported excessive flatus and borborygmi, whereas 20–30% of those ingesting 20 g/d experienced excessive flatus, borborygmi, or bloating.[92] In subsequent similar studies, the same group has reported that:
   - Among 10 volunteers ingesting 12.5 g/d of oligofructose for 12 d, only 5 reported mild bloating.[93]
   - Among 4 groups of 10 volunteers receiving increasing doses of oligofructose (0, 2.5, 10, and 20 g/d, respectively) for 12 d, digestive symptoms were reported in 37.5% of the subjects, the percentage of

complaints for excess flatus, bloating, borborygmi, and abdominal pain being 27.5, 20, 15, and 20, respectively, but a dose-effect relationship was seen only for flatus, not for the other symptoms. None of the subject reported nausea or diarrhea.[94] In conclusion, of these 3 human studies, the authors identified flatus and/or bloating as the most common symptoms that might be experienced by a minority of subjects ingesting oligofructose, with 10 g/d being a threshold dose.

3. Similarly, with doses of oligofructose ranging from 5 up to 20 g/d, other human nutrition intervention studies showed a dose-related increases in breath H_2 and mild intestinal discomfort, mainly flatulence, bloating, and borborygmi, in general, with isolated individuals experiencing somewhat more discomfort.[68,95–98]
4. Inulin at a dose of 14 g/d led to significant increases in flatulence, rumbling, stomach, and gut cramps, together with bloating in a group of 64 women in a double-blind crossover study over 4-week periods. Among the volunteers, 12% considered the flatulence severe and unacceptable. No adaptation in symptoms occurred over time.[99]
5. However, in a more recent study, Kruse et al. reported that, if the 11 subjects ingesting inulin (up to 34 g/d for 64 d) reported some signals of intestinal discomfort (essentially flatulence and bloating), these were interpreted individually in different ways, and they were ranked mild or moderate.[90]

It remains difficult to distinguish between an acceptable and nonacceptable side effect of colonic fermentation, and symptoms like flatulence or bloating are difficult to assess objectively. Moreover, then same degree of flatulence can be acceptable for one person but not for another. This is the reason why a new approach based on personal judgment has recently been developed.[100] In that approach, a food or a food ingredient is considered nonacceptable if, as evaluated by the test person himself, it causes "too much" of one of the following symptoms:

- Flatulence
- Intestinal pressure
- Intestinal noise
- Intestinal cramps

and/or diarrhoea in nonadapted volunteers ingesting increasing doses in a predetermined time period. Regarding sensitivity to intestinal fermentation of carbohydrates, the results of such tests reveal that volunteers fall into three categories in terms of the amount of nondigestible carbohydrates they tolerate:

1. Nonsensitive persons who can consume 30 g/d or more almost without undesirable (unusual) reactions
2. Sensitive persons who can consume 10 g/d almost without undesirable (unusual) reactions but can experience undesirable reactions at 20 g/d or higher
3. Very sensitive persons who can already experience undesirable reactions at 10 g/d or even lower.

Based on average reactions, these three categories represent respectively 71–94%, 5–25%, and 1–4% of 100 adult volunteers, depending on the type of food (liquid or solid) and/or the protocol of product delivery (one or two shots).[101]

5.5 DISCUSSION AND CONCLUSION

Oligofructose and inulin are completely fermented mainly, if not exclusively, in the colon (at least in humans) and, consequently, they are not recovered to any significant extent in the feces. As a result of colonic fermentation, excretion of breath H_2 is significantly increased. As discussed above (see Section 5.3.5.3), if this is a valid marker of intestinal fermentation, it cannot be considered a quantitative measurement of the extent of fermentation.

In adequate experimental models (e.g., heteroxenic rats harboring human fecal flora) it has been reported that oral ingestion of oligofructose and inulin leads to an increased production of total SCFAs and a reduction in pH in the cecum. Moreover, these data point to increased amount and/or molar ratio of butyric acid in cecum content. However, in humans no significant effects on fecal SCFAs has been reported, but, as discussed above (Section 5.3.5.3), this is not a valid model to study both quantitatively and qualitatively SCFAs production by colonic carbohydrate fermentation. Indeed, only a small proportion (probably not more than 5%) of all SCFAs produced in the large bowel is excreted in feces. In one study on rats using inulin, a high proportion of propionic acid was recorded. Although inulin, like oligofructose, is completely fermented in the colon, the degradation may be slower than with oligofructose, which might give rise to another SCFAs pattern. Whether the nature of the carbohydrate determines its fermentability in the colon is a question that has barely been addressed. Van Laere et al. produced a range of oligosaccharides with different sugar compositions and molecular sizes.[102] They tested their fermentation in a batch system with several strains of bifidobacteria, clostridia, bacteroides, and lactobacilli. Fermentability differed with oligosaccharide structure. Oligofructose, inulin, and xylooligosaccharides were extensively fermented, except by the clostridia, while few species were able to breakdown arabinoxylans under the conditions of the experiment. Linear oligosaccharides were catabolized to a greater degree than those with branched structures while bifidobacteria utilized low DP carbohydrate first, and bacteroides, those with a high DP. Metabolic collaboration among species was evident in carbohydrate breakdown. Both the structure of the carbohydrate and the bacterial species present in the ecosystem are probably important factors in controlling fermentation of carbohydrates.

Oligofructose and inulin are thus both nondigestible and fermentable carbohydrates. Like all dietary substrates that become fermented in the intestine but mostly in the large bowel, they may produce intestinal discomfort and at very high doses (30 g/d or higher) they can even become laxative (because of osmotic effect). From the studies available it can be concluded that, on average, the most frequent intestinal side effects are flatulence and bloating and that these symptoms are mild or moderate, but a small percentage (1–4%) of the adult population may experience more severe reactions. However, it must always be kept in mind that these conclusions concern all types of nondigestible but fermentable dietary substrates and not only inulin-type fructans. As a result of the assessment of the available data, it can be concluded that for a wide proportion of the adult population (probably 95% or more), a daily dose of 10 g inulin-type fructans will cause no unusual intestinal side effects. For about three quarter of these adults, even a dose of 20 g/d will be acceptable. Little information is presently available concerning the acceptability of nondigestible but fermentable carbohydrates, in general, and inulin-type fructans, in particular, in other age groups. Still, a test with oligofructose has shown that children 10 to 13 years old ingested daily doses of 3 g, 6 g, and 9 g without reporting any undesirable side effects.[103]

These various and idiosyncratic effects of inulin-type fructans on intestinal symptomatology are difficult to explain. Individuals widely vary in their responses to fermentation of carbohydrates, and the stoichiometry of fermentation differs from carbohydrate to carbohydrate, especially as a function of chain length and monosaccharide composition, suggesting that, in general, molecules with longer chain length are fermented more slowly and with less net H_2 excretion.[49] This hypothesis is supported by Brighenti et al., who compared breath H_2 production after consumption of the same amount of lactulose (short-chain molecule), inulin (medium-chain molecules) and resistant starch (long-chain molecules producing) by healthy subjects and showed they exhaled 4.7, 19.1 and 26.6 ppm H_2/h/g respectively.[104] These findings were broadly reflected by *in vitro* fermentation studies and suggest that, in general, molecules with longer chain length are fermented more slowly and with less net H_2 production than short-chain molecules.

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6 The Digestive Functions: Inulin and Oligofructose as Dietary Fiber

6.1 DIETARY FIBER: A CONCEPT IN HUMAN NUTRITION

6.1.1 History

The health benefits of fiber-rich food have been recognized for at least 2500 years. Indeed, already in 430 B.C., Hippocrates described the laxative effect of coarse wheat in comparison to refined wheat.[1] In the 19th century, a recommendation was already made to increase the fiber content in diet, and in 1920, publications by J. H. Kellogg claimed that foods rich in bran increase stool weight, promote laxation, and prevent diseases.[2] Hispley was the first to use the expression "dietary fiber" when he described the cell wall components of plants and suggested that these components of foods might protect against toxemia during pregnancy.[3] But it has only been since the 1970s that dietary fiber has become an important concept in nutrition when it was hypothesized that the development of many Western diseases, especially chronic diseases, might be due to a deficit in dietary fiber intake.[4,5] Indeed, at that time, as a result of convergent analytical, physiological, and epidemiological studies, it became clear that dietary habits relate to the prevalence of cardio-vascular diseases, obesity, diabetes, colon cancer, and various other conditions of the large bowel.[4,6,7]

6.1.2 Definition of Dietary Fiber

Dietary fiber is neither a single nor a well-defined chemical entity, but rather corresponds to a complex mixture of compounds present in plants, which strongly vary with regard to chemical structure as well as physicochemical properties. The different components of dietary fiber have miscellaneous physiological effects, also. It is therefore not surprising that no definition is yet universally accepted. Moreover, even though methods have been developed and validated for the assay of total dietary fiber, there is, presently, no universally accepted analytical method that assays all dietary fiber components.

As a matter of fact, dietary fiber is more a concept than a chemical entity. The widely accepted reference is Trowell et al.[8] who defined dietary fiber as "remnants of plant cells resistant to hydrolysis by alimentary enzymes of man" and as "plant

polysaccharides plus lignin that resist hydrolysis by human digestive enzymes." But there are many other definitions, among which just to quote a few:[9]

- "Endogenous components of plant materials in the diet that are resistant to digestion by human intestinal enzymes."[10]
- "Plant (mostly cell wall) material composed of a number of substances, mostly carbohydrates in nature, that differ in chemical, physical, and physiological properties but cannot be degraded by human digestive enzymes."[11]
- "Plant material that resists digestion by human alimentary enzymes. It includes substances of unique chemical structure, characteristic physical properties, and individual physiological effects."[11]
- "Material derived from plants and indigested by secretion of the small intestine."[12]
- "The endogenous components of plant materials in the diet that are resistant to digestion by enzymes produced by man."[13]
- "A wide range of diverse substances that cannot be digested without the action of the gut microflora."[14]
- "Plant cellular material resistant to digestion by the endogenous enzymes of humans."[2]
- "Oligosaccharides and polysaccharides and hydrophilic derivatives that are not digested and not absorbed in the upper gut of humans, including lignin."[15]

Recently, the Food and Nutrition Board (FNB) of the U.S. Institute of Medicine of the National Academy of Sciences and the American Association of Cereal Chemists (AACC) have proposed new definitions that underline resistance to digestion as being the key characteristic of dietary fiber:[16,17]

1. "Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants …"[16]
2. "Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine …"[17]

In its definition, the FNB also introduces a distinction between "dietary fiber" that is "intrinsic and intact in plants" and "added fiber" that "consists of isolated, nondigestible carbohydrates."[16] Such a distinction is rather surprising and difficult to implement practically; the report does not explain clearly how these two categories of fiber can be analyzed separately in a mixed food product that contains both dietary fiber and added fiber, especially when these two fibers are chemically identical. Furthermore, for the FNB[16] but not for the AACC,[17] the definition includes "beneficial physiological effects in humans." The AACC definition also identifies fermentation (partial or total) in the large bowel as an essential part of dietary fiber metabolism.[17] Like the Scientific Committee for Foods of the European Commission,[15] this association also includes oligosaccharides in the definition, and it exem-

plifies some of the beneficial physiological effects that are promoted by dietary fiber consumption like laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.[17]

Including oligosaccharides as one of the dietary fiber components remains, however, a focus of some debates.[18] Classically, the prefix "oligo" is used to identify oligomers (carbohydrates, peptides, and nucleotides) with 2–10 or 2–20 monomers. Regarding oligosaccharides in particular, various definitions have included anything from 2–19 monosaccharide units.[19–21] There is neither a physiological nor a chemical reason for this division.[22] Especially, when discussing dietary fiber, it must be kept in mind that, in most cases, these are naturally occurring as mixtures of both oligosaccharides and polysaccharides that form a continuum of molecular size from simple sugars up to complex polymers, sometimes with a degree of polymerization (DP) of 100,000 or more. With regard to the basic properties of dietary fiber, there is no scientific argument to consider that the properties of a component with a DP <10 or 20 differs, qualitatively, from a molecule with a DP > 20. Thus, excluding a particular compound from the dietary fiber concept simply because it has a different DP cannot be accepted, and oligosaccharides must be included.

Some authors still consider dietary fiber as only "plant cell-wall, nonstarch polysaccharides" because they believe that the value for dietary fiber, based on the measurement of these polysaccharides, really aids the consumer in choosing the type of high-fiber diet recommended in the dietary guidelines.[18,23] But the general consensus supports the view that five attributes constitute the minimum requirement for classification as dietary fiber (Figure 6.1).

As a conclusion of this discussion, the term dietary fiber will, in this book, mean "*carbohydrates (oligo- and polysaccharides) that resist both hydrolysis by mammal*

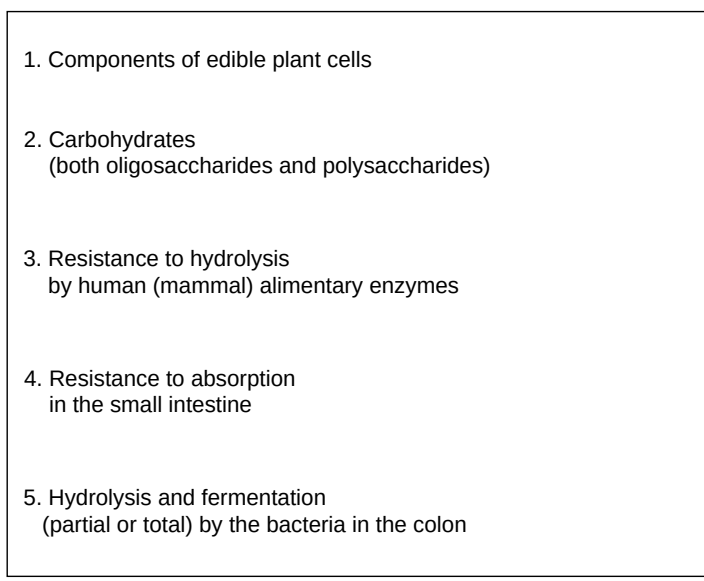

FIGURE 6.1 Major attributes of dietary fiber.

digestive enzymes and absorption in the small intestine but are, at least partly, hydrolyzed and fermented by the colonic microflora."

For the purpose of nutrition labeling and communication, the so-defined dietary fiber concept is of great value. Indeed, it is useful to distinguish between digestible carbohydrates (sugars and starches) that directly influence glycemia (the glycemic carbohydrates) and nondigestible carbohydrates that serve as substrate for the colonic microbiota and have no direct effect on glycemia (the low glycemic index carbohydrates). The former are important sources of energy, whereas the latter help maintaining regularity of gastrointestinal, especially colonic, functions and potentially contribute to well-being and health, as well as to reduce the risk of miscellaneous chronic diseases.

6.1.3 The Dietary Fiber Components

Under the entity *dietary fiber*, a great variety of food components are included that belong to plant cell polysaccharides. More than 95% of these polysaccharides are components of plant cell walls, the rest being storage carbohydrates, part of the intracellular cement, or secreted by the plant in response to an injury.[24] From a chemical point of view, these consist of miscellaneous components, i.e., cellulose, hemicelluloses, pectins, gums and mucilages, mixed-linkage β-glucans, resistant starch, inulin, and nondigestible oligosaccharides. In addition, microbial polysaccharides as well as synthetic compounds (e.g., polydextrose) are also sometimes considered as part of the dietary fiber concept. (For a more extensive description of the dietary fiber components see Chapter 5, Section 5.3.2). Except resistant starch, all these compounds have a common molecular feature; they are non-α or nonstarch oligo- and polysaccharides. With regard to solubility (or more precisely, dispersibility) in water, these components are classified as "soluble dietary fiber" if they are (soluble) dispersible in water and as "insoluble dietary fiber" if they are poorly (soluble) dispersible in water. At the moment, few labeling regulations allow this distinction on food labels. The distinction is method dependent. Moreover, as confirmed in recent recommendations, WHO/FAO[25] and the FNB[16] do not consider this to be a useful division, either analytically or physiologically. Consequently, it is recommended to abandon this categorization.

Even though they do not have a carbohydrate-type structure, lignins are also, usually, considered under the dietary fiber definition. These have a complex three-dimensional structure and are composed of phenyl propane units. In addition to polysaccharides and lignins, the human diet contains additional plant-derived materials that also resist digestion by the human digestive enzymes and can be further metabolized in the large bowel but are not included in the dietary fiber concept. These include cutin, waxes, some proteins, and some lipids, etc.

6.1.4 Analysis of Dietary Fiber

Several analytical methods have been developed for dietary fiber determination. They generally fall into one of the two categories, i.e., gravimetric analysis or component (chemical) analysis (Figure 6.2).[26]

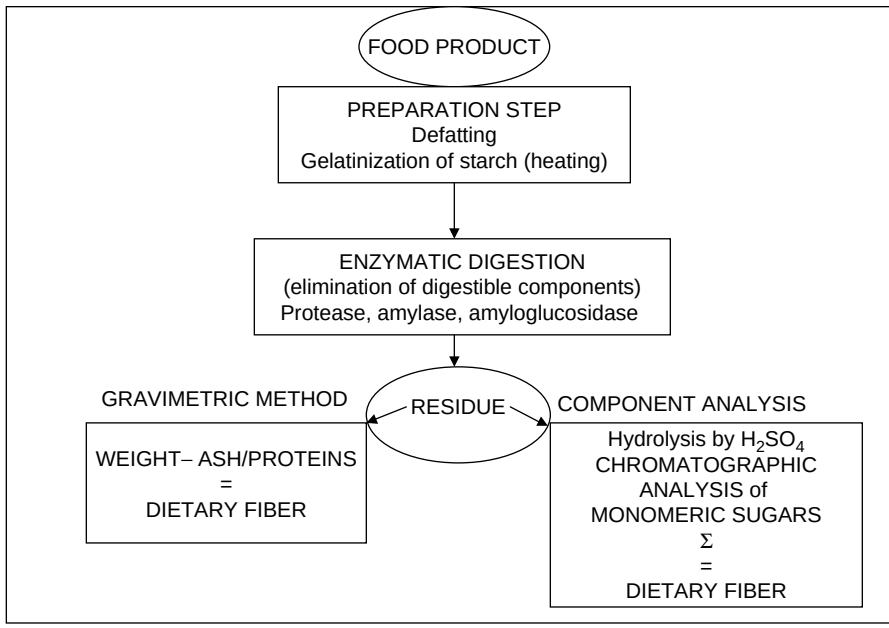

FIGURE 6.2 Basic principles of the two most common methodologies to quantify dietary fiber in food products. (Adapted from Gallaher, D. D., Schneeman, B. O., *Present Knowledge in Nutrition*, Ziegler, E. E., Filler, L. J., Jr., Eds., 7th ed., ILSI Press Washington, D.C., pp. 87–97, 1996.)

1. The gravimetric method is known as the AOAC method. It is simple and fast, but it is limited to quantify the total fiber or soluble and insoluble components.[27,28] This method is, presently, the most common method to assay the total dietary fiber in cereals, fruits, vegetables, or food products.
2. Component analysis quantifies the individual neutral sugars and the total acidic sugars (i.e., uronic acids). The total fiber content is then calculated as the sum of the individual sugars.[29]

Both these methods have their own advantages and disadvantages, and the choice of the method for dietary fiber determination automatically restricts the dietary fiber to what the method measures (Table 6.1).

This leads to illogical situations in which compounds that clearly behave as dietary fiber, (but are not measured by the method chosen) are excluded from dietary fiber labeling. There is a growing consensus that dietary fiber should not be defined by what is measured by an analytical method. Rather, a definition should refer to the essential nutritional properties of dietary fiber. Analytical methods should then be devised to accommodate this definition. Where necessary, different methods should be used for different compounds. This was clearly confirmed in the conclusions of the European Standing Committee for Food on dietary fiber.[15]

In conclusion, there is a growing consensus that dietary fiber should not be determined by an analytical method alone, but more accurate methods should be

TABLE 6.1
Advantages and Disadvantages of the Gravimetric Method vs. the Component Analysis of Dietary Fiber in Food Products

	Gravimetric Method AOAC 985.25 (AACC 32.05)	Component Analysis
Advantages:		
	• Fast	• Quantification of individual monomeric sugars
	• Simple	• Separation of neutral and acid sugars
	• Officially approved	• Separate analysis of lignin
	• Internationally validated	• Can be modified to include new fibers
	• Not requiring sophisticated equipment	
Disadvantages:		
	• Limited to total dietary fiber	• Requires more expertise
	• Applicable to separate soluble or insoluble fibers (precipitation in 80% ethanol)	• Requires complex equipments

made available and validated for any specific substance that is proposed or accepted to be a part of the dietary fiber concept.

6.1.5 Physicochemical Properties of Dietary Fiber

All dietary fiber components have different physicochemical properties. In general, these properties are determined largely by the way in which the components are linked together. It is important to note that these physical properties have often only been demonstrated *in vitro*, thus ignoring that the structure of each dietary fiber component can undergo complex modifications during passage through the gastrointestinal tract. Therefore, it is not certain that the *in vitro* data relate to the physiological properties *in vivo*. The most often cited physicochemical properties are:

- **The water-holding capacity.** This refers to the ability to retain water in the matrix. It can be measured *in vitro* by saturating the fiber with water and then removing the nonadsorbed water by either filtration or centrifugation of osmotic suction.[30,31] The shape of the molecules, their ability to pack closely together, and their solubility (dispersibility) in water are the key features controlling their water-holding capacity. Closely packed, insoluble (nondispersible) compounds (e.g., cellulose) are resistant to hydration and swelling, whereas those with disordered structures and more solubility (dispersibility) are hydrated more easily. These properties are also influenced by the particle size. In general, dietary fiber from fruit and vegetables tends to bind much more water than cereal dietary fiber. Originally, it has been suggested that components with a large water-holding capacity will have a high stool-bulking effect.[32] Unfortunately,

the water-holding capacity of a dietary fiber determined *in vitro* does not predict its stool-bulking ability, mostly because it does not take fermentation and subsequent increase in bacterial biomass into account.[33] The so-called "potential water-holding capacity" (i.e., the water-holding capacity of the dietary fiber residue and the bacterial biomass after *in vitro* fermentation) that was developed to take these factors into account is physiologically a more meaningful parameter.[34] But it is difficult to measure.[26]

- **The cation exchange capacity.** This is determined mainly by the content of carboxyl (COO^-), hydroxyl (OH), and amino (NH_3^+) groups in the sugar moieties of the oligo- and polysaccharides. *In vitro,* many different dietary fibers (e.g., pectins, because of the presence of uronic acid monomers) have been shown to bind minerals, and it has been hypothesized that these might also behave similarly *in vivo* in the gastrointestinal tract, thus impairing the absorption of some important minerals (like calcium, copper, and zinc).[34–36] The *in vivo* relevance of these data has, however, never really been demonstrated. Moreover, because of the presence of phytates in plants or crude dietary fiber preparations, the interpretation of the data, when using these sources, is difficult. But some dietary fiber components (e.g., cellulose, b-glucans, inulin) have no ionic charge, they do not bind any minerals and, as discussed later (Chapter 10), some of these nonionic dietary fiber components, particularly inulin, have even been shown to increase mineral (especially Ca and Mg) absorption.

- **Viscosity.** It is determined by the molecular weight of the polysaccharides, their capacity to interact in solution, their volume, and the presence of solid insoluble particles. When in solution, the viscosity of pectins, mixed-linkage b-glucans, and algal polysaccharides (agar and carrageenan) increase. But the importance of this effect strongly depends on the chemical structure of the dietary fiber. Insoluble dietary fiber components have practically no effect on viscosity. Once again, the actual effect of dietary fiber on the viscosity of the intestinal content is difficult to evaluate.
- **The binding capacity.** Many substances, especially bile acids, may become bound to dietary fiber components. The binding capacity depends on the shape of the molecules, the chemical nature of the surface, and the total area accessible for binding that varies with particle size. Different dietary fiber components can have strikingly different binding capacity (e.g., pectins seem to have the greatest ability to bind bile acids, whereas wheat bran has a moderate capacity and cellulose practically none). Data concerning binding ability of dietary fiber components remain, however, very controversial.[26]

In conclusion, there is not a single physicochemical property that is common to all dietary fiber components. Most of the information available comes from *in vitro* experiments, and the *in vivo* significance of the findings often remains unclear. Thus, based on the present knowledge, viscosity, water holding, binding, and cation

exchange capacities cannot be used as an exclusion or selection criterion for classification as dietary fiber. Each component in the dietary fiber concept has its own pattern of physicochemical properties. (Table 6.2)

6.1.6 Physiological Properties of Dietary Fiber: Their Effects on Upper Gastrointestinal Tract

6.1.6.1 Resistance to Digestion

Nondigestibility is, undoubtedly, the most common and the most essential basic property of dietary fiber. Almost all definitions include this as the basic characteristic of dietary fiber. This is primarily because, with the exception of resistant starch, all dietary fiber components are non-a-oligo- and polysaccharides that cannot be hydrolyzed by the small intestinal a-glycosidase in mammals. The methodologies that can be used to demonstrate resistance to digestion are described previously (see Chapter 4, Section 4.1.3). When applied to the main dietary fiber components, they have demonstrated their nondigestibility, including *in vivo* in humans, especially in ileostomy volunteers.[37,38]

TABLE 6.2
Major Physiological Properties of Dietary Fibers: Summary of their Effects on Gastrointestinal Functions

Effects of Dietary Fibers on Gastrointestinal Functions
Effects on Upper Gastrointestinal System
Resistance to digestion
Retarded gastric emptying
Increased oro–cecal transit time
Reduced glucose absorption and low glycemic index
Hyperplasia of the small intestinal epithelium
Stimulation of secretion of intestinal hormonal peptides
Effects on Lower Gastrointestinal System
Acting as food for colonic microflora
Acting as substrates for colonic fermentation
Production of fermentation end products, especially SCFAs
Stimulation of saccharolytic fermentation as opposed to proteolytic fermentation
Acidification of the colonic content
Hyperplasia of the colonic epithelium
Stimulation of secretion of colonic hormonal peptides
Bulking effect on stool production
Regularization of stool production (i.e., frequency and consistency)
Acceleration of ceco–anal transit

6.1.6.2 Effects on Upper Gastrointestinal Functions

Soluble, gel-forming (mainly pectins and guar gum), but not insoluble, dietary fiber components affect the upper gastrointestinal functions. Some, but not all, dietary fiber components affect gastric emptying, but most of the evidence is indirect, and the few direct studies are conflicting or difficult to interpret.[39] The major determinant of this effect is, most probably, viscosity because viscous dietary fiber components may delay gastric emptying by increasing the resistance of the gastric juice to be pressed through the pylorus.[40,41] But negative results have also been reported, and, moreover, the influence of viscosity in gastric emptying rate has been questioned.[42]

The gel-forming dietary fiber components may also create a viscous environment in the small intestine, thereby increasing the thickness of the unstirred water layer and consequently hindering diffusion (and thus absorption) of macronutrients. Such an effect is also likely to slow down the transit.

Both gastric emptying and small intestinal transit time are also under the control of gastrointestinal hormonal peptides (see Chapter 2, Section 2.3), and recent data indicate that some fermentable dietary fiber components like inulin stimulate the secretion of such peptides, thus indirectly modulating these parameters.

Reducing gastric emptying as well as increasing the viscosity of the small intestinal content may limit the absorption of glucose by slowing down its delivery to and its absorption from the small intestine.[43] Viscous dietary fiber components (e.g., guar gum and pectins) flatten the glucose response curve and reduce postprandial glycemia and insulinemia, thus helping to improve glucose tolerance and insulin sensitivity.[44–46] But other dietary fiber components, such as cellulose and wheat bran, have no effect on glucose absorption.

Pectin has also been shown to increase maltose absorption in the ileum, an effect that correlates with an induction of brush-border enzymes resulting from hyperplasia of small intestinal mucosa.[47] Such hyperplasia is part of the physiological adaptation to high-fiber diet, which leads to an increase in epithelial cell turnover, in length and weight of the small intestine, as well as in depth of crypts.[11,47,53] This is likely to be a trophic effect mediated by short-chain fatty acids (SCFAs) that are the end products of the fermentation of fermentable dietary fiber components,[54–57] but not by inert bulking agents.[14,56] Moreover, highly fermentable dietary fiber components might also affect small intestinal epithelial cell turnover, as well as glycemia and insulinemia, via their effects on the production of gastrointestinal hormonal peptides like GIP and GLP-1 (see Chapter 2, Section 2.3).

6.1.7 Physiological Properties of Dietary Fiber: Their Effects on the Large Bowel

6.1.7.1 Colonic Fermentation

Most, if not all, large bowel functions are affected by microbial fermentation, for which the dietary fiber components are important substrates.[58–60] Indeed, although they are resistant to hydrolysis and digestion in the stomach and the small intestine, some dietary fiber components are, partly or totally, hydrolyzed and fermented by the anaerobic bacteria (among the colonic microflora), resulting in the formation of

gases, SCFAs (acetate, propionate, and butyrate), and lactate as terminal electron acceptors. To some extent, the molar ratio between these metabolites is characteristic of the dietary fiber component that is fermented.[61–64] Establishing the exact pattern of the production of SCFAs from dietary fiber components remains, however, a very difficult task. Indeed, such a study cannot be done *in vivo* in humans because the fecal SCFAs do not equal the colonic ones. Moreover, carbohydrate fermentation takes place primarily in the cecum and in the proximal colon, and the SCFAs are very efficiently taken up and metabolized by the colonocytes and absorbed to reach the systemic circulation.[65–68]

Consequently, the composition of the SCFA pool in the feces collected from human volunteers fed a fiber-rich diet does not really represent the products of colonic fermentation, and such data should be interpreted with great caution.

The patterns of dietary fiber fermentation have thus been studied mostly in *in vitro* systems (for a review of these systems, see Chapter 5, Section 5.3.5) using either fecal slurries or content taken from different parts of the large bowel of victims of fatal accidents. Such *in vitro* studies have demonstrated that pectins, hemicelluloses, and gums[61,69,70] are, largely, if not quantitatively, fermented whereas insoluble dietary fiber components like cellulose[61,70] or wheat bran are not. With regard to SCFAs production, pectin fermentation produces mainly butyrate and acetate, whereas resistant starch shifts the short chain fatty acids production mainly to butyrate. Arabino-galactan or guar gum increases overall SCFAs production, but hardly affects the ratio of acids produced.[71,72]

From a qualitative point of view, hydrolysis and fermentation control the composition of the products and, consequently, their effects on colonic mucosa (hyperplasia, endocrine activity, etc.), colonic content (pH), and large bowel functions (stool production), as well as the composition of the colonic microflora (see Chapter 9). One key effect seems to be the pH reduction that, indirectly, influences the composition of the intestinal flora (e.g., less potentially pathogenic clostridia when pH is more acidic), the solubility of bile acids, the absorption of the SCFAs as well as minerals (indirectly), and the protonic dissociation of ammonia and other amines.

It has become clear that the colonic microflora plays a very important role in human health and well being. In that respect, one of the most beneficial effects of the intestinal fermentation of dietary fiber components is to keep the intestinal flora in a metabolically active and typically saccharolytic state, preventing it from shifting towards proteolysis that is known to produce toxic (carcinogenic) metabolites.

As in the small intestine (see Section 6.1.6.2), the colonic epithelial cell proliferation is stimulated by fermentation of carbohydrates.[56,57,70,73,74] This effect is most likely to be mediated by butyrate that affects cell growth and differentiation, and increases the proliferative index at the bottom of the crypts.[75,76]

In conclusion, the fermentation by the intestinal microflora is considered a crucial property of dietary fiber. Many dietary fiber components are fermented, at least partly, in the large intestine. By providing sufficient fermentable carbohydrate (dietary fiber) substrates, the production of unwanted proteolytic metabolites can be reduced or even avoided.

6.1.7.2 Bowel Habit

Consumption of a dietary fiber-rich diet affects bowel habit both quantitatively and qualitatively, typically fecal weight, stool form, and consistency, stool frequency, and transit time (for a general discussion of bowel habit and transit time, see Chapter 2, Section 2.2.3 and Chapter 2, Section 2.2, respectively).

According to Cummings, dietary fiber is "the only dietary component to have been shown, consistently and over many generations, to control bowel habit."[18] By combining the results of 11 published studies (covering 26 separate dietary periods and a total of 206 persons), in which the dietary fiber (all types) intake has been measured carefully, accurate stool collection was made, and other dietary components were kept constant, this author has clearly shown that an increase in dietary fiber intake increases stool weight (Figure 6.3).

Based on the regression equation that fits these data, it can be predicted that an increase of 1 g in daily dietary fiber intake will increase stool weight by ±5 g/d. If all dietary fiber components are to produce such an increase, the change will be due to different types of fibers. Again, by compiling the stool weight data from nearly

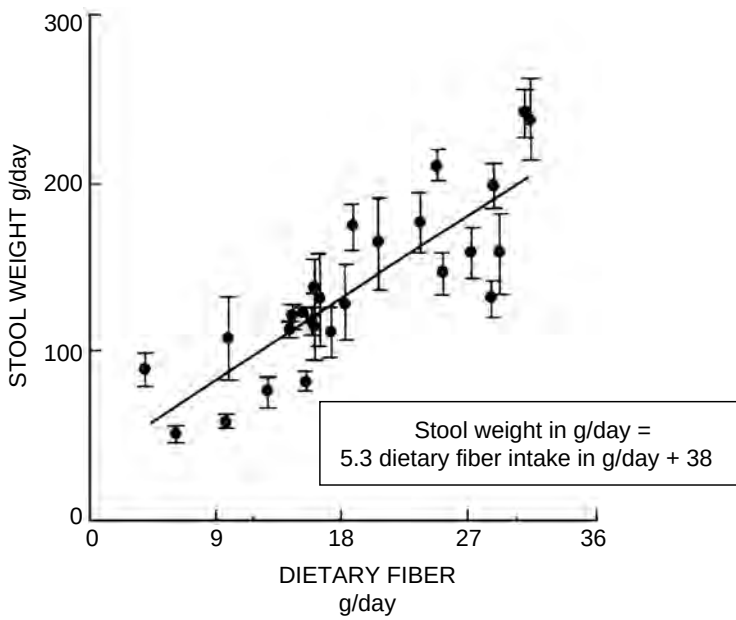

FIGURE 6.3 Correlation of daily stool weight and daily dietary fiber (nonstarch polysaccharides) intake in healthy subjects (n = 206) eating controlled diets with different amounts of dietary fiber (Reproduced from Cummings, J. H., *The Large Intestine in Nutrition and Disease*, Danone Chair Monograph, Institut Danone, Brussels, Belgium, 1997 with the permission of the Institute Danone, Belgium; and adapted from Cummings, J. H., Bingham, S. A., Heaton, K. W., Eastwood, M. A., Fecal weight, colon cancer risk, and dietary intake of non-starch polysaccharides (dietary fibre), *Gastroenterology*, 103, 1783–1789, 1992.)

120 papers and detailing 150 separate studies (reporting quantitative data on changes in stool weight by comparing at least one control and one test period), Cummings [18,78,79] has calculated the average increase in stool output, expressed as a stool bulking index:

Bulking index = g of increased stool (wet weight) production/g of dietary fiber component-fed

These values, which are significantly different ($p < .001$), are shown in Figure 6.4. The insoluble dietary fiber components that are not fermented or only poorly fermented (i.e., wheat bran, cellulose, and oat fiber) are the most effective stool bulking agents with +5.4, 3.5, and 3.4 g stool/g dietary fiber component-fed, respectively. Gums and mucilages are also very effective at +3.7 g stool/g dietary fiber

DIETARY FIBER	Average & Range of BULKING INDEX (g increase in stools per g of intake)	Range of DAILY INTAKE (g)
INULIN-Type FRUCTANS	1.7 (1.5–2.1)	15–50
PECTIN	1.7 (0.4–2.3)	6–36
GUMS	1.6 (0.3–5.0)	5–35
CELLULOSE	3.1 (2.4–3.5)	12–25
WHEAT BRAN	3.3 (0.8–7.2)	16–36
CORN	3.4 (2.9–4.6)	17–29
PSYLLIUM	4.2 (0.9–6.6)	5–20
ISPAGHULA	4.5 (3.2–6.3)	7–25
OAT BRAN	5.0 (3.8–5.4)	2–12

FIGURE 6.4 Comparison of the bulking index of inulin-type fructans (calculated from Gibson, G. R., Beatty, E., Wang, X., Cummings, J. H., Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin, *Gastroenterology*, 108, 975–982, 1995; Kleessen, B., Sykura, B., Zunft, H. J., Blaut, M., Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons, *Am. J. Clin. Nutr.*, 65, 1397–1402, 1997; Den Hond, E., Geypens, B., Ghoos, Y., Effect of high performance chicory inulin on constipation, *Nutr. Res.*, 20, 731–736, 2000) and various dietary fibers (adapted from Cummings, J. H., *The Large Intestine in Nutrition and Disease*, Danone Chair Monograph, Institut Danone, Brussels, Belgium, 1997; Cummings, J. H., The effects of dietary fiber on fecal weight and composition, in: *CRC Handbook of Dietary Fiber in Human Nutrition*, Spiller, G. A., Ed., 2nd ed., Boca Raton, FL: CRC Press, 263–349, 1993).

component-fed whereas pectin, as a representative of a highly fermented component, is less effective at +1.2 g stool/g dietary fiber component-fed. Diets rich in fruits and vegetables, known as the major natural sources of dietary fiber components, are remarkably effective at +4.7 g stool/g dietary fiber component-fed.

An increase in stool weight leads to increased stool frequency that is accepted as an indirect proof for a stool bulking effect. For the consumer, an increase in stool frequency is generally regarded as one of the most important and best understood benefits of dietary fiber. Laxative effects and bowel habit in general were considered as the best parameters to establish "Daily Recommended Values" for dietary fiber in the U.K.[80] Similarly, Bagheri and Debry have suggested using wet weight of stool as a basis for determining a recommended dose of dietary fiber to achieve regular intestinal transit.[81] The mechanism of these effects on stool production depends on the physical property of the dietary fiber component considered:

- The dietary fiber components that are hardly fermented (e.g., wheat bran, oat bran, and cellulose) have a direct bulking effect and adsorb water, thus increasing fecal volume and stool weight. They also affect intestinal transit.
- The dietary fiber components that are fermented in the large bowel disappear as such from the intestinal environment and are converted to gases, SCFAs, and bacterial biomass, the latter in itself contributing to an increase in fecal mass.[60,82,83] An increase in stool volume can also occur due to trapping of gases and retention of water.[84] In addition, the SCFAs produced during the fermentation affect bowel movement and consequently have an indirect effect on transit time.[85]

In conclusion, fecal bulking and increase in stool frequency are considered to be two of the basic nutritional properties of dietary fiber. The dietary fiber components that are less readily fermented influence fecal bulking more strongly than easily fermented dietary fiber components such as gums and pectins.

6.2 INULIN AND OLIGOFRUCTOSE AS DIETARY FIBER

6.2.1 Inulin and Oligofructose, and the Concept of Dietary Fiber

As discussed above, the five basic attributes of a dietary fiber are:

- It is a component of an edible plant cell.
- It is a carbohydrate (both oligosaccharides and polysaccharides).
- It resists hydrolysis by human (mammal) alimentary enzymes.
- It is resistant to absorption in the small intestine.
- It undergoes hydrolysis and fermentation (partial or total) by the bacteria in the large bowel.

The question now is: Do inulin and oligofructose have these basic attributes? And the answer is that inulin and oligofructose:

1. Are part of edible plants (see Chapter 3, Section 3.2.3).
2. Are carbohydrates that are composed of a mixture of various oligosaccharides, or oligosaccharides and polysaccharides (see Chapter 3, Section 3.3.1 and Section 3.3.2).
3. Resist hydrolysis by human digestive enzymes (see Chapter 4, Section 4.3.1).
4. Do not appear to be significantly absorbed in the small intestine, except possibly for the very short-chain oligosaccharides (di- and trisaccharides).[86] Even if such small molecular weight oligosaccharides are absorbed, they are not hydrolyzed inside the body, and they are excreted as such in the urine as is intravenously injected inulin (DP 30) that is classically used in medical practice for measuring either extracellular fluid volume or the renal glomerular filtration rate. It has been reported that approximately 1% of oligofructose that disappears from the small intestine is recovered in urine.[87]
5. Are hydrolyzed and completely fermented by the colonic microflora, and are oxidized to produce gases and SCFAs (see Chapter 5, Section 5.4).

Thus, inulin and oligofructose can be described as carbohydrates (oligo- and polysaccharides) that resist both hydrolysis by mammal digestive enzymes and absorption in the small intestine but are, at least partly, hydrolyzed and fermented by the colonic microflora. They should be classified as dietary fiber and labeled as such on consumer food products.

6.2.2 Inulin and Oligofructose, and the Analysis of Dietary Fiber

The next question is: Do the classical methods for dietary fiber analysis (see Section 6.1.4) quantify inulin and oligofructose?

Oligofructose is not measured by the AOAC[27,28] or Englyst[29] dietary fiber methods and inulin is only partly measured. They do not (or only partly) precipitate in aqueous ethanol, a step used to collect the analyte in both methods. But, as discussed above and based on the results of a collaborative validation ring test, AOAC has adopted the fructan method (method number 997.08) that specifically allows the accurate quantitative determination of inulin and oligofructose in foods.[88] This method can be combined with the standard AOAC Total Dietary Fiber method to quantify the total amount of fiber used for food labeling.[89,90]

Thus, in agreement with the growing consensus, an accurate method is made available and validated for measuring inulin and oligofructose as part of dietary fiber.

6.2.3 Inulin and Oligofructose, and the Physicochemical Properties of Dietary Fiber

Being a mixture of relatively short-chain molecules (up to DP 60–65), inulin and oligofructose have no water-holding capacity. However, being water soluble and

hydroxyl-rich molecules, they exert an osmotic effect that is likely to be the origin of the laxative effect that occurs at high doses.[91]

Inulin has a rather low viscosity (less than 2 mPa for a 5% w/w solution in water), but oligofructose is not viscous. However, as described previously (see Chapter 3, Section 3.3.3), when it is thoroughly mixed with water or another aqueous liquid, submicron crystalline inulin (especially inulin HP) particles form a tridimensional gel network, resulting in a white creamy structure with a short spreadable texture that can easily be incorporated into foods to replace up to 100% fat.[92] Large amounts of water are immobilized in this network, which assures its physical stability. Moreover, inulin works in synergy with most gelling agents such as gelatin, alginate, k- and i-carrageenans, gelling gum, and maltodextrins. It also improves the stability of foams and emulsions.[93]

Being composed of neutral sugar moieties (glucose, but mainly fructose), inulin and oligofructose have no cation exchange capacity. This, however, does not preclude the classification of inulin and oligofructose as dietary fiber. Based on present knowledge, viscosity, and water-holding, binding, and cation exchange capacities cannot be used as an exclusion or selection criterion for classification as dietary fiber. Each component in the dietary fiber concept has its own physicochemical properties (see Section 6.1.5).

6.2.4 Inulin and Oligofructose, and the Effects of Dietary Fiber on the Gastrointestinal Tract

6.2.4.1 Resistance to Digestion

In addition to what has already been discussed earlier concerning the resistance to digestion (see Chapter 4, Section 4.3.1.5 and Section 6.2.1), it is important to mention here that oligofructose tolerance tests, even if performed after 2 to 3 months of oligofructose feeding, do not show any increase in glycemia or insulinemia, thus demonstrating that the oligosaccharides are not hydrolyzed in the upper gastrointestinal tract.[94]

6.2.4.2 Inulin and Oligofructose, and Upper Gastrointestinal Functions

During their passage through the upper intestine, inulin and oligofructose may influence the digestion process and thus metabolic responses.

In rats fed a diet containing 10% oligofructose for 3 months, there was a reduction in sucrose–maltose tolerance levels. This result, together with a reduction in intestinal sucrase–maltase activity, might indicate a slowing down in the digestion of disaccharides.[95] Similarly, Buddington et al. have shown in mice that inulin and oligofructose (10% in diet) lower the rates of glucose transport and absorption of leucine, proline, and glycylsarcosine.[96] Such effects are, however, not seen in streptozotocin-treated (diabetic) rats fed a diet containing 20% oligofructose for 2 months.[97] Moreover, and contrary to what has been reported for some dietary fiber components,[98] inulin or oligofructose have no influence on starch[94,99] or glucose

(glucose load test)[99] absorption in human. Moreover, Ellegärd et al. have reported no effect of inulin on small intestinal handling of nitrogen, fat, starch, or minerals (Ca, Mg, and Zn) in ileostomy volunteers.[100]

With regard to gastrointestinal transit time, only one study in rats has reported a shortening of the oro–anal transit time by oligofructose (25 and 50% at a dose of 10 and 20% in the diet, respectively). In human upper gastrointestinal tract, Bach-Knudsen and Hesov have shown that transit time of inulin (as measured in ileostomy volunteers) is shorter (4.9 or 3.4 h for 10 and 30 g, respectively) than that of nonstarch polysaccharides (6–8 h).[101] In healthy volunteers, Rumessen et al. have shown that inulin is transported more slowly than lactulose, with inulin having an orocecal transit time of 2.7, 1.75, and 1.45 h for single oral doses of 5, 10, and 20 g, respectively, and the transit time for lactulose being 0.9 h.[94]

Like other fermentable dietary fiber components, oligofructose increases the length and weight of the small intestine and small intestinal mucosa.[95,96] In an experimental protocol (using 5-week-old male Sprague-Dawley rats) that separated the proximal from the distal small intestine, Ohta et al. have reported that oligofructose (5 and 10% w/w in diet for 10 d) significantly ($p < 0.05$) increased (+20% and +30%, respectively) the weight of the small intestinal mucosa but had no significant effect on mucosal proteins.[102] In another protocol (using 4-week-old sham-operated or gastrectomized Sprague-Dawley male rats) aimed at testing the preventive effect of oligofructose on osteopenia, the same group has reported no effect of oligofructose (10% w/w in diet for 10 d) on mucosa nor on mucosal proteins in the small intestine (both proximal and distal).[103]

Moreover, because of their effects in the large bowel, inulin-type fructans might affect the production of gastrointestinal hormonal peptides and consequently the hormonal regulation of gastrointestinal motility as well as systemic metabolic processes (see Chapter 2, Section 2.2).

6.2.4.3 Colonic Fermentation of Inulin and Oligofructose

Inulin and oligofructose are rapidly and quantitatively fermented by colonic microflora (see Chapter 5, Section 5.4). However, because of the differences in chain length (see Chapter 3, Section 3.3.5 for a detailed description of these differences), oligofructose is more rapidly fermented than inulin, and long-chain inulin (inulin HP) is more slowly fermented than inulin.[104] Such a difference in fermentation rate might be of interest to differentiate some of the physiological effects of these dietary fiber components. Indeed, it can be hypothesized that the low molecular weight oligofructose monomers (DP 2–8) are completely fermented in the cecum and the proximal (ascending) segment of the colon whereas the longest chain oligomers (up to DP 60) that exist in inulin (DP 2–60), especially in long-chain inulin (inulin HP DP 20–60), have the capacity to progress along the transverse and possibly to reach the distal (descending) segments of the colon before being completely fermented. Interestingly enough, a special mixture of oligofructose (DP 2–7) and long-chain inulin (inulin HP DP 20–60) has been developed by the industry (oligofructose-enriched inulin Synergy 1). *In vitro* fermentation data demonstrate that in a three-stage fermentation system that mimics the three segments of the colon, a saccharo-

lytic fermentation does, indeed, occur all along the three vessels (segments), whereas oligofructose and inulin are fermented only in the first and the first–second vessels (segments) in the presence of the mixture, respectively (Gibson, personal communication).

The increase in production as well as the change in molar ratio of SCFAs in the cecum as a result of inulin and oligofructose fermentation has been discussed earlier (see Chapter 5, Section 5.4.1 and Chapter 5, Section 5.4.2). In summary, such a fermentation increases the concentration of SCFAs by 1.7 times and almost doubles the production of butyrate.

It has also been reported that oligofructose feeding reduces the production of putrefactive substances known as the products of amino acids fermentation.[105,106]

6.2.4.4 Inulin and Oligofructose, and Lower Gastrointestinal Functions

SCFAs, especially butyrate, play an essential role in maintaining colonic mucosa integrity by acting on metabolism, proliferation, and differentiation of different epithelial cell types.[57] Moreover, it has recently been hypothesized that certain bacteria, especially lactobacilli, directly influence epithelial cell functioning.[107]

Because their cecocolonic fermentation produces high amount of SCFAs, a high relative proportion of butyrate as well as lactate (see Chapter 5, Section 5.4.1.2), inulin, and oligofructose cause an acidification (–0.4 to –1.4 and in average –0.9 pH unit) of the cecal content.[72,108,118] In addition, Ohta et al. have reported that the pH of the colon lumen is similarly reduced (–1.2 pH unit).[110] Wolf et al. have shown that the decrease in pH is dose dependent, being –0.43, –0.85, and –1.1 when the concentration of oligofructose in the diet of rats is 1, 3, and 5%, respectively.[115] Bruggencate et al. have similarly reported a dose-dependent decrease in the pH of the cecal content after feeding rats with a diet supplemented with either 3% (–0.9 pH unit) or 6% (–1.8 pH units) oligofructose.[119]

Moreover, inulin-type fructans are likely to modulate several cecocolonic function, especially, those associated with epithelium integrity (Table 6.3).

The hypothesis has barely been tested, and no evidence has yet been found in humans. However, the weight of the cecal wall of rats harboring either a human or a conventional microflora and fed a diet containing 4 to 10% inulin or oligofructose was significantly increased (1.2–3.4 times).[72,112,114,116,121] Similar effects have been reported in rats after gastrectomy, a surgery that is known to modify the intestinal microflora.[120,122] In an experimental protocol (using 5-week-old male Sprague-Dawley rats) that separated the proximal from the distal small intestine, Ohta et al. have reported a significant ($p < 0.05$) increase in the weight of the small intestinal mucosa and mucosal proteins after feeding with oligofructose (5 and 10% w/w in diet for 10 d).[102] With the lowest dose (5% w/w), the effect was significant both for the mucosa (1.8 times) and the mucosal proteins (1.8 times) only in the cecum, but with the highest dose (10% w/w), it was significant for the mucosa (2.2 times) and for the mucosal proteins (1.9 times) in the cecum and only for the mucosa (1.5 times) in the colon. In another protocol (using 4-week-old sham-operated or gastrectomized Sprague–Dawley male rats) aimed at testing for

TABLE 6.3
Summary of the Changes in Cecal Parameters in Rats Fed with Inulin and Oligofructose

Rats			Treatment			Short Chain Fatty Acids (SCFAs)		Pattern of SCFAs Acetate/Propionate/Butyrate		
Flora	n[1]	Age	Weeks[2]	Oligo-fructose (%)	Inulin (%)	mmol/g	Increase, in times	Control	Treatment	Refs.
CV[3]	10	AD[4]	2	6		36.5	1.4x	72/14/14	71/9/20 (1.4x)[6]	112
	12	GR[5]	3		5	84.5	1.7x	64/23/12	45/33/22 (1.8x)	108
					10	84.5	1.8x	64/23/12	42/38/20 (1.7x)	108
HF[7]	8	AD	3		10	36.5	1.8x	66/18/14	65/7/34 (2.4x)	107
	6		5	4	10	31.5	1.8x	58/22/15	64/13/20 (1.3x)	72
	5	GR	4		10	28.5	1.2x	64/21/7	64/22/12 (1.7x)	113
	6		8			37.0	1.5x	63/19/8	56/15/27 (3.4x)	109
Mean						51	1.7x	65/19/12	58/20/22 (1.9x)	

[1]n is the number of rats.
[2]weeks is the number of weeks of treatment.
[3]CV = conventional flora.
[4]AD = adult rats.
[5]GR = growing rats.
[6]The number in () is the fold increase in the proportion of butyrate in the pool of SCFAs.
[7]HF = human flora.

the preventive effect of oligofructose on osteopenia, the same group has reported that feeding oligofructose (10% w/w in diet for 10 d) significantly increased the weight of the mucosa (2.1 times and 1.4 times in the cecum and the colon, respectively) and of the mucosal proteins (2.8 times and 2.1 times in the cecum and the colon, respectively) in the large bowel.[103]

In one experiment that compared the effects of three doses of inulin, a dose–effect relationship was reported, showing an increase in cecal wall weight and a reduction in cecal pH.[109] In the same experiment, cecal blood flow was also increased by inulin treatment and the effect was dose dependent. In germ-free rats, inulin did not affect the weight of the cecum, the cecal pH, the total amount, or the concentration of acetate, the only SCFA identified (Figure 6.5).[121]

Similarly, in the large intestine of neonatal pigs, the fermentation of oligofructose increased the height and leading edge of the mucosa crypt, as well as the epithelial cell density and proliferation, thereby preventing colonic epithelial atrophy observed in piglets fed an elemental diet.[123]

In heteroxenic rats harboring a human fecal flora and fed with an inulin diet as compared to a standard diet, jejunal as well as cecocolonic epithelia had higher villi, deeper crypts, more goblet cells per crypt, and an increased mucin layer. This effect

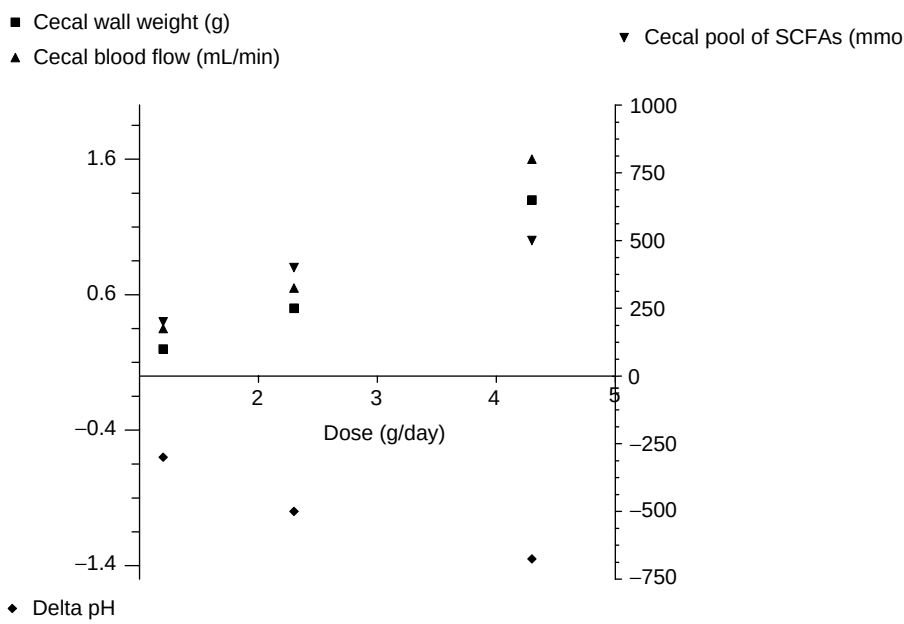

FIGURE 6.5 Dose effect of inulin on rat cecal wall weight, cecal blood flow, cecal pool of SCFAs, and cecal pH. (Adapted from Levrat, A. M., Rémésy, C., Demigné, C., High propionic acid fermentation, and mineral accumulation in the cecum of rats adapted to different levels of inulin, *J. Nutr.*, 121, 1730–1737, 1991.)

was not seen in germ-free rats. However, the authors concluded that it shows an indirect effect of inulin and its fermentation by the intestinal bacteria.[124]

In the same experimental model, rats fed an inulin diet, as compared to a standard or a sucrose containing diet, the cecum content as well as the cecocolonic epithelia contained higher amounts of sulphomucins and lower amounts of sialomucins.[114,124] This effect is interesting to consider because sulphomucins are, in general, associated with a higher level of protection, and their proportion is decreased in several intestinal diseases such as inflammation or certain forms of cancer. These effects may explain the stimulation of mucosa repair in colitis in rats given oligofructose.[125] In these rats, treatment with oligofructose (1 g/d) inhibited the anorexia and weight loss associated with the onset of inflammation and reduced the extent of epithelial damage, assessed by macroscopic examination and measurement of myeloperoxidase activity (specific enzyme marker of polymorphonuclear neutrophil primary granules). Finally, in conventional rats, Delzenne et al. reported that oligofructose feeding (10% in diet) doubled the concentration of putrescine in the cecal content and increased the concentration of putrescine, spermine, and spermidine in the cecal tissue.[126] In line with that last effect, Rémésy et al. had previously reported a rise in the crypt column height and in the activity of ornithine decarboxylase, a key enzyme in polyamine synthesis.[127] Such changes in polyamines in cecal mucosa might correlate with the hyperplasia of the mucosa. Similar effects on cecal polyamines have been reported in rats fed with guar gum and pectin. In comparing germ-free vs. conventional rats, these authors demonstrated that such an increase in polyamines originates in colonic bacteria.[128]

6.2.4.5 Effects of Inulin and Oligofructose on Bowel Habit

As a consequence of rapid and quantitative fermentation in the large bowel that leads to an increase in bacterial biomass, inulin and oligofructose affect bowel habit. In rats, the weight of feces is increased, on an average, 1.4 times after feeding a diet containing inulin or oligofructose (Table 6.4). A similar increase (1.3 and 1.6 times) in fecal output has also been shown in Beagle dogs fed a diet containing oligofructose (4%) or inulin (8%).[129]

In humans, the stool-bulking index of inulin (about 2 g of stool weight increase per g of ingested substrate) and oligofructose (1.2–1.5 g/g) is close to that of other fermentable dietary fiber components, such as pectins (1.3 g/g) or guar gum (1.5 g/g) (for a review, see Cummings;[18] Cherbut;[125] Nyman[130]).

As expected, the effect on stool weight depends on the dose and on the amount of other fibers in the diet.[131,132] In a controlled (including food composition) study, Gibson et al. have reported an increase in stool weight in humans after consuming inulin and oligofructose.[133] In postmenopausal women, oligofructose (2 ¥ 5 g/d) significantly increased both wet and dry fecal weights (+42 and +30%, respectively) corresponding to an increase of ±3.5 g/g of oligofructose ingested.[134]

In addition, inulin and oligofructose intake has constantly been reported to increase fecal water content, another factor that contributes to increased stool

TABLE 6.4
Summary of Data Demonstrating the Stool-Bulking Effect of Inulin and Oligofructose in Rats

n[1]	Treatment	Increase in Fecal Output	References
5	Oligofructose 10%		
	12 d	1.40x	137
	17 d	1.25x	
	27 d	1.25x	
5	8 d		
	18 d	1.30x	
	24 d	1.25x	
	29 d	1.40x	
15	Oligofructose 20%	1.45x	
	17 d		
		1.65x	
6	Oligofructose 10%		
	42 d	1.40x	138
	Oligofructose 20%		
	42 d	2.15x	
10	Oligofructose 10%		
	50 d	1.30x	139
	Inulin 10%		
	50 d	1.40x	
6	Oligofructose 5%		
	7 d	1.20x	140
	Inulin 5%		
	7 d	1.15x	
	Oligofructose/Inulin (50/50) 5%		
	7 d	1.20x	
MEAN		1.50x	

[1]n is the number of rats/group.

weight.[131,132,134–136] Inulin has also been shown to stimulate bowel movements and to normalize stool frequency, especially in slightly constipated subjects.[133,135]

6.2.5 Conclusion

Inulin and oligofructose are plant carbohydrates resisting digestion in the upper gastrointestinal tract and fermenting in the colon. By increasing fecal biomass and water content of stools, they improve bowel habits. Likely due to their specific fermentative properties, they affect several functions involved in colonic mucosa protection and repair, which may contribute to reduce the risk of intestinal diseases. For all these reasons, inulin and oligofructose should be undoubtedly part of the dietary fiber complex. In addition, they do have characteristic features

different from other fibers. Therefore, they may contribute in a significant way to a well-balanced diet by increasing the fiber content, by improving the diversity of the fiber sources, and by their specific effects on several gastrointestinal functions.

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7 Inulin and Oligofructose as Low-Calorie Carbohydrates

7.1 INTRODUCTION

From a nutrition perspective, the energy content of a food is an important parameter. It is of particular interest to consumers who are aware that their lifestyles make them prone to diseases like obesity and diabetes. They may have been advised to reduce energy intakes, especially by purchasing food products labeled as "reduced in calorie," "low in calorie," or "light." It is necessary for manufactures to have the correct energy values for the different food components in such labeled food products. In many countries, information on the energy provided by a particular food product is required, as is the information on the composition of the various nutrients it provides. Such information is mandatory on prepackaged food products in the U.S., but in the European Union (EU) only certain nutrition claims require this labeling.

The energy content of a food product equals the amount (in grams) of each component or group of components (i.e., carbohydrates, protein, fat, dietary fiber, and polyols, etc.) multiplied by their respective energy conversion factors (that are eventually specified in laws or directives) and totalling these amounts. These conversion factors are expressed in kilocalories per gram (kcal/g) or kilojoules per gram (kJ/g), 1 kcal being equivalent to 4.184 kJ.[1]

In the Western countries (principally Australia, New Zealand, Canada, the EU, the U.S., and Switzerland), the standard for energy conversion for carbohydrates is the Atwater factor, i.e., 4 kcal/g or 17 kJ/g.[2] The recently revised value is 3.75 kcal/g or 15.7 kJ/g.[3] For the carbohydrates that resist absorption and digestion in the upper gastrointestinal tract but are completely or partly fermented in the intestine (i.e., the dietary fibers and the nondigestible oligosaccharides; see Chapter 6, Section 6.1.2), no universally accepted energy value exists. The proposed conversion factors are 0, 2.4, or even 4 kcal/g (0, 10, or 17 kJ/g), depending on the degree of their colonic fermentation.[4] Modern research technology and methods have revealed newer dietary fibers and nondigestible oligosaccharides and also precise energy conversion factor values.[5] Nevertheless, it must be kept in mind that various methods may yield results that differ due to natural variation and experimental error. It is the aim of the present chapter to review the data available and to propose a specific energy conversion factor for inulin-type fructans.

7.2 METHODOLOGIES TO ASSESS ENERGY VALUE OF INULIN-TYPE FRUCTANS

In food science, to characterize the energy content of a food component or ingredient, different terms can be used.[6] For the purpose of the present discussion that focuses on the energy value of a nondigestible carbohydrate (see Chapter 4, Section 4.4 and Table 7.1), these terms are gross energy (H_c) and net energy (NE):

where

$$NE = H_c \times f. \quad (7.1)$$

In Equation (7.1), "f" is the "fractional availability of energy" from the portion of the nondigestible carbohydrate that is fermented. In practice, f equals the amount (number of mols) of adenosine triphosphate (ATP) found in the body from the metabolism of the end products (essentially the short-chain fatty acids [SCFAs]) of fermentation of 1 mol of the nondigestible carbohydrate, whereas compared with 1 mol of glucose (or eventually fructose as in the case of inulin-type fructans) is absorbed in the small intestine and fully oxidized in cellular metabolism inside the body.

$$f = (1 - a - b - c) \times g \quad (7.2)$$

In Equation (7.2):

a = fractional efficiency of conversion to fecal energy
b = fractional heat of fermentation
c = fractional efficiency of gas production
g = fractional efficiency of SCFAs used to produce ATP

In the case of nondigestible oligosaccharides, and according to Livesey,[7] the values that can be recommended for these factors are 0.2, 0.05, 0.05, and 0.85, respectively. The aim of the following discussion is to adapt these factors to inulin-type fructans.

To establish precise values for these factors and to assess the caloric value of inulin-type fructans, the following questions need to be answered:

TABLE 7.1
Definition of Energy Terms[5,6]

Gross energy or heat of combustion H_c	Heat energy released by complete combustion of the nondigestible carbohydrate, i.e., heat of combustion (17.2 kJ/g)
Net energy NE	Gross energy corrected for fractional availability of energy from the portion of the nondigestible carbohydrate that is fermented (f)

1. What is the percentage of the ingested dose (and thus the energy intake) that is absorbed or hydrolyzed in the gastrointestinal tract?
2. What percentage of the ingested dose is fermented by the intestinal microflora?*
3. What is the efficiency of microbial biomass production, and how much of the C atoms and energy in inulin-type fructans are lost during the fermentation in the intestine?
4. What is the ATP yield (number of mols of ATP produced) of the metabolism of the fermentation end products by the host?

Answering the last two questions is, by far, the most difficult part. The fermentation process is complex and likely to be dependent on the composition of the intestinal microflora which varies among individuals. One approach is the "theoretical" approach.[8] It is based on the hypothesis that inulin-type fructans are specifically metabolized by bifidobacteria (see Chapter 9 for more discussion of that hypothesis) that are known to have a unique carbohydrate metabolic pathway. Bifidobacteria lack both aldolase (EC 4.1.2.13) and glucose-6-phosphate $NADP^+$ oxidoreductase (EC 1.1.1) but, instead, use phosphoketolases (EC 4.1.2.22 and 4.1.2.9) in the so-called "bifid shunt" (see Chapter 5, Section 5.3.3.2 and Figure 5.4).[9] The theoretical approach, then, uses the following steps and subsequent calculations (Figure 7.1):

1. Calculate the value, Equation (7.3), for fructose (and thus inulin-type fructans) oxidation by bifidobacteria according to the stoichiometry of the bifidus pathway.[10]
2. Calculate the value, Equation (7.4), for the fermentation of fructose (and thus inulin-type fructans) by the mixed colonic microflora, by reference to the metabolic pathways that further oxidize the end products of the metabolism of this carbohydrate by bifidobacteria (especially lactate and pyruvate)[11,12] (see Chapter 5, Section 5.3.3 and Section 5.3.4).
3. Based on Equation (7.4), calculate the percentage of the C atoms from the fermented fructose that are recovered in SCFAs, lactate, and CO_2.
4. Calculate the percentage of C atoms produced by fructose fermentation and metabolism of end products that are used for bacterial growth.
5. By combining steps 3 and 4, calculate the overall balance of fructose fermentation by the colonic microflora in terms of SCFAs, lactate, CO_2, and bacterial biomass.
6. Based on published information, estimate the percentage of SCFAs and lactate that are absorbed and reach the host's metabolic pools.

* In the present discussion, the expression "fermentation by intestinal microflora" is used to take into account the fact that the small intestine, even in humans but certainly in most pets and domestic animals, is not germ-free and thus may, along with the large bowel, contribute to fermentation of nondigestible food components.

7. Based on metabolic charts including both catabolic and anabolic pathways for the major SCFAs (i.e., acetate, propionate, and butyrate), calculate the yield of ATP.
8. Summing up of all the information, calculate the energy value of fermented fructose (and thus inulin-type fructans).

A similar approach has been taken by an expert group of FASEB/LSRO[13] for evaluating the energy content of polyol. These experts have applied the factorial method which uses the following formula:

$$NE = (A - B) ¥ (1 - C) ¥ (1 - D) ¥ E \quad (7.3)$$

In this equation:

- A = the energy (kcal or kJ) present in the intestine as fermentable substrate
- B = the energy excreted in feces
- C = the proportion of C atoms going into bacterial biomass
- D = the loss of C atoms and energy due to fermentation
- E = the efficiency of utilization of the fermentation end products by the host compared to glucose

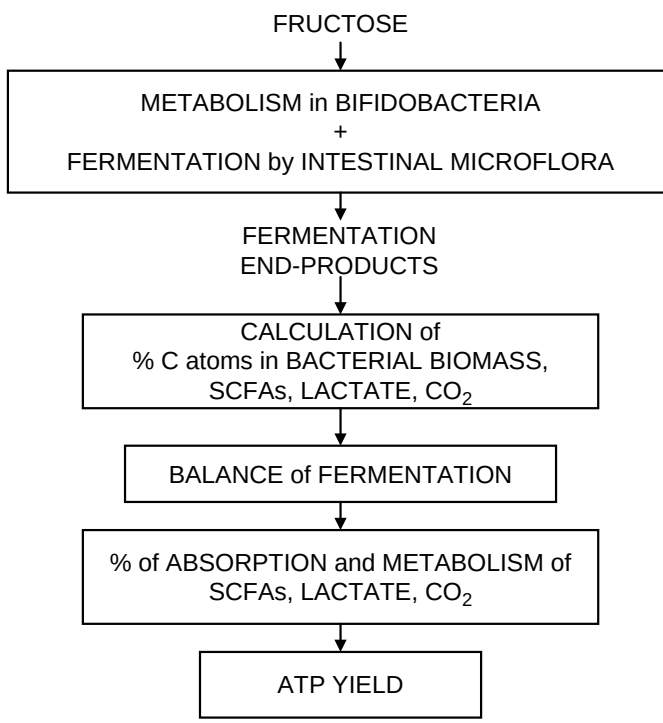

FIGURE 7.1 Theoretical approach to assess the energy content of inulin-type fructans.

7.3 ASSESSMENT OF ENERGY VALUE OF INULIN AND OLIGOFRUCTOSE: RESULTS AND DISCUSSION

As extensively reviewed and discussed above (Chapter 4, Section 4.3) and (Chapter 5, Section 5.4), inulin-type fructans are resistant to absorption and hydrolysis in the stomach and the small intestine; they are completely fermented by the intestinal microflora (mostly in the large bowel) and are not excreted in feces. Only an insignificant proportion (0.12%/24 h) has been recovered in urine of human volunteers fed a low molecular weight oligofructose.[14] Inulin-type fructans are thus not absorbed nor digested by the acid of the stomach or the mammalian digestive enzymes, but they are completely fermented by the intestinal bacteria. In the discussion above and in the proposed approaches to assess the caloric value of inulin-type fructans, the percentage of the ingested dose that is fermented by the intestinal microflora equals 100%. The key questions concern:

1. The stoichiometry of the metabolism by bifidobacteria
2. The stoichiometry of the fermentation by the intestinal microflora
3. The efficiency of microbial biomass production
4. The ATP yields of the metabolism of the fermentation end products by the host

7.3.1 Stoichiometry of Metabolism by Bifidobacteria

By reference to the stoichiometry of the bifidus pathway,[10] the equation of the metabolism of the hexose (essentially fructose) units of inulin-type fructans by bifidobacteria is Equation (7.4):

$$6\,C_6H_{12}O_6 + 17\,ADP + 17\,P_i \rightarrow 17\,ATP + 4\,HCOO^- + 11\,CH_3COO^- + 2\,CH_3CHOHCOO^- + 2\,CH_3CH_2OH \quad (7.4)$$

7.3.2 Stoichiometry of Fermentation by Intestinal Microflora

In the complex intestinal microflora, bifidobacteria is far from being the only microorganism. Some of the metabolites they produce (especially lactate and pyruvate) are thus further oxidized, as shown in their principal microbial, metabolic pathways.[11,12]

$$2\,CH_3COCOO^- + 2\,NADH + 2\,H^+ \rightarrow 2\,CH_3CHOHCOO^- + 2\,NAD^+ \quad (7.5)$$

$$2\,CH_3COCOO^- + 4\,NAD(P)H + 4\,H^+ + 2\,ATP \rightarrow 2\,CH_3CH_2COO^- + 4\,NAD(P)^+ + 2\,ADP + 2\,P_i + 2\,H_2O \quad (7.6)$$

$$2\,CH_3COCOO^- + ADP + P_i + H_2O\ CH_3\,CH_2\,CH_2COO^- + ATP + 2CO_2 \quad (7.7)$$

The overall equation, Equation (7.8), for the fermentation of fructose units by the mixed intestinal microflora is thus:

$$6\,C_6H_{12}O_6 + 14\,ADP + 14\,P_i \rightarrow 14\,ATP + 9\,CH_3COO^- + 2\,CH_3CHOHCOO^- + 2\,CH_3CH_2COO^- + CH_3CH_2CH_2COO^- + 2\,CO_2 + 2\,H_2O + 14\,H^+ \quad (7.8)$$

Based on Equation (7.7), it can be calculated that from the C atoms of a fructose unit in inulin-type fructans:

- 78% are recovered in the SCFAs, essentially acetate (64%), propionate (21%), and butyrate (15%)
- 15% are recovered in lactate
- 5% are recovered in ethanol, formate, and CO_2

These figures compare reasonably well with those obtained using the generalized colonic fermentation equation derived by different authors, the major difference being the relatively high proportion of acetate (Table 7.2).[12,15–21]

7.3.3 Efficiency of Microbial Biomass Production

However, the above analysis does not account for the fact that the fermentation of hexose also provides bacteria with maintenance energy as well as ATP and substrates (essentially acetyl-S-CoA and to a lesser extent succinic acid)for biosynthetic path-

TABLE 7.2
Comparative Stoichiometry of SCFAs Production by Anaerobic Bacteria Fermenting 1 mol of a Hexose Monomer in an Oligosaccharide as Reported in the Scientific Literature

Acetate	Propionate	Butyrate	C-atoms[1] as SCFAs (%)	References
1.39 (75)[2]	0.32 (17)	0.14 (7.5)	72	12
0.91 (65)	0.26 (19)	0.23 (16)	59	16
1.07 (62)	0.38 (22)	0.28 (16)	73	15
1.03 (60)	0.41 (24)	0.28 (16)	74	18
1.32 (74)	0.32 (18)	0.14 (18)	70	20
1.13 (65)	0.35 (20)	0.26 (15)	72	17
1.35 (66)	0.40 (20)	0.30 (14)	85	19
1.50 (76)	0.33 (17)	0.15 (7)	78	8
Mean 1.21	0.35	0.22	73	

[1]C-atoms of hexose monomers recovered (%) as SCFAs.
[2]Numbers in () is the relative percent.

ways necessary for growth and proliferation.[10,17,20,21] According to De Vries and Stouthamer,[10] Baldwin,[22] Soergel,[17] and Cummings,[23] the fermentation of 1 mol of hexose supports the production of 37 g, 25–31 g, 15–60 g, or 40–57.5 g of bacterial biomass (dry weight), respectively (Figure 7.2).

In vitro experiments have shown that the fermentation of oligofructose by human fecal slurries yields 40% C atoms as SCFAs, 15% as lactate and 5% as CO_2, thus leaving some 40% for bacterial (mainly bifidobacteria) growth.[24] A similar figure has been reported by Baldwin,[22] Payne,[25] Isaacson et al.,[26] and Cummings et al.,[27] for the growth of mixed populations of bacteria fermenting a hexose substrate.

In vivo experiments with rats fed a diet containing 10–20% oligofructose have shown that the increased fecal excretion (dry weight) during a 24-h period corresponded to 41–47% of the C atoms ingested as oligofructose being incorporated into new bacteria.[8] By applying similar calculations on data reported by Nakae et al.[28] and Nyman et al.[29] who fed rats mixtures of dietary fibers, the C atoms recovered in fecal bacterial biomass account for 45–55% and 39–54%, respectively.

Taken together, this information indicates that, in terms of C atoms, the intestinal fermentation of 1 mol fructosyl equivalent from inulin-type fructans produces:

- 40% SCFAs
- 15% lactate
- 5% CO_2
- 40% bacterial biomass (mainly bifidobacteria)

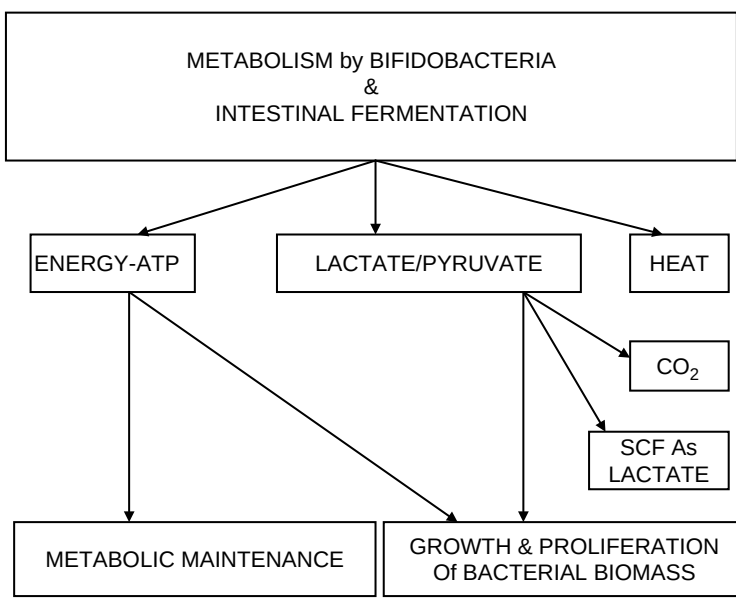

FIGURE 7.2 Energy and C-atoms balance chart of fermentation of fructose by intestinal microflora.

The 40% figure for the yield of SCFAs is similar to that reported by Cummings et al.[27] who have quantitatively calculated that the fermentation of 10 g nonstarch polysaccharides in the human hind gut is likely to produce some 75 mmol of SCFAs which, based on the recovery of C atoms, corresponds to a yield of approximately 45%. Such a figure falls within the extreme values proposed by Baldwin,[22] and Bernier and Pascal,[30] and it is close to the values proposed by Bär.[19] Moreover, assuming that 40–45% of the C atoms of 1 mol of fermented fructose unit in inulin-type fructans are used to build up bacterial biomass is compatible with the ATP cost of the process (0.1–0.2 mol ATP/2–6 g bacterial biomass).[31] In molar terms, this means that the fermentation of 1 mol fructose unit in inulin-type fructans gives about 1 mol SCFAs and about 0.3 mol of lactate. By reference to Table 6.3, the relative distribution of the SCFAs is 58% or 0.58 mol acetate, 20% or 0.2 mol propionate, and 22% or 0.22 mol butyrate.

7.3.4 ATP Yield of the Metabolism of the Fermentation End Products by the Host

Both CO_2 and bacterial biomass are excreted. They do not participate in the cellular metabolism of the host, and they are calorie free. This is certainly not the case for SCFAs and lactate that are absorbed and used by host's cells to produce energy and serve as anabolic substrates. The questions that remain to be solved are thus:

1. What proportion of these molecules is absorbed and how much is excreted?
2. What is the efficiency in terms of ATP production and utilization of their cellular metabolism as compared to fructose?

7.3.4.1 Absorption and Excretion of SCFAs and Lactate

The intestinal absorption of SCFAs is a very efficient process that takes place mostly in the cecum and the colon.[30–33] Only 5–10% are excreted in feces.[31,33] Of 90–95% of SCFAs absorbed, majority of butyrate is used by colonocytes for maintenance energy;[34,35] the totality of propionate is metabolized in the liver whereas acetate serves as metabolic substrate both for the liver (50–70%) and peripheral tissues (mainly muscles).[36,37]

Lactic acid is also likely to be absorbed. Moreover, many different intestinal bacteria metabolize it. It has been estimated that some 50% of L-lactate (the only isomer produced by bifidobacteria) will reach the systemic circulation.[8]

7.3.4.2 Cellular Metabolism of SCFAs and Lactate and ATP Yield

SCFAs are metabolized at three major sites in the host body.

1. Cells of the ceco-colonic epithelium that use butyrate as a major substrate for maintenance-energy producing pathways
2. Liver cells that fully metabolize residual butyrate and propionate via gluconeogenesis and 50–70% of acetate
3. Muscle cells that generate energy from the oxidation of the residual acetate

Even though little information is available on the metabolism of L-lactate originating from intestinal carbohydrate fermentation, it is likely that it serves as substrate both for gluconeogenesis and lipogenesis; part is also excreted in urine.

The host cells' metabolic pathways that use SCFAs and lactate originating from intestinal carbohydrate fermentation are yet not fully understood. Nevertheless, the relative efficiency of utilization of their energy content compared to glucose or fructose can be estimated, on a theoretical basis, by using literature information.[38] A theoretical approach has been published and is summarized by Roberfroid et al.[8]

Figure 7.3 shows an average ATP yield of 14 mol from the cellular metabolism of a typical mixture of 0.9–0.95 mol SCFAs and 0.075 mol L-lactate resulting from the fermentation of 1 mol of inulin-type fructan followed by its absorption through the intestinal wall. As the ATP yield from the complete metabolic oxidation of glucose or fructose is 38 mol ATP,[38] the net energy content (see Equation 7.1) of inulin-type fructan is 14/38 ¥ 100 = 36.8% that of glucose or fructose, i.e.,

$$3.75 \text{ ¥ } 0.368 = 1.4 \text{ kcal / g or } 5.8 \text{ kJ / g}$$

Similar figures have been reported for the ATP yield of SCFAs resulting from the intestinal fermentation of polyols.[19]

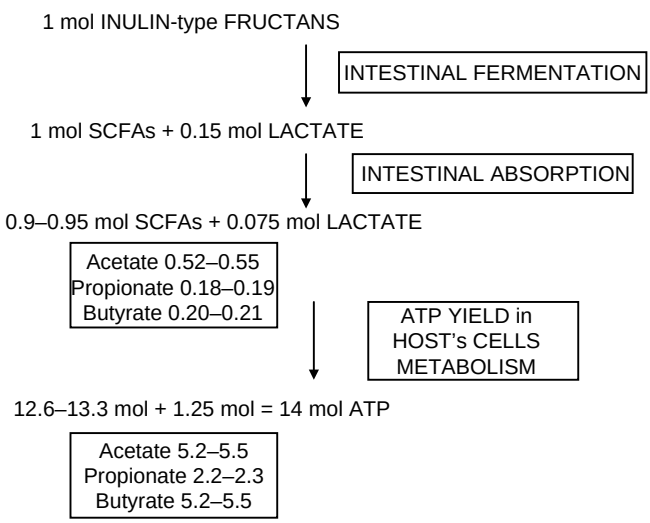

FIGURE 7.3 Theoretical calculation of ATP yield in the host cells' metabolism of SCFAs and lactate, originating from intestinal fermentation of inulin-type fructans.

7.4 INULIN AND OLIGOFRUCTOSE AS LOW-CALORIE CARBOHYDRATES: CONCLUSION

Being fermented in the intestine, especially the large bowel, rather than being absorbed in the small intestine, a fructose unit of inulin-type fructan has a net energy content that is only 37% that of a digested fructose. This corresponds to an energy content of 1.5 kcal/g or 6 kJ/g as compared to 3.75 kcal/g or 15.7 kJ/g.

Applying the Equation (7.3) and using, for each factor, the value given in the FASEB/LSRO factorial method (see Section 7.2, Equation (7.3)) similar energy content is obtained:[13]

$$\begin{aligned} &NE = (A - B)\ (1 - C)\ (1 - D) \times E \\ &\text{where } A = 3.75 \text{ kcal / g or } 15.7 \text{ kJ / g; } B = 0; \ C = 0.15 - 21; \\ &D = 0.25 - 30 \text{ and } E = 0.70. \\ &\text{Thus,} \\ &NE = 3.75\ (15.7) \times [1 - (0.15 \text{ or } 0.21)] \times [1 - (0.25 \text{ or } 0.3)] \times 0.7 \\ &NE_{\text{INULIN/OLIGOFRUCTOSE}} = 1.45 - 1.65 \text{ kcal / g or } 6.1 - 7 \text{ kJ / g} \end{aligned} \qquad (7.3)$$

Using [U-^{14}C]-labeled synthetic oligofructose and a radiochemical balance study in humans, Hosoya et al. have established a caloric value of 1.5 kcal/g or 6.3 kJ/g.[39]

In addition to the uncertainty factors discussed above and used in these energy calculations to account for the efficiency of the fermentation process and the metabolism of the fermentation end products by host cells, it must be kept in mind that inulin and oligofructose may have additional gastrointestinal as well as systemic effects that may influence the energy balance of the host. In the context of this discussion, it is worth mentioning that inulin-type fructans may inhibit hepatic lipogenesis (see Chapter 11) and, as shown in rats, may induce a reduction in body fat deposition.[40,41] Like other dietary fibers that cross the small intestine without being digested, inulin-type fructans may affect the small intestinal transit time of other nutrients.[42,43] Preliminary data in mice have indeed shown that inulin and oligofructose influence some of the absorptive functions (lower rates of glucose transport and absorption of leucine, proline, and glycyl-sarcosine) of the small intestine.[44] Such an effect, if at all present, might well result in an increased transfer of these nutrients in the large bowel, thus reducing the energy value of the whole diet or some of its macroconstituents. Moreover, these food components may interfere with the digestion of proteins and fats.[45] The value given above for the net energy content of inulin-type fructans is thus acceptable and likely to be at the upper limit. That value is well in line with the recommendation that for the purpose of food labeling, all carbohydrates, which are more or less completely fermented in human colon, be given an energy value of 1.5 kcal/g or 6.3 kJ/g.[45] It should be kept in mind that the daily intake of energy as in these carbohydrates (especially nondigestible oligosaccharides) is likely to remain relatively small, less than 10% and

probably often not more than 5% of total daily energy intake.[46] Thus, and from a scientific point of view, it is not essential, and probably even not possible, to give, for each such carbohydrate, a precise energy value.[4] Discussing methodologies as well as reported energy values for food ingredients, Livesey et al.[5] have challenged the 1.5 kcal/g or 6.3 kJ/g value for inulin and oligofructose. These authors proposed that all carbohydrates that undergo fermentation be given a universal energy value of 2 kcal/g or 8.4 kJ/g. However, because of the arguments that have been given, the 0.5 kcal/g or 1.9 kJ/g difference is nutritionally insignificant.

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8 Inulin-Type Fructans and Gastrointestinal Functions: Conclusions and Perspectives

As discussed in Chapter 2, the gastrointestinal tract has a large number of important physiological functions. With regard to these functions, inulin-type fructans are probably relatively unique, mostly because of their chemical nature and the combination of their effects.

Chemically they are not polysaccharides but mainly oligosaccharides. Moreover, they are not composed only of very short-chain oligosaccharides but, rather, contain short-, medium-, and long-chain compounds with DP ranging from 2 to 60–65 and DP_{av} from 4 to 20. In the Synergy®-type products, two distinct populations of low- and high-molecular-weight compounds are mixed, thus creating a unique type of oligosaccharides. Being oligo- not polysaccharides, the inulin-type fructans affect gastrointestinal functions not because of their physicochemical properties but rather because of their biochemical and physiological attributes.

Because of the b configuration of the osidic linkages inside the fructooligosaccharides chain, all inulin-type fructans resist hydrolysis in the upper part of the gastrointestinal tract. Moreover, and with the exception of the very small-molecular-weight components (DP 2 or eventually DP 3) that are found primarily in the oligofructose produced by enzymatic synthesis (see Chapter 3, Section 3.3.1), they are not absorbed. Thus, and even though they are mainly composed of fructose, they have no effect on fructosemia, the concentration of fructose in the blood. Indeed no fructose is available for absorption, provided the product does not contain residual free fructose or sucrose that can be hydrolyzed by intestinal sucrase. That issue is relevant as it is important for individuals who are genetically unable to handle fructose because of an enzyme deficiency and who are forced to eat a fructose-free diet. Even if very low-molecular-weight compounds are absorbed, they will not influence fructosemia because no fructosidase exists inside the human body and because these short chains are excreted in the urines.

During their passage through the upper part of the gastrointestinal tract, the inulin-type fructans may well influence transit time as well as digestion and absorption of different macronutrients and micronutrients. However, data are too scarce to draw conclusions, and that question needs further research. At least, and as shown in ileostomy volunteers, they do appear to influence neither mineral absorption nor protein digestion in the small intestine. Of special interest and still unanswered is

the question of their eventual effects on the rise in glycemia and or insulinemia that follow the ingestion of digestible carbohydrates like starches or sucrose. Once again, if such effects exists, the mechanism will not be physicochemical in nature (as with classical dietary fiber) but, rather, it will involve biochemical or physiological processes.

Thus, inulin-type fructans resist digestion in the upper gastrointestinal tract, and they reach the colon intact as they are eaten. They are classified as "colonic foods" or foods that feed the large bowel and the microflora it contains.[1] In the cecum/colon, and because of the population of bacteria colonizing it, inulin-type fructans are hydrolyzed, most likely inside the bacterial cells and primarily inside the bifidobacteria, and rapidly ferment to produce short-chain fatty acids, lactate, and gases. The final pattern of production of these molecules is the result of metabolic interactions between different genera of bacteria in complex metabolic processes that are not fully identified. The major site of hydrolysis and fermentation is likely to be the proximal colon where the major populations of saccharolytic microflora are located. Because of the presence, in inulin-type fructans, of molecules with different chain lengths, it is very possible that fermentation continues in the transverse and, eventually, in the distal segments. It has thus been hypothesized that the compounds with the longest chains are fermented all along the full length of the large bowel, thus supporting growth and proliferation of saccharolytic bacteria (especially bifidobacteria, see Chapter 9) in the distal segment where, usually, proteolytic microorganisms dominate. Furthermore, using a mixture of short and long chains of fructooligosaccharides as in the industrial product oligofructose-enriched inulin Synergy 1 might favor such a progressive fermentation pattern. Only preliminary data in a three-chamber *in vitro* fermentation system inoculated with human fecal slurries (Gibson, personal communication) give some support to that hypothesis. The question of the colonic sites of fermentation of the low- vs. medium- and high-molecular-weight fructans in inulin remain largely unanswered but it is an important question, especially in relation to the mechanism of some of the effects that will be reviewed in upcoming chapters.

Being nondigestible but highly fermentable, inulin-type fructans are dietary fiber. Indeed, they fit with the definition adopted in this book (see Chapter 6, Section 6.1.2):

> *Carbohydrates (oligo- and polysaccharides) that resist both hydrolysis by mammal digestive enzymes and absorption in the small intestine but are, at least partly, hydrolyzed and fermented by the colonic microflora.*

The five basic attributes of a dietary fiber are:

- Components of edible plant cell
- Carbohydrates (both oligosaccharides and polysaccharides)
- Resistance to hydrolysis by human (mammal) alimentary enzymes
- Resistance to absorption in the small intestine
- Hydrolysis and fermentation (partial or total) by the bacteria in the large bowel

Additionally, inulin and oligofructose:

- Are part of edible plants (see Chapter 3, Section 3.2.3)
- Are carbohydrates that are composed of a mixture of either oligosaccharides, or oligosaccharides and polysaccharides (see Chapter 3, Section 3.3.1 and Section 3.3.2)
- Resist hydrolysis by human digestive enzymes (see Chapter 4, Section 4.3.1.5)
- Do not appear to be significantly absorbed in the small intestine
- Are hydrolyzed and completely fermented by the colonic microflora and are oxidized to produce gases and short chain fatty acids (see Chapter 5, Section 5.4).

They are classified as dietary fiber and must be labeled as such on consumer food products. Moreover, AOAC International has validated and adopted the "fructan method" (method number 997.08) that specifies the accurate quantitative determination of inulin and oligofructose in foods.[2] This method can be combined with the standard AOAC total dietary fiber method to quantify the total amount of fiber used for food labeling

Being fermented in the large bowel, inulin-type fructans influence colonic functions. They improve stool production, both quantitatively and qualitatively. Quantitatively, they increase stool weight with a bulking index of 1–2 g of additional stool per gram of ingested inulin which is equivalent to the bulking index of other fermentable dietary fibers. Qualitatively, and perhaps more importantly, they improve stool frequency and stool consistency, especially in constipated individuals. Once again, the mechanism of these effects is not physicochemical in nature but rather due to fermentation and subsequent stimulation of growth and proliferation of intestinal bacteria.

Fermentation also produces SCFAs that are efficiently absorbed and reach the systemic circulation where they may exert miscellaneous metabolic regulations. SCFAs are thus good candidates to explain some of the systemic effects of inulin-type fructans that will be reviewed in the upcoming chapters. Of particular interest is the observation that, as a result of the fermentation of inulin-type fructans, the pattern of SCFAs production is significantly modified in favor of butyric acid, probably the most physiologically active short-chain fatty acid.

Fermentation of inulin-type fructans in the large bowel also induces changes in colonic epithelium stimulating proliferation in the crypts, increasing the concentration of polyamines and changing the profile of mucins. Such observations in experimental models need further investigations and might be key to understanding the mechanism of the physiological effects of inulin-type fructans. Additionally, and as discussed later, modulation of endocrine as well as immune functions in the colonic epithelium might be other important topics. Moreover, and up to now, interest has focused only on colonic fermentation, but even if the number of bacteria in the small intestine is certainly much lower than in the large bowel, it cannot be excluded that part of the dose of oligofructose or even inulin is already metabolized by small intestinal bacteria, thus influencing small intestinal physiology. Furthermore, other

mechanisms like binding to receptors at the surface of small intestinal epithelial cells have not yet been investigated but might well exist as it has been reported for mannooligosaccharides.

From a nutrition labeling perspective, inulin-type fructans are not only dietary fiber, they are also low-calorie carbohydrates. That is because they are nondigested and fermented. The energy content has been evaluated, and it is of the order of 1.5 kcal/g or 5.6 kJ/g. Due to low daily intake (<20 g/d) that question does not deserve further investigation or discussion. That value is perfectly in line with recommended value for all nondigestible carbohydrates.

In conclusion of this discussion of gastrointestinal functions, data demonstrate that inulin-type fructans are natural food ingredients that may significantly contribute to a well-balanced diet by increasing fiber content, by improving the diversity of the fiber sources, and by their specific effects on several gastrointestinal as well as systemic functions. Indeed, and as stated by Cummings, a gastroenterologist who revisited the physiological role of the colon:[3] "Anaerobic fermentation dominates large bowel function. It affects every process including salt and water absorption, pH, epithelial cell metabolism, motility and bowel habit and colonization resistance, in addition to providing products which are absorbed and reach the liver and peripheral tissues." (See Chapter 2, Section 2.2.1.4.)

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* In Chapter 8, the list of references is limited, but the reader is invited to review the reference lists of Chapter 3 through Chapter 7 for a more extensive bibliography.

9 Inulin-Type Fructans and the Modulation of the Intestinal Microflora: The Prebiotic Effect

9.1 INTRODUCTION

9.1.1 Concept of Colonic Health

Among the physiological functions that will benefit most from the development of functional foods and for which enhanced function claims are substantiated, the gastrointestinal functions are certainly in the forefront. A very promising area of study is modulation of the activity of the colon, which is increasingly recognized as playing an essential role in maintaining health and well-being, as well as in reducing the risk of diseases.[1,2] Disturbances of the colon's functions may lead to dysfunction not only in the intestine but also in the whole body. The classical view that the human colon is an organ that absorbs salt and water, and provides a mechanism for the orderly disposal of the waste products of digestion is no longer appropriate. Indeed, the colon has a major role in digestion (as achieved by microbial fermentation) through the salvage of energy, and possibly nitrogen, from carbohydrate and protein not digested in the upper intestine. It also plays important roles in:[1,3–5]

- Absorption of minerals and vitamins
- Production and absorption of fermentation end products like the short-chain fatty acids and lactate
- Protection of the body against translocation of bacteria
- Protection of the body against the *in situ* proliferation of pathogens
- Endocrine functions (via the gastrointestinal peptides)
- Regulation of intestinal epithelial cell growth and proliferation
- Immune function

The concept of *colonic health* has thus emerged and is becoming a major target for functional food development in the area of enhanced function claims.[6] Besides, its important physiological and immunological functions, the colon is subject to miscellaneous diseases from acute infections (diarrhea or constipation) to chronic diseases such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), or cancer.[1] Thus, through the modulation of the colonic functions, functional foods can potentially reduce the risk of some diseases.

9.1.2 Concept of Balanced Colonic Microflora

The microflora that symbiotically colonizes the large bowel is key to keeping the colon (and thus the whole body) healthy. A major determinant in that role is the composition of the microflora that:

- Is largely determined by the flora that establishes at and immediately after birth
- Can be modulated by specific compounds in the diet
- May change during a life time, becoming more and more complex as we age[7]

To support health and well-being, and to reduce the risk of various diseases, it has been hypothesized that the gut microflora must remain a balanced microflora. That concept implies that the intestinal (especially the colon) microflora must be composed predominantly (in numbers) of bacteria recognized as potentially health-promoting (like lactobacilli, bifidobacteria, and fusobacteria), to prevent, impair, or control the proliferation of the potentially pathogenic and harmful microorganisms (like *Escherichia coli*, clostridia, veillonellae, and candida).[2] But that does not mean that the microorganisms in this last category are useless and must necessarily be eliminated. Indeed, the colonic microflora is a complex "ecosystem" with a wide variety of potential interactions between the different populations of microorganisms (see discussion in Chapter 13). Thus, it cannot be excluded that some interactions between potentially health-promoting and potentially harmful bacteria and microorganisms do, in fact, play a role in maintaining health and well-being, and in reducing the risk of some diseases. It is, thus, quite possible that some populations of potentially harmful or even pathogenic bacteria are necessary, provided they remain small compared to the health-promoting species. This is particularly true for the species that are recognized as being both potentially health-promoting and potentially harmful. We are still in a phase of exploration of the colonic microflora in terms of its composition at species, as well as strain levels. However, we still largely ignore most of the activities of these microorganisms, such as the interactions, exchanges, and complementarities that exist between the different genera, species, and strains (Figure 9.1)

All the same, the fact cannot be ignored that dysfunction in the other organs of the body might influence the composition of the colonic flora and, as a consequence, the colonic functions.

Thus, it is not surprising that in functional food science the concept of "colonic foods" (see the following box) has attracted the interest of both the scientific community, and the food, food ingredient, and food supplement industries.

Colonic Foods: Foods designed to reach the large bowel and feed its microflora, especially the health-promoting genera or species.

This concept is also at the core of the hypothesis that dietary strategies might be developed to improve colonic health, and thus, indirectly, health and well-being of the host, as well as the host's ability to reduce the risk of various diseases. Indeed,

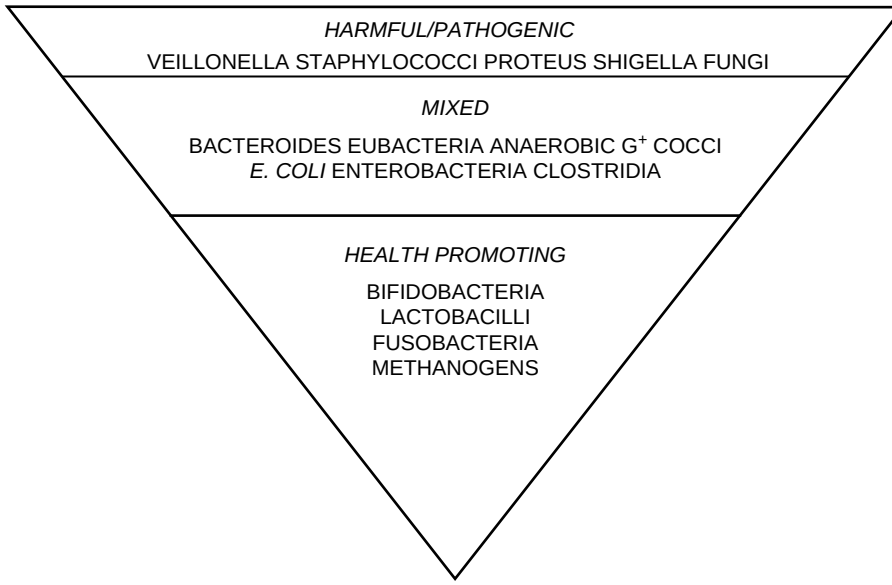

FIGURE 9.1 Classification of the major bacteria in human feces. (Adapted from Cummings, J. H., *The Large Intestine in Nutrition and Disease*, Danone Chair Monograph, Institut Danone, Brussels, Belgium, 1997; Gibson, G. R., Roberfroid, M. B., Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics, *J. Nutr.*, 125, 1401–1412, 1995.)

through modulation of the composition of the colonic microbiota (i.e., by stimulating the growth of health-promoting bacteria and suppressing, or at least reducing, the number of potentially harmful microorganisms.), it must be possible to influence large bowel functions and to act, indirectly, on host health and well-being, as well as on the risk of various diseases.[8] Such a strategy includes the consumption of prebiotics aimed towards the stimulation of the growth of potentially beneficial bacteria, considering that the ultimate aim of supplementation of the human diet with prebiotics is the beneficial management of gut microbiota.

9.2 PREBIOTICS: DEFINITION AND REQUIREMENTS FOR SCIENTIFIC SUBSTANTIATION

The term, "prebiotic" was first defined as: "A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health."[2] Since its introduction, the concept of prebiotics has attracted much attention, stimulating scientific as well as industrial interest. However, a prebiotic effect has been attributed to too many food components, sometimes without due consideration to the criteria required. In particular, many food oligosaccharides and polysaccharides (including dietary fiber) have been claimed to have prebiotic activity, although not all dietary carbohydrates are prebiotics.[9]

Such classification requires a scientific demonstration that the ingredient:

1. Resists gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption
2. Is fermented by intestinal microflora
3. Selectively stimulates the growth and activity of intestinal bacteria associated with health and well-being

Although each of these criteria is important, the third, concerning the selective stimulation of growth and activity of bacteria, is the most contentious and difficult to fulfill. It requires anaerobic sampling followed by reliable and quantitative microbiological analysis of a wide variety of bacterial genera such as total aerobes and anaerobes, bacteroides, bifidobacteria, clostridia, enterobacteria, eubacteria, and lactobacilli. A reported fermentation in pure cultures of single microbial strains, or an increase in a limited number of bacterial genera in complex mixtures of bacteria (e.g., fecal slurries) either *in vitro* or *in vivo,* cannot be accepted as demonstrating a prebiotic effect. This is because such a limited approach does not take into account the high complexity of the gut microflora and the numerous bacterial interactions that exist therein. By taking into consideration the stimulation of bacterial activity, patterns of production of organic acids and other fermentation products, gases, and enzymes have been used. However, these have not yet been fully validated as biomarkers of specific bacterial genera.

As with functional foods or ingredients, the final demonstration of the prebiotic effect must be carried out *in vivo,* through appropriate human nutritional feeding trials and supported by sound science.[10] Moreover, the methodologies used must be validated.

Clearly, such a demonstration has convincingly been conducted for only a limited number of food ingredients or supplements. As a matter of fact, a recent review of the data available has concluded that, out of some 20 compounds for which prebiotic attribute has been claimed in scientific literature (as up to April 2003), only three meet the requirements for prebiotic classification.[9] These three products are:

- Inulin-type fructans
- (Trans)-galactooligosaccharides
- Lactulose (but it is, almost exclusively, used as a laxative drug)

As a conclusion of their review, a slightly modified definition of the term, "prebiotic" has also been proposed: A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and activity in the gastrointestinal microflora that confers benefits upon host well-being and health.[9]

Thus, prebiotic fermentation should be directed towards bacteria seen as health promoting, with indigenous lactobacilli and bifidobacteria currently being the preferred targets, and with special attention to the bifidobacteria that are considered as the main health-promoting group in gut microflora.[11] Indeed, *Bifidobacterium* sp. dominate the intestinal microflora of breast-fed infants, and they are thought to play an important role in the improved health and development of breast-fed infants as

compared to those that are formula fed. The beneficial effects of bifidobacteria are thought to be:

- Protection from enteric infection
- Lowering of intestinal pH by formation of acids after assimilation of carbohydrates
- Suppression of putrefactive and pathogenic bacteria
- Production of vitamins
- Activation of intestinal function, assistance of digestion, and absorption
- Stimulation of the immune response

Because bifidobacteria are susceptible to oxygen and heat, their application in foods as probiotics has been limited in comparison with the lactobacilli. Therefore, there has been much interest in food-grade bifidogenic factors (especially the prebiotics) that endure normal processing and show effectiveness in the human body after ingestion. Among these, inulin and oligofructose are legally classified as food ingredients in all countries in which they are used and are well accepted for food use without limitations.[12] These are even considered model prebiotics. It is indeed the nutritional effects of inulin and oligofructose that are at the origin of the concept.

However, the demonstration of a prebiotic effect is very much dependent on the availability of adequate methodologies that are easily applicable, relevant to the end points, and validated for the identification and characterization of food ingredients or supplements that are able to modulate the composition and maintain or improve the balance of the gut (especially the colon) microflora.

9.3 METHODOLOGIES FOR THE STUDY OF THE COMPOSITION OF THE GUT MICROFLORA

Perhaps because of their fastidious requirement for anaerobiosis, but more likely due to their complex nutritional requirements, about 60% of the intestinal bacterial community cannot be cultivated in the laboratory even when excellent bacteriological culture methods are used.[13] However, as the field of colonic food, but more specifically that of prebiotic, has developed, so have the methodologies for investigating flora compositional changes.

Much of the early (and some of the current) studies were performed on pure cultures that involve the selection of a range of strains of different genera (i.e., *Bifidobacterium* spp., *Lactobacillus* spp., and other bacteria such as *Bacteroides* spp., *Clostridium* spp., *Eubacterium* spp., and *E. coli*) and testing for their ability to ferment the test compound (see Chapter 5, Section 5.3.5). The problem with this approach is, of course, that the strains selected cannot truly be considered as representative of the colonic microbiota. This is further compounded in some studies as authors have used a wide range of bifidobacteria and lactobacilli but only one or two strains of the "undesirable" species. Such studies cannot establish that the test carbohydrate is selectively metabolized and should be used for initial screening purposes only.

A more meaningful *in vitro* method to ensure a representative range of bacterial species for studying prebiotic oligosaccharides uses a fecal inoculate that is exposed to the test material in batch or multichamber culture systems (see Chapter 5, Section 5.3.5.2). A study of the changes in populations of selected genera or species can then establish whether or not the fermentation is selective. The use of feces probably gives an accurate representation only of events in the distal colon, but, still, it is largely accepted as a surrogate for the colon content. However, in discussing the data, it must be kept in mind that more proximal areas will have a more saccharolytic nature, and both the composition and activities of the microbiota indigenous to the colon are variable, dependent upon the region sampled (for a more extensive discussion see Chapter 5, Section 5.3.5.1). This has been confirmed through studies on sudden-death victims, where the colon contents were sampled shortly following death.[14,15] The complex gut models, which replicate different anatomical areas, attempt to overcome this and should be used in concert with human trials.

Whatever samples are analyzed, whether freshly collected feces or *in vitro* cultured fecal inoculate, different methodologies are available to identify and quantify the bacteria, at the genus, species, and, eventually, strain level, and to demonstrate changes induced by modulation of the composition of the microflora. These belong to two categories (Table 9.1):

- Culture on selective media
- Molecular-based methodologies

TABLE 9.1
Principal Methodologies Employed to Enumerate Colonic Bacteria

Method	Advantages	Disadvantages
Selective culturing and biochemical characteristics	Straightforward, relatively inexpensive, a large number of replicates can be carried out	Operator subjectivity, only applicable to culturable bacteria, selectivity of media is ambiguous, metabolic plasticity of organisms may introduce error
Fluorescence *in situ* hybridization (FISH)	Can be used on unculturable as well as culturable bacteria, highly specific	Can only probe for known bacteria, more time-consuming than culture procedures
Polymerase chain reaction (PCR)	High-fidelity, reliable, allows placement of previously unidentified bacteria, can be used for unculturable bacteria	Expensive, time-consuming, some bias in the PCR process
Direct community analysis	Culture-independent, the diversity of the entire samples can be elucidated	Some loss of bacterial diversity due to the bias introduced by PCR
Denaturing/temperature gradient gel electrophoresis (D/TGGE)	Rapid, can be used for both culturable and unculturable bacteria	Qualitative rather than quantitative, bias introduced by the PCR process may lead to a loss of diversity

9.3.1 Culture on Selective Media

Until recently, the identification and quantification of bacteria in the colonic microflora, at the genus or (eventually) at the species level, were almost entirely based on culture on selective media, to cope with phenotypic characteristics. Traditionally, this has been accomplished by culturing on a range of purportedly selective agars, followed by morphological and biochemical tests designed to confirm culture identities.[16] This approach is adequate to establish that a prebiotic selectively enriches defined "desirable" organisms and depletes "undesirable" organisms, but that it does not give a true picture of the population changes that occur. This is unavoidable while using selective culture because it is estimated that, qualitatively, only about 50–60% of the diversity present in the human colon have yet been characterized using culture on selective media.[17] But still, from a quantitative point of view, most, if not all, of the major genera and species (in terms of number of colony-forming units per gram of feces or cultured fecal inoculate) are known, and selective culture media are available to culture them.

In addition, these methodologies are time consuming, laborious, and costly, and, often, unreliable. Their use in studies aimed at demonstrating a modulation of colonic microflora composition by a colonic food remains scarce mostly because of the difficulty to include significant numbers of volunteers, as well as because of the requirement to collect samples in anaerobic conditions to preserve the obligatory anaerobes.

9.3.2 Molecular Methodologies[9,18]

A much more reliable approach involves the use of molecular methods of bacteria identification. These have advantages over culture-based technologies in that they have improved reliability and can encompass the full flora diversity. Indeed, the advent of molecular biology has revolutionized the identification as well as the quantification of microorganisms.[7] In particular, the sequencing of the highly specific bacterial ribosomal RNA (rRNA) has provided a very powerful tool for determining the evolutionary interrelationships of microorganisms.[19] They have spurred the discovery and recognition of new biodiversity, especially in the gut microflora. In contrast to traditional taxonomy based on phenotypic traits, ribosomal RNA-based taxonomy reflects natural evolutionary relationships among organisms[20] because ribosomal RNAs are excellent molecules for measuring evolutionary relationships among organisms.[21]

The prokaryotic ribosomes contain the 50 S subunit composed of about 34 proteins, as well as 5 S rRNA (120 bases) and 23 S rRNA (about 2900 bases); and the 30 S subunit composed of about 21 proteins and 16 S rRNA (about 1500 bases). Mostly because the degree of conservation differs considerably within the various regions, the 16 S rRNA has been the most widely investigated molecule to develop the phylogeny of prokaryotes. Analyses of rRNA sequences have revealed short signature sequences that are unique to species, generic, or suprageneric groups of organisms, enabling their phylogenetic placement and identification. In particular, the 16 S rRNA sequence analyses performed in the European project (FAIR CT97-3035) have greatly advanced information on the true genetic diversity of the

microorganisms in the human gut.[7] Based on the knowledge of these signature sequences, various molecular and (sometimes quantitative) procedures have been developed to study as well as to characterize changes in the composition of gut microflora. These procedures use the following molecular techniques:

- *In situ* hybridization
- Polymerase chain reaction (PCR)
- Direct community analysis
- Denaturing/temperature gradient gel electrophoresis

9.3.2.1 Fluorescence *In Situ* Hybridization[22,23]

Modern techniques are now available whereby quantitative bacterial enumeration can be carried out using a quick, culture-independent and reliable method known as fluorescence *in situ* hybridization (FISH). The FISH technique involves the use of group (and in some cases, species) specific oligonucleotide probes that target discrete discriminatory regions of the rRNA molecule. By targeting highly conserved areas of the rRNA, specific groups of bacteria can be distinguished from others in a mixed culture.

A host of phylogenetic probes is currently available and being validated for the enumeration of fecal bacteria, while more are being designed and validated.[23] Groups targeted include *Bacteroides* spp.,[24] *Bifidobacterium* spp.,[25] *Lactobacillus/Enterococcus* spp.,[26] and *Eubacterium* spp.[23,27]

Besides being a relatively quick technique, this method also removes the ambiguity that is a prominent feature of traditional selective agars. Additionally, FISH provides a means through which hitherto unculturable bacterial species of the gut may be investigated because this is a culture-independent technique and, therefore, does not require prior, often anaerobic, growth of an organism upon laboratory media.[28]

The FISH method is the most commonly used method to quantitatively analyze changes in the composition of the dominant groups of bacteria (lactobacilli, bacteroides, bifidobacteria, eubacteria, *E. coli*, clostridia, etc.), induced by prebiotic feeding. The other molecular methodologies are more qualitative, and their main interest is in the investigation of the composition of the fecal flora. Hence, they are only briefly described here. Table 9.1 summarizes the principal techniques along with some of their advantages and disadvantages.

9.3.2.2 Polymerase Chain Reaction

Bacterial ribosomes offer the means by which identification of the fecal bacteria can be made at a molecular level. The genes coding for the 16 S ribosomal subunits are comprised of both conserved and variable regions, and sequencing of the 16 S rRNA gene enables bacterial identifications to be made by using PCR to amplify segments of this gene to a level whereby their sequence can be subsequently determined.[29]

9.3.2.3 Direct Community Analysis

This process characterizes the 16 S rRNA diversity of the sample of interest. The total bacterial DNA is extracted from the sample and 16 S rDNA genes are amplified via PCR (using universal primers).[17] The purified amplification products are subsequently cloned into *E. coli*, and clones containing the 16 S rDNA inserts are sequenced and identified by comparison to database 16 S rDNA sequences.

9.3.2.4 Denaturing or Temperature-Gradient Gel Electrophoresis

Denaturing-gradient gel electrophoresis (DGGE) or temperature-gradient gel electrophoresis (TGGE) is used to separate amplified DNA fragments of the same size based on the extent of the sequence divergence between different polymerase chain reaction (PCR) products.[30] A whole community PCR is carried out and partial 16 S rDNA sequences amplified from the different bacterial species present based on the decreased electrophoretic mobility of the partially melted double-stranded DNA molecule in polyacrylamide gels containing either a temperature or chemical denaturant gradient.[30] Identification can be carried out either by excising fragments from the gel and sequencing them, or by comparing their motility with that of known control sequences. As with FISH, both culturable and unculturable populations can be characterized, and this relatively rapid technique also offers the potential of monitoring gut flora over time.[31]

9.4 INULIN-TYPE FRUCTANS CLASSIFY AS PREBIOTIC: SCIENTIFIC SUBSTANTIATION

Inulin and oligofructose are the most studied and well-established prebiotics.

As previously mentioned, inulin and oligofructose escape digestion in the upper gastrointestinal tract and reach the large intestine virtually intact; they are thus colonic foods (see Section 9.1.2). But, in addition, they act as prebiotics, as has been shown in the many studies that investigated the effects of inulin and oligofructose on the human gut microbiota both *in vitro* and *in vivo*. Selective stimulation of growth of the beneficial flora, namely, bifidobacteria and, to a lesser extent, lactobacilli and possibly eubacteria has also been reported. The prebiotic effect of inulin and oligofructose is thought to be due to their selective fermentation by the bifidobacteria that produce an intracellular inulinase necessary to hydrolyze the b-(2,1) osidic linkages between the fructose units.

9.4.1 Experimental Evidence

9.4.1.1 *In Vitro* Data

The data on *in vitro* fermentation of inulin and oligofructose in anaerobic batch culture inoculated with pure genera or strains of different bacteria have already been reported and discussed (see Chapter 5, Section 5.4.1.1, Table 5.4, and Table 9.2). The main conclusion is that most bifidobacteria species ferment inulin, oligofructose,

TABLE 9.2
Studies Carried Out Demonstrating the *In Vitro* Selective Fermentation of Inulin in Pure, Mixed Batch, and Mixed Continuous Cultures

Study	Observations	Investigators
Examining the growth of bifidobacteria on different types of oligofructose in pure culture. Eight species tested, as well as species of clostridia, bacteroides, enterococci, and *E. coli*	Linear oligofructose had more of a bifidogenic effect than larger mol wt molecules and branched chain varieties. Bifidobacteria species showed a preference for fructans compared to glucose	Gibson and Wang[38]
Species of bifidobacteria (*longum*, *breve*, *pseudo-catenulatum*, and *adolescentis*) were tested in pure culture for their ability to ferment oligofructose	*B. adolescentis* was seen to grow best and was able to metabolize both short and long chain oligofructose	Marx et al.[43]
The ability of bifidobacteria and lactobacilli to grow on MRS agar containing oligofructose was investigated	7 out of 8 bifidobacteria and 12 out of 16 lactobacilli were able to grow on agar containing oligofructose	Kaplan and Hutkins[42]
Batch culture using fecal inocula to ferment inulin, oligofructose, pectin, starch, polydextrose, and fructose	Bifidobacteria most increased with oligofructose and inulin whereas populations of *E.coli* and clostridia were maintained at relatively low levels	Wang and Gibson[36]
Batch culture using fecal inoculum to ferment oligofructose, branched fructan, levan, and maltodextrin	FISH revealed that branched fructan had the best prebiotic effect, followed by oligofructose	Probert and Gibson[46]
Continuous culture fermentation to study fermentation of oligofructose	Selective culturing showed that bifidobacteria and, to a lesser extent, lactobacilli preferred oligofructose to inulin and sucrose. Bacteroides could not grow on oligofructose	Gibson and Wang[39]

and glucose equally well, whereas for all the other bacterial genera tested, glucose is a more efficient substrate for growth than inulin-type fructans that were fermented by bacteroides but not by *E. coli* or *Clostridium perfringens*. All the bifidobacteria species tested for their capacity to ferment inulin and oligofructose do it well except for *Bifidobacterium animalis*, which is more effective in fermenting oligofructose than inulin, and *Bifidobacterium bifidum*, which utilizes neither inulin nor oligofructose.[32–46] However, as discussed previously (see Section 9.2), fermentation of inulin and oligofructose in pure cultures of different genera, species or strains of

bacteria is not proof for prebiotic effect. More complex models have to be used. One such model is the single-stage continuous culture systems containing human fecal bacteria, used by Gibson and Wang, and Dal Bello et al. to demonstrate the bifidogenic effect of oligofructose and inulin.[38,39,47] Human feces were also used as inoculum for single-stage continuous culture experiments that demonstrated the selective promotion of the growth of bifidobacteria (+2 $\log_{10}$). Experiments with a three-stage continuous culture model of the human colon further confirmed the bifidogenic effect of oligofructose, and demonstrated that, in parallel with the increase in the number of bifidobacteria, the numbers of *E. coli* and *C. perfringens* were significantly reduced.[38,39,47,48] Similarly, using molecular techniques to quantify the bacterial populations, Sghir et al. demonstrated in a continuous culture system that inulin and oligofructose selectively stimulated the growth not only of bifidobacteria but also of lactobacilli.[49] Also, in batch cultures, Dal Bello et al. reported that the addition of oligofructose, but not inulin, resulted in enhanced growth of *C. perfringens*.[47]

9.4.1.2 *In Vivo* Data

Like *in vitro* work, *in vivo* studies have also been carried out using animal models. The effect of the prolonged intake of oligofructose has been studied in rats fed either a low-fiber diet (basal) or the basal diet supplemented with 9 g per 100 g of oligofructose daily for periods of 2, 8, or 27 weeks.[50] Supplementation with oligofructose led to an increase in lactic acid bacteria after 2 weeks without changing total anaerobic bacterial levels. However, the majority of the effects were abolished by weeks 8 and 27 of oligofructose consumption.[50]

As discussed above, a particularly interesting experimental model to investigate changes in gut microflora, that might be relevant to humans, is a germ-free rat associated with a human fecal flora (see Chapter 5, Section 5.3.5.3). In such a model, a selective stimulation of growth of bifidobacteria was induced in the intestinal microflora by oligofructose. Lactobacilli were also increased in rats fed only oligofructose, or a mixture of oligofructose and inulin. The same mixture also led to a reduction in the number of clostridia.[51–54] The same model was used by Kleessen et al. who compared the effect, on the gut microbial ecology, of inulins with different chain lengths, i.e., oligofructose, inulin HP, and oligofructose-enriched inulin Synergy 1 (see Chapter 3, Section 3.3.1 for a detailed description of these products).[52] These authors also investigated, in detail, the changes in microflora composition in the cecum, the colon, and the feces, and they used the FISH technique to quantify the bacteria. This extensive study gives very interesting information that can be summarized as follows:[55]

- Changes in counts of bacteria belonging to the *Clostridium coccoides–Eubacterium rectale* cluster in all three microflora (cecum, colon, and feces), with Synergy 1 (+1–1.2 $\log_{10}$, $p < 0.05$) being more efficient than inulin HP (+0.6–0.9 $\log_{10}$, $p = 0.06$) and oligofructose (+0.3–0.5 $\log_{10}$, not significant)

- Increase in counts of bifidobacteria by oligofructose in colonic (+1.2 $\log_{10}$, $p < 0.05$) and fecal (+0.9 $\log_{10}$, $p = 0.05$), but not cecal microflora — not shown by inulin HP (inulin even reduced the counts in the cecum [1.2 $\log_{10}$, $p < 0.05$]) or Synergy 1
- Increase in counts of lactobacillus by Synergy 1 in colonic (+1.5 $\log_{10}$, $p < 0.05$) and cecal (+1.5 $\log_{10}$, $p < 0.05$), but not fecal microflora, and by oligofructose in feces (+1.2 $\log_{10}$, $p < 0.05$) — not shown by inulin HP
- Increase in counts of enterococcus by oligofructose in feces (+1.6 $\log_{10}$, $p < 0.05$)
- Reduction in counts of *Clostridium histolyticum* and *C. lituseberense* groups by oligofructose and Synergy (–0.6–1.3 $\log_{10}$, $p < 0.05$)
- No significant differences in numbers of bacteroides-prevotella and Enterobacteriaceae.

The most important finding probably is the increase in counts of bacteria belonging to *C. coccoides–E. rectale* cluster. At the end of the treatment period, these microorganisms increased, as a proportion of the total flora, from 13 to 63%, displacing bacteroides-prevotella as the predominant cecal bacteria.[55] This is especially important because the *C. coccoides–E. rectale* cluster contains most of the butyrate-producing microorganisms. The increase in their counts following inulin feeding might, thus, explain the increase in the proportion of butyrate in SCFAs resulting from its intestinal fermentation (see Chapter 5, Section 5.4.2).

In an experiment comparing the effect of different nondigestible oligosaccharides (oligofructose, lactulose, resistant starch, and dextrin) on gut microecosystem in growing rats, Bielecka et al. did see a slight (+0.3 $\log_{10}$), but not significant, increase in fecal bifidobacteria after oligofructose feeding (10% w/w in fiber-free diet for 4 weeks).[56] In the same experiment, lactulose and, surprisingly, resistant starch had a significant prebiotic effect (+1.35 and 1.2 $\log_{10}$, respectively).[56] But, the number of bifidobacteria in the feces of rat fed the control diet (containing 10% cellulose as dietary fiber) was unusually high for rats, i.e., $10^{9.2}$ cfu/g. These authors also report a significant ($p < 0.05$) increase in the number of spores of anaerobic saccharolytic bacteria in rats fed only oligofructose but not the other nondigestible oligosaccharides. But the meaning of that observation is not discussed in the paper. In a second paper, the same authors, however, report a highly significant ($p < 0.001$) prebiotic effect of oligofructose (5% w/w in diet) in rats (+1.6 $\log_{10}$ in fecal bifidobacteria).[57]

In an experiment comparing gastrectomized and sham-operated rats, Sakai et al. reported an increase in total bacteria and lactobacilli, as well as a higher frequency and higher numbers of bifidobacteria in cecal microflora by oligofructose (7.5% w/w in the diet) in both conditions.[58] Whereas in the rats fed the control diet bacteroides were the predominant genus, in oligofructose-fed rats it was lactobacilli. In mice fed a high-fat, fiber-free diet, inulin (10% w/w in the diet) stimulated overall bacterial growth and fermentation, increased the number of bifidobacteria, and increased the residual concentration of lactic acid in the cecum.[58]

In addition, by applying culture-independent techniques (nonselective DNA-based analysis, percent guanine + cytosine-based profile[59] combined with 16 S ribosomal DNA sequencing), Apajalahti et al. have analyzed the total cecal microbial

community. Data reveal some bacterial population shifts of hitherto unknown bacterial species.[60] However, these techniques remain qualitative and do not allow quantitative evaluation of the relative importance, in terms of numbers, of these unknown genera in the whole cecal microflora.

In conclusion, the experimental data both *in vitro* and *in vivo* demonstrate that inulin and oligofructose selectively stimulate the growth of health-promoting bacteria in the colonic microflora, and are thus prebiotic. These changes are probably not limited to the lactic acid bacteria (bifidobacteria and lactobacilli), but might concern other species like *C. coccoides–E. rectale* cluster known to produce butyrate as well as hitherto unknown bacteria.[55,60] But these data also call attention to the facts that not all inulin derivatives have, qualitatively, the same effects on intestinal microflora and that for each inulin-type fructan, the different segments of the large bowel (including the feces) might be differently influenced. However, as in humans, only fecal samples are available for analysis, the latter observation will be difficult to confirm.

By combining the responses of each volunteer's fecal samples to one substrate, Rycroft et al. reported a significant ($R^2 = 0.868$; $p < 0.01$) correlation between the initial bifidobacteria population and the increase in numbers of bifidobacteria during the 24-h batch culture.[61]

All these data fully justify testing of the prebiotic effect of inulin-type fructans in human nutrition intervention trials that are, indeed, the ultimate and necessary step to fully justify a prebiotic claim.

9.4.2 Human Data

Human trials with oligofructose and inulin include those with a controlled diet and a free diet, as well as crossover feeding trials, although the dose, substrate, duration, and volunteers, as well as bacteriology vary[26,35,62–77] (see Table 9.3).

In the *in vivo* trials, there were large variations between the subjects in their microflora compositions (analyzed by applying both classical culture and molecular techniques) and in their responses to the substrates (particularly between Western and Eastern [both European and North American]) subjects. Another general observation was the decrease in bifidobacteria once administration of the oligofructose and inulin ceased.[26,67–69,74–75,77–79] Even though they reported data on total bacterial and bifidobacteria counts only (thus not complying with the first criterion for the demonstration of a prebiotic effect), papers by Bouhnik et al. are included for the sake of completeness.[72,78] All the other studies have quantified not only bifidobacteria but also other genera like bacteroides, lactobacilli, clostridia, fusobacteria, and eubacteria, reporting no change, a decrease or even an increase in these populations.

The selective stimulation of bifidobacteria by inulin and oligofructose has been investigated in a 45-d study of 8 healthy male human subjects, using a protocol that is the gold-standard reference for the demonstration of a prebiotic effect.[68] Volunteers were fed strictly controlled diets supplemented with 15 g/d sucrose for the first 15 d, followed by 15 g/d oligofructose for a further 15 d. Four volunteers went on to consume 15 g/d inulin for the final 15 d of the study. Total bacterial levels remained unaffected, but both oligofructose and inulin caused dramatic changes in the com-

TABLE 9.3
Information on Published Human Nutrition Studies Designed to Test for Prebiotic Effect of Inulin-Type Fructans

Daily Dose, g	Duration, Weeks	Number of Volunteers	Age Category of Volunteers	Effects of Prebiotic on Bacteria Other than Bifidobacteria	Refs.
8	2	23	Elderly	Not significant	34 35
8	5	6	Adult	Not significant	64
4	2	10	Adult	Not reported	66
8	2	38	Adult	Decreased clostridia	67
15	2	8	Adult	Decreased clostridia Decreased bacteroides Decreased fusobacteria	68
15	2	4	Adult	Not significant	68
12.5	2	20	Adult	Not reported	78
20	2	17	Elderly	Not significant	71
5	1	8	Adult	Not reported	72
10	1	8	Adult		
20	1	8	Adult		
8	5	8	Adult	Not significant	70
5	3	8	Adult	Decreased clostridia Increased bacteroid	74
6.6	3	31	Adult	Not significant	75
8	4	9	Adult	Not significant	76
9	2	10	Adult	Increased bacteroid	26
8	3	19	Elderly	Decreased *E. rectalis*	77

position of the fecal microflora, as shown by the significant increases (+0.7 and 0.9 $\log_{10}$, respectively) in fecal bifidobacteria, whereas bacteroides, clostridia, and fusobacteria all decreased during oligofructose supplementation and gram positive cocci were reduced during inulin supplementation.

The other published human studies that have demonstrated the prebiotic effect of inulin-type fructans are:

1. Mitsuoka et al. reported the first human study demonstrating that by giving 8 g/d of oligofructose for 2 weeks to 23 elderly hospitalized human volunteers, it was possible to increase the numbers (+0.8 and 0.9 $\log_{10}$ at day 4 and 14, respectively), as well as the frequency of identification (from 87 to 100%), of bifidobacteria in feces. The other populations of bacteria (veillonellae, lactobacilli, enterobacteriaceae, streptococci, and clostridia) were not modified.[35]
2. Hidaka et al. performed a similar study both in a group of hyperlipidemic outpatients (n = 6; daily dose = 8 g oligofructose for 5 weeks) and in a group of chronic failure patients compared with a group of healthy

volunteers (n = 10 and 6, respectively; daily dose = 8 g oligofructose for up to 1 year). In the first trial, the number (+0.5 $\log_{10}$), and frequency of presence (from 8.75 to 33%) of bifidobacteria increased, whereas enterobacteriaceae, streptococci, and lactobacilli completely disappeared from the feces. The populations of bacteroides and eubacteria did not change. In the long-term trial with chronic failure patients, the counts (+1.1 $\log_{10}$) as well as the frequency (from 2.5 up to 22.7%) of bifidobacteria had already increased after 1 month of oligofructose feeding, and remained at the same level through the 12 months of administration. But it must be outlined that 3 out of the 10 volunteers had zero or very low levels of bifidobacteria in feces, thus giving an artificially low average value for the t = 0 reference value. In the healthy volunteers who all had bifidobacteria in their feces, oligofructose feeding only slightly (and, probably, not significantly) increased the count of bifidobacteria. In these two groups, no other bacterial populations were modified.[64]

3. Williams et al. reported a significant increase in bifidobacteria levels (+1 $\log_{10}$) and frequency (from 1.3 up to 6.8%) in 10 healthy adult humans, as well as an increase in lactobacilli in 6 out of 10 volunteers (who had a very low level of lactobacilli before the test), after eating oligofructose at a dose of 4 g/d for 2 weeks.[66]
4. Rochat et al. have studied the effect of oligofructose (8 g/d) given for 2 weeks to 38 healthy volunteers on indigenous fecal *Bifidobacterium* spp. The ingestion of oligofructose increased (+0.9 and +1.3 $\log_{10}$, respectively) fecal counts of bifidobacteria and reduced the counts of clostridia (–0.3 and –1 $\log_{10}$, respectively) after both 1 and 2 weeks, whereas the counts of enterobacteriaceae and bacteroides were not modified.[67]
5. Buddington et al. studied the influence of oligofructose supplementation on the fecal flora of 12 healthy adult humans. Subjects were fed a controlled diet for 42 d that was supplemented with 4 g/d oligofructose between days 7 and 32. The controlled diet increased bifidobacteria levels, but the highest increases were observed during oligofructose supplementation.[69]
6. Kleessen et al. investigated the effect of eating an inulin-supplemented diet for 19 d on fecal flora in 10 elderly constipated patients. Inulin was initially administered at a 20 g/d dose for days 1 to 8; this was gradually increased to 40 g/d during days 9 to 11 and was maintained at these levels until the end of the study. These authors observed a significant increase in bifidobacteria, whereas a decrease in enterococci and enterobacteria numbers occurred.[71]
7. Kruse et al. investigated the effect of inulin on fecal bifidobacteria in 8 healthy humans. The subjects consumed a typical Western diet (45% energy as fat and 40% energy as carbohydrate) followed by a reduced fat diet (30% energy as fat), using inulin as fat replacement (maximum amount of inulin consumed was 34 g/d). The controls followed identical diets, but without inulin supplementation. The effect on fecal flora was

monitored using the FISH technique. Inulin significantly increased fecal bifidobacteria (+1.2 $\log_{10}$) but it did not change the total bacterial counts.[79]

8. Bouhnik et al. assessed how different doses (0, 2.5, 5, 10, and 20 g/d) of oligofructose affected the fecal bifidobacteria counts in a 7-d study of 40 healthy human volunteers. They reported that the lowest dose (2.5 g/d) had no effect; a dose of 5 g/d had an intermediate effect (+0.9 $\log_{10}$), whereas the two largest doses (10 and 20 g/d) had similar and maximum effects (+1.3–1.5 $\log_{10}$) for increased bifidogenesis.[72]
9. Brighenti et al., in a placebo-controlled study designed to test for the effect of inulin (9 g/d in breakfast cereals for 4 weeks) on lipid parameters (see Section 11.3.2.1), also controlled the composition of the fecal microflora. They reported that, even though the level was very high in the placebo group (10.66 $\log_{10}$ cfu/g), inulin still significantly increased the number of bifidobacteria (10.99 $\log_{10}$ cfu/g). Such an increase represents a huge number of "new" bifidobacteria, i.e., $+6.10^{10}$ bacterial cells/g of feces.[80]
10. Menne et al. tested the hypothesis that $F_{py}F_n$ components of oligofructose produced by enzymatic hydrolysis of inulin are as effective as the $G_{py}F_n$ components (see Chapter 3, Section 3.2.2 and Section 3.3.1 for the description of these molecules) in selectively stimulating the growth of bifidobacteria. During a controlled feeding study, 8 volunteers consumed 8 g/d of $F_{py}F_n$-rich oligofructose preparation (Raftilose® L-60) for 5 weeks. After 2 and 5 weeks of consuming oligofructose, all the volunteers had significantly ($p < 0.01$) increased numbers of bifidobacteria in feces, whereas the populations of total anaerobes, bifidobacteria, lactobacilli, bacteroides, coliforms, and *Clostridium perfringens* were not modified.[73]
11. Rao demonstrated that a low dose of oligofructose (5 g/d) added to the diet of 8 healthy volunteers for 3 weeks had already significantly increased the number of fecal bifidobacteria (+1.0 $\log_{10}$) after 11 d. Total anaerobe (+0.6 $\log_{10}$) and bacteroides (+0.5 $\log_{10}$) also increased, but the populations of anaerobes and coliforms did not change.[74]
12. Tuohy et al., in a double-blind, placebo-controlled cross-over study of 31 healthy human volunteers, analyzed the prebiotic effects of biscuits containing a blend of partially hydrolyzed guar gum and oligofructose. The effects were confirmed using the FISH method for the quantification of fecal bacteria. A significant increase in bifidobacteria numbers occurred, whereas the numbers of bacteroides, lactobacilli, clostridia, and total cells remained at similar levels throughout the study.[75]
13. Tuohy et al. carried out a study to determine the effect of inulin HP (see Chapter 3, Section 3.3.1 for a description) on human fecal flora composition. After a control period of 2 weeks during which they were asked to add 8 g/d of maltodextrins to their usual diet, nine healthy volunteers consumed a supplement of 8 g/d of inulin HP for 14 d. The FISH method was used to enumerate the predominant groups of gut bacteria. A small, but statistically significant ($p < 0.05$), increase was observed in bifidobac-

teria (+0.2 $\log_{10}$ from $10^{8.8}$ to 10^{9}) and, to a lesser extent, in clostridia (+0.4 $\log_{10}$ from $10^{7.2}$ to $10^{7.6}$). Inulin HP had no effect on populations of total bacteria, *Bacteroides* spp., lactobacilli, or enterococci.[76]

14. Harmsen et al. used both the FISH and the DGGE methods to investigate the effects of inulin HP (see Section 3.3.1 for a description) on the gut microflora of 10 healthy adult volunteers. Even though the initial level of bifidobacteria was quite high (0.9 ¥ 10^{10} cfu/g of feces), inulin HP still significantly stimulated their growth (2.3 times, up to 2.1 ¥ 10^{10} cfu/g of feces). Inulin HP also decreased the numbers of the *Eubacterium rectale–Clostridium coccoides* group (from 6.8 down to 4.8 ¥ 10^{10} cfu/g of feces), but it did not modify the numbers of total bacteria, bacteroides, and eubacteria low in G + C . In terms of the percentage of fecal bacteria, the bifidobacteria increased from 4.6 to 10%, whereas the *E. rectale–C. coccoides* group decreased from 15.6 to 10.1%. DGGE analysis used to investigate the bifidobacteria species composition revealed that inulin intake had no significant effect on that parameter even though each of the 10 volunteers had unique banding patterns of bifidobacteria that remained stable throughout the trial.[26]
15. Guigoz et al. designed a study to confirm the prebiotic effect of oligofructose in 19 elderly nursing home patients who were asked to consume, every day, 2 ¥ 4 g of the product for 3 weeks. Classical bacteriology culture methods were applied to enumerate fecal bifidobacteria, lactobacilli, bacteroides, enterobacteriaceae, enterococci, and *Clostridium perfringens*. The numbers of bifidobacteria were very low at the beginning of the test period (10^{6} cfu/g of feces), and they increased dramatically (+2.8 $\log_{10}$) after 3 weeks of oligofructose consumption to reach a level that is usually observed in adults ($10^{8.4}$ cfu/g of feces). The numbers of bacteroides also increased (+0.7 $\log_{10}$) but lactobacilli, enterobacteriaceae, enterococci, and *Clostridium perfringens* were not affected by the ingestion of oligofructose.[77]

In a synbiotic-type approach, Bouhnik et al. studied the effect of a fermented milk product (12 d of treatment) containing *Bifidobacterium* spp. with or without inulin on the fecal bacteriology of six healthy volunteers for each test. The authors observed that the addition of the bifidobacterium-fermented milk substantially increased bifidobacteria levels after 12 d (+1.3 $\log_{10}$), but the addition of 18 g/d inulin to this formulation did not enhance the effect significantly (+1.6 $\log_{10}$). This study measured only bifidobacteria and total anaerobes but no other bacterial genera, thus precluding any conclusion with regard to the selective stimulation of growth of the bifidobacteria. Moreover, the volunteers receiving the fermented milk alone and those receiving the fermented milk with inulin were different people, thus making any comparison difficult. Also, the effect of the fermented milk (+1.3 $\log_{10}$) alone on the bifidobacteria population was already quite high, making a further increase by inulin difficult.[81]

The efficacy of prebiotics has also been evaluated with a view to their administration to formula-fed infants.[82–85] All these studies report an increase (up to 3.3 $\log_{10}$ cfu/g feces) in the counts of bifidobacteria and lactobacilli in feces of infants fed a formula milk supplemented (0.4 or 0.8% w/v) with a mixture of inulin HP and galactooligosaccharides (10/90 w/w). However, in testing the effect of three doses (1, 2, or 3 g/d) of oligofructose alone, Guesry et al. did not see any stimulation of bifidobacteria growth in infants.[86]

9.5 INULIN-TYPE FRUCTANS AS PREBIOTICS: DISCUSSION AND PERSPECTIVES

9.5.1 Qualitative Aspects of the Prebiotic Effect

In all the nutrition trials so far reported that have tested for the effect of inulin-type fructans on human fecal flora, the increase in the number of bifidobacteria (expressed as cfu/g of feces):

- Becomes significant and reaches its maximum, probably, in less than a week
- Remains as long as the intake of the prebiotic continues
- But, afterwards, progressively (within 1 or 2 weeks) disappears when that intake stops

Such observations are well in line with the hypothesis that the prebiotic acts as a selective substrate for the fermentation of the bifidobacteria that become stimulated to grow.

At present, sampling for microbiological analysis is limited to feces only. Even if it is generally accepted that feces is a surrogate for the colonic content, this certainly limits our understanding of what is happening inside the colon and, more specifically, inside the different segments that are known to differ in their environmental (pH, mineral and water content, etc.) conditions and in their physiological functions.[1] Moreover, even though the bacterial population is certainly much lower there than in the large bowel, the fact remains that the small intestine, and especially the distal ileum, is also colonized by bacteria, the proliferation of which can be well supported by prebiotics; also, feces is, most probably, not a good surrogate to study that population and its modulation by prebiotics . There is, thus, a need to develop new models and new methodologies to allow for sampling in these parts of the gastrointestinal tract.

Another topic of growing interest is the bacterial flora colonizing surfaces in the large intestine, especially the mucosa, the mucus layer, or eventually the particulate materials in the colonic lumen.[87] Indeed, a few studies using either biopsy or resected samples have demonstrated the presence, in the colonic mucosa, of a microflora with a specific composition different from that of the luminal colonic microflora.[88] In an *ex vivo* protocol in which 15 healthy volunteers selected from the colonoscopy waiting list had been asked to supplement their usual diet with oligofructose-enriched inulin Synergy 1 (15 g/d) for 2 weeks, preliminary data reported in an abstract form

have shown an increase in both bifidobacteria and lactobacilli counts in the mucosa (+1 and 0.5 $\log_{10}$ cfu/g of mucosa, respectively).[89] Using the model of rats harboring a human fecal flora, Kleessen et al. have similarly reported that feeding an inulin-supplemented diet significantly increased (16 times) mucosal bifidobacteria numbers (cells/mm^2 of mucosal surface), even though the stimulation was not significant in the intestinal lumen.[90] As discussed by these authors, these findings suggest "specific bacterial populations that occupy an ecological niche such as the epithelial surface or the mucus layer are distinct from those in the gut lumen." Moreover, the findings also suggest that the adhesion of bifidobacteria to the mucus might be strain specific and might depend on the presence of specific substrates like mucins or prebiotics.[91] It can thus be hypothesized that the prebiotic effect of inulin-type fructans concerns both the luminal and the mucosa-associated microflora. It can further be speculated that the microorganisms of the mucosal microflora play specific roles in the protection of the mucosal epithelium, and that changes in the composition of that intestinal environment may influence the functions of the epithelium, such as nutrient absorption, endocrine activities, immunological functions, etc.

9.5.2 Quantitative Aspects: The Prebiotic Index

Two questions that have attracted (too much!) attention (mostly from marketing people!) concern the quantitative aspects of the prebiotic effect. These questions can be formulated as follows:

1. Are the different inulin-type fructans equally effective?
2. Can a dose–effect relationship be established?

To answer these questions, a kind of meta-analysis has been performed based on the results of the studies described in Table 9.3, and on a few others that have appeared in abstract form only, have been published as part of the proceedings of a conference, or have been given to the author as a personal communication. The criteria for including these studies in the analysis were that the available report should have included at least the:

1. Daily dose of the prebiotic
2. Nature of the prebiotic, i.e., inulin or oligofructose
3. Number of volunteers
4. Number of bifidobacteria per gram of feces, both at the beginning and at the end of the supplementation period

These data are presented in Table 9.4 and include calculations that are usually not used when discussing the results of a prebiotic test.

Indeed, classically (and rightly so) in such studies, the microbiological data are presented as $\log_{10}$ cfu/g of feces and the prebiotic effect is then expressed as (**D**), the "crude" increase (or +X $\log_{10}$ cfu/g) of feces. (For example, if the initial and the final numbers of bifidobacteria are 8.8 and 9.5 $\log_{10}$ cfu/g, the prebiotic intake has increased the population of bifidobacteria by 0.7 $\log_{10}$ cfu/g.) This parameter does not correlate with the daily dose (**A**) of the prebiotic (r = 0.06; not significant).

But the real meaning of the "crude" increase is generally misinterpreted. Certainly, if the initial population of bifidobacteria is 8, 9, or 10 $\log_{10}$ cfu/g, increasing it by 0.7 $\log_{10}$ cfu/g will not have the same meaning in terms of the number of "new" bifidobacteria cells that have appeared because of the prebiotic treatment. In the example, the prebiotic treatment will have caused the appearance of 5×10^8, 5×10^9, and 5×10^{10} "new" bacterial cells, respectively, or 100 times more cells in the last case than in the first.

It is thus necessary to calculate the absolute numbers of new bacterial cells that have appeared as a consequence of the prebiotic treatment. Such numbers can be expressed either as (**E**) or as $\log_{10}$ values (**F**). But, once again, the daily dose (**A**) of the prebiotic does not correlate with these numbers (**E** and **F;** $r = 0.06$ and 0.09, respectively; NS). The reason is that an important parameter, i.e., the initial number of bifidobacteria (**B**) is not taken into account. In the first report of a prebiotic effect, Hidaka et al. have already argued that the initial numbers of bifidobacteria (expressed as $\log_{10}$ cfu/g of feces or **B**) influence the prebiotic effect, on observing an inverse correlation between these numbers and their crude increases after oligofructose feeding.[34] Roberfroid et al.,[41] Rycroft et al.,[61] and Rao[74] have reached the same conclusion, which is also supported by the data in Table 9.4 (Figure 9.2; $r = .76$; $p < .01$). However, the correlation holds true only for crude increases and not for absolute increases in cfu/g of feces (**F**; $r = 0.12$ $p > 0.10$).

To further discuss the prebiotic effect, we propose to use the absolute values of new cells to calculate a prebiotic index, which can be defined as:

> Prebiotic index: "The increase in bifidobacteria expressed as the absolute number (E) of "new" cfu/g of feces divided by the daily dose (A in g) of inulin-type fructan ingested in each individual human nutrition trial."[92]

Based on the available data (Table 9.4), it can be concluded that the prebiotic index of inulin-type fructans is on the order of a few 10^8 cfu/g (average = 4 ± 0.82; range, 0.3–13) and that it is comparable for the different types of inulin, especially oligofructose and inulin (averages being 4×10^8 cfu/g and 5×10^8 cfu/g, and ranges, 0.5–12 $\times 10^8$ cfu/g and 0.3–13 $\times 10^8$ cfu/g, respectively).

As indicated by one experimental study, different types of inulin molecules might, however, affect differently the bacterial populations that colonize different segments of the gastrointestinal tract, especially the different segments of the colon, as well as the different habitats in the colon (e.g., the mucosa or the mucosal layer).[55] But this needs further investigations that require the development of new methodologies.

Other parameters related to the prebiotic effect that could be of interest are the increase in total daily fecal excretion of bifidobacteria *per se* and per gram of inulin-type fructan ingested. But, unfortunately, only one out of the 22 publications available so far has given the 24 h fecal output of volunteers (the paper by Gibson et al.[68]); the calculated increases in bifidobacteria (total and per gram, respectively) are $+32 \times 10^{10}$ cfu/24 h or $+2 \times 10^{10}$ cfu/24 h/g oligofructose, and $+142 \times 10^{10}$ cfu/24

TABLE 9.4
Summary of Quantitative Data on Prebiotic Effect of Inulin-Type Fructans — Results from All Human Intervention Studies Available

A Dose, g/d	Number of Volunteers	B Log_{10} cfu/g T_0	C Log_{10} cfu/g T_{max}	D C–B	E cfu/g T_{max} –cfu/g T_0	F Log_{10} D	Prebiotic Index E/A log_{10} E/A	Refs
10	5	8.8	9.5	0.7	25.3 ¥ 10^8	9.4	2.50 ¥ 10^8 8.4	63
6	9	8.7	9.8	1.1	55.0 ¥ 10^8	9.7	9.20 ¥ 10^8 8.96	62
8	23	8.8	9.7	0.9	44.6 ¥ 10^8	9.6	5.57 ¥ 10^8 8.75	35
8	6	9.0	9.5	0.5	22.0 ¥ 10^8	9.3	2.75 ¥ 10^8 8.44	64
12.5	20	7.9	9.1	1.2	11.9 ¥ 10^8	9.1	0.95 ¥ 10^8 7.98	78
8	12	8.5	9.2	0.7	12.8 ¥ 10^8	9.1	1.60 ¥ 10^8 8.2	65
4	10	8.3	9.3	1.0	18.0 ¥ 10^8	9.25	4.50 ¥ 10^8 8.65	66
8	38	7.7	9.0	1.3	9.5 ¥ 10^8	8.98	1.19 ¥ 10^8 8.07	67
15	8	8.8	9.5	0.7	25.6 ¥ 10^8	9.41	1.70 ¥ 10^8 8.23	68
15	4	9.2	10.1	0.9	110 ¥ 10^8	10.04	7.30 ¥ 10^8 8.86	68
4	12	9.4	9.86	0.46	47.0 ¥ 10^8	9.65	11.7 ¥ 10^8 9.07	69
2.75	11	8.0	9.0	1.0	10.0 ¥ 10^8	9.0	3.64 ¥ 10^8 8.56	70
20	17	7.9	8.8	0.9	5.6 ¥ 10^8	8.75	0.28 ¥ 10^8 7.45	71
		8.8	9.2	0.4	9.5 ¥ 10^8	8.96	0.47 ¥ 10^8 7.67	
5	8	8.1	9.0	0.9	8.7 ¥ 10^8	8.94	1.75 ¥ 10^8	72
10	8	8.0	9.5	1.5	31.0 ¥ 10^8	9.49	8.24	
20	8	8.2	9.5	1.3	30.4 ¥ 10^8	9.48	3.10 ¥ 10^8 8.5	
							1.52 ¥ 10^8 8.18	
9	12	10.66	10.99	0.33	600 ¥ 10^8		66 ¥ 10^8 9.78	80
8	8	8.6	9.4	0.8	21.0 ¥ 10^8	9.32	2.63 ¥ 10^8 8.42	73

-- continued

TABLE 9.4 (CONTINUED)
Summary of Quantitative Data on Prebiotic Effect of Inulin-Type Fructans — Results from All Human Intervention Studies Available

A Dose, g/d	Number of Volunteers	B Log_{10} cfu/g T_0	C Log_{10} cfu/g T_{max}	D C–B	E cfu/g T_{max} –cfu/g T_0	F Log_{10} D	Prebiotic Index E/A log_{10} E/A	Refs
5	8	8.8	9.8	1.0	57.6 ¥ 10^8	9.76	11.5 ¥ 10^8 9.06	74
6.6	31	9.1	9.6	0.5	27.4 ¥ 10^8	9.44	4.15 ¥ 10^8 8.62	75
8	9	8.8	9.0	0.2	3.6 ¥ 10^8	8.55	0.45 ¥ 10^8 7.65	76
9	10	9.9	10.3	0.4	119 ¥ 10^8	10.07	13.2 ¥ 10^8 9.12	26
8	10	5.6	8.4	2.8	2.5 ¥ 10^8	8.40	0.38 ¥ 10^8 7.58	77
Total	**296**							
Mean[1] (± sem)							**4.0 ¥ 10^8 (0.82 ¥ 10^8)**	

[1]This mean does not include the value calculated based on the Brighenti et al. (1999) data that are unusually high.

h or +9.5 ¥ 10^{10} cfu/24 h/g of inulin, respectively. By assuming that, on an average, the 24 h fecal output is 100 g, and that the bulking indexes of oligofructose and inulin are 1 and 2, respectively (see Chapter 6, Section 6.2.4.5), values of the same parameters for all the studies in Table 9.4 are estimated as 36 ¥ 10^{10} cfu/24 h and 4.5 ¥ 10^{10} cfu/24 h/g of inulin-type fructans, respectively.

9.5.3 Conclusions and Perspectives

As discussed above, inulin-type fructans are nondigestible oligosaccharides that are also very efficiently fermented by the anaerobic colonic microflora. Additionally, and as a result of selective fermentation, bifidobacteria (and, possibly, a few other genera) are preferentially stimulated to grow, causing significant changes in the composition of the gut microflora by increasing the number of potentially health-promoting bacteria and, eventually, by reducing the number of potentially harmful species. Thus, inulin-type fructans meet all the criteria introduced in the preceding text, and so they are prebiotic. They are also the most extensively tested food ingredient and, for that reason, they need to be considered as the "model prebiotic." As reviewed recently, the only other compounds that fulfill the criteria for prebiotic classification are the food ingredient transgalactooligosaccharides and the drug lactulose.[9] For a few other food ingredients, i.e., isomaltooligosaccharides, lactosucrose, xylooligosaccharides, soybean oligosaccharides, and glucooligosaccharides, though preliminary and promising data are available, the data are insufficient to classify

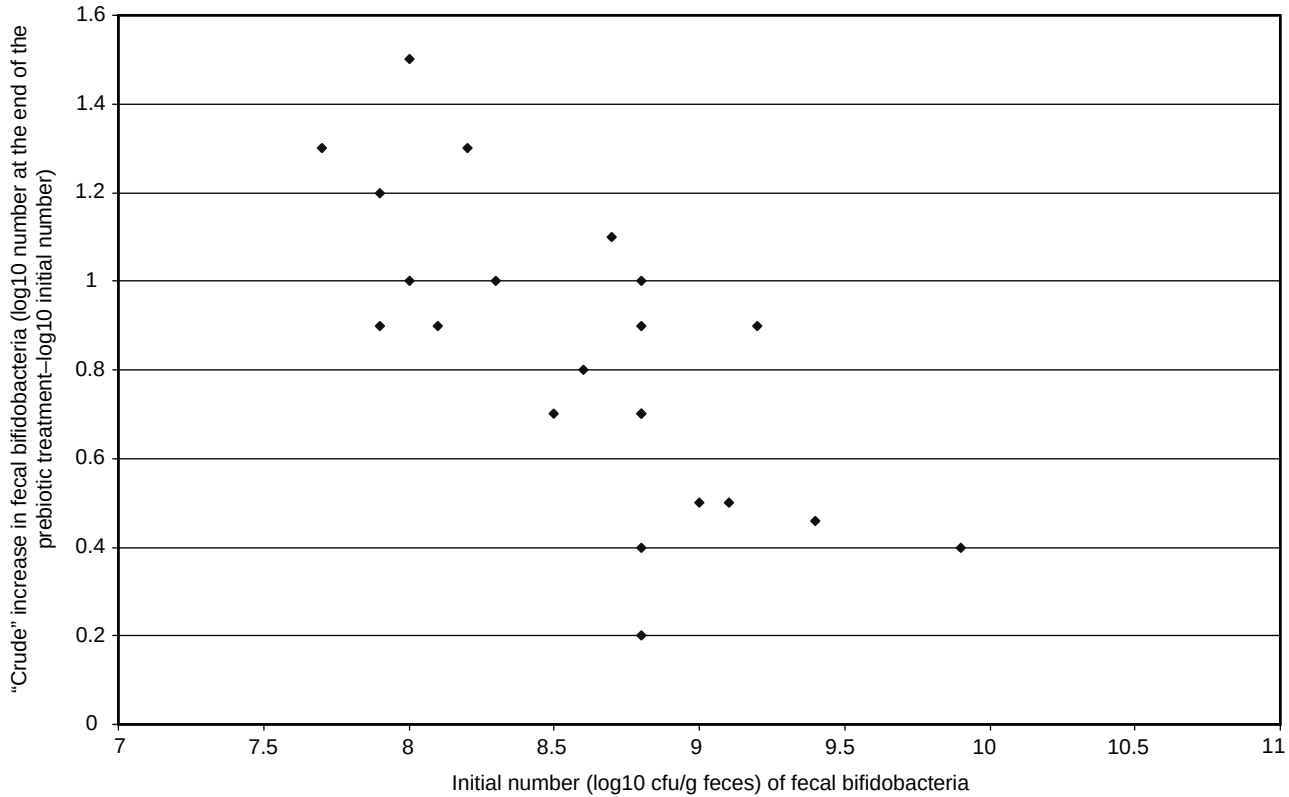

FIGURE 9.2 Correlation between the initial number (log10 cfu/g feces) and the "crude" increase in the number of fecal bifidobacteria (log10 number at the end of the prebiotic treatment period–log10 initial number).

them as prebiotic.[9] Prebiotic potential has also been claimed for several other oligosaccharides. However, at present, evidence pointing towards any prebiotic effect is too sparse to justify their classification as prebiotic. These compounds include gentiooligosaccharides,[61] lactose,[91] mannan oligosaccharides,[93,94] oligodextrans,[95] oligosaccharides from melibiose,[96] pectic-oligosaccharides,[97] and resistant starch and its derivatives. [98–100]

Some preliminary data support the hypothesis that the prebiotic effect of inulin-type fructans might not be limited to bifidobacteria but might also affect other populations of bacteria (like lactobacilli, members of the *Clostridium coccoides–Eubacterium rectale* cluster, or even species or genera that are not yet fully characterized.[55,60] The likelihood of other bacteria also being the target for a prebiotic effect must be put in perspective with our increasing understanding (thanks to new molecular methodologies) of the bacterial diversity in the gut microflora. Indeed, the more we identify and characterize the bacterial genera, species, and even strains that make up the intestinal microflora, the more we will be in a position to describe, both qualitatively and quantitatively, changes in that composition and, consequently, to understand how the myriads of bacterial cells in the intestine interact and how they contribute to and modulate intestinal (especially colonic) physiology. Prebiotics will then become unique tools to create, both in experimental animals and in humans, colonic microflora with "controlled" compositions that will then be correlated with specific physiological conditions. But data are still too preliminary to speculate on these perspectives. The present discussion has thus concentrated on the effect of inulin-type fructans on bifidobacteria, i.e., the so-called *bifidogenic effect*.

At the present stage of knowledge, both oligofructose and inulin are equally active as prebiotics, but it cannot be excluded that inulin-type fructans with different chain lengths or different mixtures of these compounds can influence differently the microflora in the different segments of the intestine, especially in the large bowel. Because these different parts of the gastrointestinal tract are likely to have different physiological roles, specifically located prebiotic effects of particular compounds or mixtures might be of great physiological importance.[1]

The prebiotic effect of inulin-type fructans appears rapidly and lasts for as long as the fructans are ingested. But studies so far performed are limited in time (up to a few months), and it would be of interest to test the effect of much longer administration periods, e.g., up to a few months or even a few years.

The daily dose of the prebiotic is not a determinant of the prebiotic effect; even if, in one group of volunteers with relatively similar initial counts of fecal bifidobacteria, a limited dose–effect relationship has been established.[72] The daily dose does not correlate with the crude or absolute increase in bacterial cells. The major factor that quantitatively controls the prebiotic effect is the number of bifidobacteria per gram of feces the volunteers have before the supplementation of the diet with inulin-type fructans begins. This parameter correlates inversely with the crude increase in fecal bifidobacteria. At the population level it is, thus, the fecal flora composition (especially the number of bifidobacteria) characteristics of each individual that determines the efficacy of a prebiotic and not necessarily the dose itself. The ingested prebiotic stimulates the whole indigenous population of bifidobacteria to growth, and the larger that population, the larger the number of new bacterial

cells appearing in feces. The "dose argument" (often used for marketing some prebiotics) is, thus, not straightforward and cannot be generalized because, as supported by scientific data, the factors controlling the prebiotic effect are multiple. The "dose argument" can be misleading for consumers and should not be allowed.

One important question, still unanswered, is the effect of a prebiotic (especially inulin-type fructans) not on the numbers of bacteria (especially bifidobacteria) but rather, on the activities associated with these bacteria. Indeed, the health benefits for the host are part of the definition ("confers benefits upon host well-being and health"),[2,9] and these benefits are directly dependent on what these bacteria do, how they interact with other bacteria, and how they modulate intestinal functions. Miscellaneous activities of bacterial enzymes such as glucuronidase, glucosidases, and nitroreductase; metabolites such as SCFAs; or end products of the fermentation of amino acids, mucins, or sterols (especially, primary and secondary bile acids) have been measured and shown to vary (increase or decrease) after consuming prebiotics. But the validity of these parameters still remains to be established, especially with regard to their value as biomarkers of colonic, and, eventually, of host health and well-being or of disease-risk reduction. In this context, the effects of inulin-type fructans on the parameters reported so far are contradictory and difficult to interpret.[56,69,71]

The concept of prebiotics is only 8 years old, has already attracted and stimulated research in many fields such as nutrition and medical sciences. In particular, the prebiotic effect of inulin-type fructans has been confirmed in numerous *in-vitro* experiments and in laboratory as well as human trials. New developments in molecular microbiology will allow more similar studies specifically targeted at answering important unsolved questions. In particular, they will help determine health applications, and explain mechanisms of effect. A further desirable attribute of prebiotics is the ability to act ever in the most distal region of the colon, known to be the site of origin of several chronic diseases including colon cancer and ulcerative colitis. There is thus, currently, much scientific interest in developing prebiotics that target this region of the colon. A mixture of inulin-type fructans known as oligofructose-enriched Synergy 1 has been developed with that aim.

In the paper introducing the concept of prebiotics, Gibson and Roberfroid also suggested that combining a prebiotic with a probiotic in a "synbiotic" approach could open new perspectives.[2] But, up to now, the synbiotic concept has not really been tested. In *in vitro* experiments designed to test the inhibitory effect of probiotics on the growth of human intestinal pathogens (*E. coli, Campylobacter jejuni, and Salmonella enteritidis*), Fooks and Gibson showed that compared with other carbohydrates (lactulose, lactitol, dextran, and starch), inulin-type fructans (alone or combined with xylooligosaccharides) were observed to strongly support the inhibitory activity.[101] In a rat model, Bielecka et al. showed that combining a probiotic (10^9 *Bifidobacterium* spp. per rat) and oligofructose (5% w/w in diet) did not improve the prebiotic effect.[57] The human study reported by Bouhnik et al. has already been discussed (see Section 9.4.2).[81] A few other synbiotic protocols have also been used in experimental carcinogenesis in rats, in the human EU-funded SYNCAN project,[102] and in the development of animal feed. These data will be discussed in the chapters covering these topics.

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10 Inulin-Type Fructans and the Intestinal Absorption of Minerals

10.1 INTRODUCTION

Bone mass is the result of a constant balance between breakdown and formation under the control of several factors that can be grouped into two categories:

1. Genetics, ethnicity, gender, age, and body (frame) size that cannot be modified
2. Hormonal status (especially sex and calcitropic hormone status), lifestyle factors (including diet), physical activity and levels, and smoking and alcohol consumption patterns that can be modulated or changed

The interaction of these factors influences both the development of bone during childhood and adolescence until it reaches its peak mass at maturity and its subsequent slow loss later in life. Genetic factors probably account for up to 80% of the bone mass variation in the population.[1] Even though lifestyle factors, especially diet and physical activity, have a smaller influence than genetics on bone mass, those can be modified and targeted to modulate the achievement of maximum genetic potential peak bone mass during adolescence, as well as the subsequent rate of bone loss.[2]

The age-related changes in bone mass parallel those occurring in the bone mineral mass composed primarily of calcium, phosphorus, magnesium, and zinc. By maturity, these bone mineral masses are approximately 1200 g Ca, 500 g P, 15 g Mg, and 0.5 g Zn, i.e., 99%, 80%, 60%, and 30% of the total body mineral content.[3] Among all these factors Ca and Mg also play major roles because they are required for normal growth and development of the skeleton.[4,5] Moreover, both Ca and Mg are key physiological factors that regulate and participate in a number of essential metabolic processes.

10.2 THE PHYSIOLOGY OF CALCIUM

10.2.1 Calcium Metabolism[6]

Ca is an essential nutrient. It is absorbed in the intestine, it is used to build bones, and, to a lesser extent, teeth. It maintains plasma and extra- and intracellular pools, and it is excreted via the feces, the urine, and to some extent from the skin and the hair (Figure 10.1).

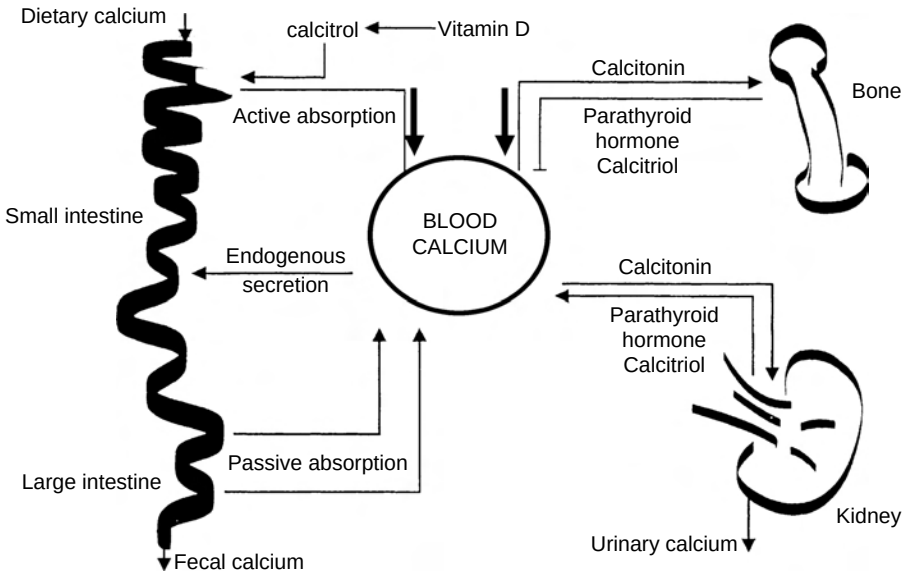

FIGURE 10.1 Schematic representation of Ca homeostasis in the human body.

The mineralized tissues contain 99.9% of all body Ca, and the remaining 0.1% is found in plasma and extracellular fluids, as well as inside all types of eucaryotic cells. In plasma, total Ca consists of 3 fractions (i.e., about 45% as protein-bound; about 10% as citrate, phosphate, and bicarbonate salts; about 45% as free Ca ions). The normal plasma concentrations of total and ionized Ca are 2.3–2.5 and 1.1–1.3 m*M*, respectively. The Ca in plasma, extracellular fluids, and cells plays major roles in modulating the activity of a wide range of enzymes and transport or regulatory proteins, especially in mediating vascular contraction and vasodilatation, muscle contraction, nerve transmission, and glandular secretion.[7] The physiology of Ca is under close homeostatic control with processes such as absorption, excretion and secretion, and storage in bone being involved in maintaining the concentration of ionized Ca in the plasma within a tightly regulated range. This tight regulation of plasma Ca concentration is achieved through a complex physiological system comprising the interaction of the calcitropic hormones such as the parathyroid hormone, 1,25 dihydroxycholecalciferol (or 1,25 dihydroxy-vitamin D3), and calcitonin, with specific target tissues (kidney, bone, and intestine) that serve to increase or to decrease the entry of Ca into the extracellular space. The secretion of these hormones is governed wholly, or in part, by the plasma concentration of ionized Ca, thus forming a negative feedback system. Parathyroid hormone and 1,25 dihydroxycholecalciferol are secreted when plasma Ca is low, while calcitonin is secreted when plasma Ca is high.

The Ca pools in the body are in balance when absorption equates with losses. During growth, more Ca is added to the pools than is lost, and the balance is positive; in adult life absorption and losses are equal; with increasing age, losses are not anymore compensated for by absorption, and the balance progressively becomes negative.

Two main absorption processes exist in the intestine, namely an active and a passive (or simple diffusion) absorption process. The active transport is essentially localized to the upper duodenum but a small part also takes place in the colon. It operates already at low Ca concentration, and it is mediated via the metabolic product of vitamin D (i.e., 1,25-dihydroxycholecalciferol) which stimulates the synthesis of a calcium-binding protein (Ca-binding proteins) that carries the Ca across the gut wall. The passive absorption which involves paracellular as well as transcellular moves through and between the mucosal cells, occurs in all parts of the gut, but mainly in the large bowel. It accounts for most Ca absorption when Ca intake is adequate or high[8] and helps salvage Ca that has escaped absorption in the small intestine. It is controlled by osmolarity in the gut lumen and in the extracellular fluids, but also by the intraluminal pH, by the presence of specific compounds (e.g., SCFAs), by the concentration of Ca in the gut lumen, and eventually by the presence of a Ca carrier protein, namely calbindin. It is independent of age or vitamin D intake. The passive intestinal paracellular Ca flux can increase if the space between cells, i.e., between the tight and gap junctions increases.[9] The colonic component of Ca absorption (±10%) is independent of the efficiency of the small intestinal absorption, and thus it is proportionately larger in low as compared to high small intestinal absorbers.[10] It is also influenced by the metabolic activity of the colonic flora that controls Ca speciation and solubility on the one hand and mucosal transport pathway on the other hand.[11]

Total Ca absorption efficiency adapts to physiological conditions (higher during growth and adolescence and pregnancy but lower after 50 years of age) as well as to intake (greater at low levels of Ca intake). Usually approximately 30–35% of Ca in food is absorbed but it can be higher or lower, depending on food composition but also probably on genetic factors.

The fecal Ca pool represents the biggest proportion of excreted Ca. It is composed of the nonabsorbed dietary Ca plus the endogenous Ca that is secreted in the gastrointestinal tract (from saliva, pancreas, bile, or intestinal mucosa). Once Ca has reached the bloodstream, the main excretion route is the urine (plus very small losses through hair and skin, and in sweat) but the kidney reabsorbs 98–99% of all Ca that it filters. The amount of Ca excreted in the urine varies considerably with age (reduced excretion in old age), sex (men excrete more than women, who excrete more after than before menopause) but also with dietary habits (high with high intakes of sodium and protein but low with high intakes of phosphorus).

10.2.2 Calcium Intake and Bone Health

The Ca found in mineralized tissues, bones, and teeth (99.9% of all body Ca) is in the form of a phosphate salt, namely hydroxyapatite or $Ca_{10}(PO4)_6(OH)_2$ (together with a small component of carbonate salt) that provides rigidity and structure.[5]

During skeletal growth and maturation, i.e., until the early 20s in humans, Ca accumulates in the skeleton at an average rate of 150 mg/d. During maturity, the body, and therefore the skeleton, is more or less in Ca equilibrium. From the age of about 50 in men and from menopause in women, bone balance becomes negative and bone is lost from all skeletal sites. This bone loss is associated with a marked

rise in fracture rates in both sexes, but particularly in women. Adequate Ca intake is critical to achieving optimal peak bone mass during adolescence and modifying the rate of bone loss associated with ageing.[12]

The major disease risk associated with inadequate bone health is osteoporosis, a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture.[3] Because of the increased risk of fragility fractures (particularly hip, wrist, and spine fractures), osteoporosis is a major health problem, especially in Europe and North America. From incidence rates of fragility fracture at one of these three sites in North America, Melton et al. have estimated that the lifetime risk among white women and men aged 50 years is 40% and 13%, respectively.[13] Moreover, bone fractures are often associated with considerable morbidity and hip fractures may lead to an overall reduction in survival of around 15%.

In a public health strategy to improve well-being and health and to reduce the risk of disease, the development of maximal bone mass during growth and reduction of loss of bone later in life are thus two important targets. A large number of macro- and micronutrients have been proposed as possible determinants of bone health but most approaches have concentrated on dietary Ca. At the same time, calcium is the most likely bone nutrient to be inadequate in terms of dietary intake.[14]

In recent years, convincing evidence has emerged with respect to effects of dietary Ca on bone health in all age groups[15] and the findings of many of these controlled Ca intervention trials have been reviewed.[7,16–18]

The major conclusions of these trials are as follows:

1. In children and adolescents:
   - A positive effect of Ca intake on bone mass has been reported in intervention and cross-sectional studies.[19–21]
   - Dietary Ca intake in childhood and adolescence was positively related to bone mineral density in young women.[22]
   - Ca supplementation, typically of 1 to 2 years' duration, have shown a higher rate of accrual of bone mass (approximately +1.5% as measured by bone mineral content or bone mineral density) when Ca intake is increased.[20,23–29]
2. In premenopausal women:
   - A meta-analysis (based on 33 published studies) concluded that there was an overall association between dietary Ca intake and bone mass.[30]
   - Increasing dietary Ca intake above that usually consumed was shown to have benefits for the development and maintenance of bone health, and to reduce the risk of osteoporosis in later life.[31]
3. In postmenopausal women:
   - Increasing dietary Ca intake does not prevent bone loss but rather reduces the rate of bone loss to some extent. However, the effectiveness of Ca varies by skeletal site, by menopausal age, and with usual Ca intakes.[7] For example, an increase in Ca intake for women during the first 5 years of menopause (the period of most rapid bone loss) does not retard bone loss from trabecular regions of the skeleton, including

those most vulnerable to osteoporotic fracture[32–34] but reduces the risk of bone loss in cortical regions.[32–36]
- Women who are more than 5 years past menopause tend to be more responsive.[37–40]
- Women with very low Ca intakes generally gain more from Ca supplementation than do women with higher than usual Ca intakes.[33,34]
- Increasing Ca intake above 750 mg,[39] 800 mg,[41] or 1000 mg,[32] reduces loss of bone mineral from cortical-rich sites, such as the proximal radius, femoral neck, and total body. But increasing Ca intake has little effect on spinal-bone mineral in older women.[38,40,41]

But, and besides the number and the quality of all these studies, the real meaning of the effects of Ca on bone (especially the long-term significance) is still unknown.[17] Especially, and in the absence of longitudinal studies of sufficient duration, it is not clear whether additional Ca intake throughout early life still results in increased peak bone mass in adulthood. This question is of great significance since peak bone mass in adulthood is predictive of bone mass, and therefore osteoporosis risk, in later life.[42] Moreover, the significance of the reduction in the rate of bone loss observed in the Ca supplementation studies in postmenopausal women is still debated. A meta-analysis of Ca supplementation trials confirmed that Ca supplementation reduces bone loss, but the effects were only significant in the first year of supplementation.[43]

10.2.3 Calcium Requirements and Recommendations

The development and maintenance of bone is the major determinant of Ca needs. The highest proportion of body Ca is in bone that functions as the major reservoir. However, Ca is a "threshold" nutrient, i.e., at suboptimal intakes, the ability of the body to store Ca as bone tissue is limited by the intake of Ca, but increasing Ca intake above that required as optimum does not further increase the stores.[44] The quantitative requirement of Ca, unlike that of most other nurients, does not relate to a metabolic function. Moreover, and because of the small metabolic pool of Ca (less than 0.1% in the extracellular fluid compartment) relative to the large skeletal reserve, for all practical purposes, metabolic Ca deficiency probably never exists, at least not as a nutritional disorder. If there is a chronic Ca deficiency resulting from a continual inadequate intake or poor intestinal absorption, circulating Ca concentration is maintained via three compensatory mechanisms that are all triggered by an increased synthesis of parathyroid hormone:

- An increased rate of bone resorption
- An increased reabsorption of Ca in the kidney distal tubules
- A stimulation of the production of 1,25 dihydroxycholecalciferol that increases intestinal Ca absorption

Because of the importance of the metabolic roles of Ca, these compensatory mechanisms are very efficient and function within minutes to hours.[2,31]

The increased rate of bone resorption in chronic Ca deficiency is one of several important causes of reduced bone mass that may cause osteoporosis.[7,12,45] Indeed, the cumulative effect of Ca depletion on the skeleton over many years contributes to the increasing frequency of osteoporotic fractures with age.[31]

Because of the variation in body growth at different ages, Ca requirements vary throughout an individual's life, with greater needs during the periods of rapid growth in childhood and adolescence. Requirements also become higher during pregnancy and lactation, and in later life when bone resorption increase. All this is reflected in recommended Ca intake values that vary with age. Both genetic (e.g., bone architecture and geometry and responsiveness of bone to hormones) and environmental (e.g., dietary constituents and the degree of mechanical loading imposed on the skeleton in everyday life) factors influence Ca requirements.[44] Because of their effects on urinary Ca losses, high intakes of both sodium and protein increase dietary Ca requirements.[44,46,47]

Because of different interpretations of available human Ca balance data, there is considerable disagreement on human Ca requirements and thus on recommended Ca intakes made by different expert authorities that vary widely. For example, expert committees in the U.S. and EU have established very different recommendations for Ca intake.[7,15]

By assuming that reduced bone mass (as measured by bone mineral density or bone mineral content) is a good indicator of low Ca status, it has been possible to sample various populations and estimate that this situation is common in western countries.[48] Because life expectancy in these countries is increasing, it is anticipated that this shall be true for an even larger proportion of the population in the future.[13] However, it should be noted that there are a number of contributory factors to this besides dietary Ca deficiency (e.g., altered hormonal status associated with amenorrhoea or menopause and physical inactivity). Moreover, estimates of Ca intakes from foods usually provide an underestimate of Ca intake due to underreporting of food intakes in self-reported food consumption surveys that usually do not include the contribution of supplements, medicines, or drinking water. Nevertheless, and based on recommended intakes for the individual countries, it has been estimated that, even in a number of Western countries where recommended intakes are relatively low, a significant proportion of some population groups fails to achieve the recommended Ca intakes. For example:

- In Germany about half of adult women and one third of adult men have Ca intakes lower than recommended.[49,50]
- In Ireland, over 50% of females aged 12 to 18 years fail to achieve the recommended Ca intake.[51]
- In Italy, 50% of elderly subjects (>60 years) do not meet the recommended allowance for Ca.[50]
- In the Netherlands, a significant proportion (8–25%) of adult males and females fail to achieve even 80% of the recommended allowance for Ca.[50]
- In Switzerland, a large proportion of adult women fail to achieve the recommended Ca intake.[50]

- In the U.K. and for females, 13–18% of ages 14–34 and 8–15% of those over 65 years have habitual Ca intakes less than the lower reference nutrient intake, a level below which intake is almost certainly deficient.[17]
- In the U.S., most females aged 9–18 years and from 31 years onwards fail to achieve the recommended Ca intake.[52]

10.2.4 Improving Calcium Intakes and Calcium Bioavailability in the Population[18]

The dietary deficiency of Ca identified in some population groups may be addressed in a number of ways. This includes changing eating behavior at the population level by increasing the consumption of foods that are naturally rich in Ca (e.g., milk and milk products), Ca fortification of foods consumed by target groups, or increasing Ca intakes from Ca supplements. These may be seen as complementary rather than alternative strategies, and each has advantages and disadvantages.[31] For example, it is notoriously difficult to achieve changes in the diet of entire populations, and thus persuading individuals to consume more dairy produce represents a considerable challenge. The use of Ca supplements can be effective in increasing Ca intake in individuals who consume them regularly, but it has limited effectiveness at the population level due to the poor compliance with supplement use.[31] Ca-fortified food products could provide additional choices for meeting Ca requirements; however, attention should be paid to the selection of products so that they reach the target groups (i.e., those population groups who have the greatest difficulty in meeting Ca requirements). Moreover, Ca intake cannot be increased unlimitedly because, as recommended by the National Institutes of Health (NIH) Consensus Conference on Optimal Calcium Intake, "practices that might encourage total calcium intake to approach or exceed 2000 mg/d seem more likely to produce adverse effects and should be monitored closely."[12] That topic has recently been reviewed by Whiting and Wood who cautioned that supplemental foods should "prevent overconsumption of calcium while still promoting improved calcium nutriture…."[53]

Besides the amount of Ca in the diet, the absorption of dietary Ca in foods is also a critical factor in determining the availability of Ca for bone development and maintenance. Thus, and because dietary factors that alter Ca absorption also modify Ca retention,[54] there is a need to identify food components and/or functional food ingredients that may enhance Ca absorption in order to optimise Ca bioavailability from foods.[54,55] As stated by Weaver and Liebman:

> Increasing para cellular [*sic*] absorption is promising because it is not limited by becoming saturated, it is vitamin D independent, and it occurs throughout the length of the intestine in contrast to active absorption which is dominant in the duodenum. [º] Ideal compounds would be those that could be incorporated into Ca-containing food to enhance absorption of Ca but would have only transient effect, so that transfer of undesirable organisms and ions would be minimized.[54]

A number of food constituents have attracted attention as potential enhancers of Ca absorption like lactose, casein phosphopeptides,[56,57] and nondigestible

oligosaccharides.[11,18] According to Berrocal et al., the phosphopeptides have the capacity to chelate Ca and to prevent the precipitation of its phosphate salts, thus helping to maintain a high concentration of soluble Ca in the intestinal lumen.[58] There is some experimental, but only limited, human evidence that casein phosphopeptides increase Ca absorption.[42,44,59,60] Therefore, the significance of these phosphopeptides for enhancing Ca absorption in humans remains unclear. Ziegler and Fomon showed that Ca absorption in human infants was significantly higher from a soy-based infant formula containing lactose as compared to a similar formula containing a mixture of starch hydrolysate and sucrose.[61] Enhancement of Ca absorption by lactose has also been reported in rats.[62–64] However, studies on the effect of lactose on Ca absorption in human adults generally have failed to demonstrate this effect. Miller, in a critical review of this area, concluded that it is likely that lactose enhances Ca absorption in human infants and in rats, while, at levels normally present in milk, lactose does not have a significant effect on Ca absorption by healthy adults consuming normal diets.[65] Recently, Van den Heuvel et al. have reported that consumption of lactulose (5 or 10 g/d) or transgalactooligosaccharides (20 g/d) increased Ca absorption in postmenopausal women in a dose-responsive manner.[66]

Increasing Ca absorption (either directly or indirectly via an increase in Ca intake) also affects bone turnover, e.g., by down-regulating bone resorption[67] and leads to increased Ca retention.

10.3 THE PHYSIOLOGY OF MAGNESIUM[68,69]

10.3.1 Magnesium Metabolism

Mg, the second intracellular cation, is an essential nutrient that plays a role in most metabolic pathways as well as in the ionic equilibrium of the cell membranes. It is absorbed in the intestine, it is used to build bones, it maintains the plasma, extra- and intracellular pools, and it is excreted via the urine. The mineralized tissues contain 60–65% of all body Mg (±25 g) and, of the remaining 35–40%, ±30% is found in muscle and ±7% inside the eukaryotic cells whereas plasma and extracellular fluids contain only 1% of total body content. In plasma total Mg consists of 3 fractions (i.e., about 30% as protein-bound, 13% as salts or chelates, and about 55% as free Mg ions). The normal plasma concentrations of total and ionized Mg are 0.7–0.9 and 0.4–0.45 m*M*, respectively. The intracellular Mg plays major roles in controlling the activity of a wide range of coenzymes (especially ATP) and enzymes (±300 enzymes are Mg dependent) as well as transport or regulatory proteins. Mg deficiency is associated with neuromuscular excitability, muscular discomfort changes in phosphocalcic metabolism, and potassium deficit. Various hormones (e.g., parathyroid hormone, calcitonin, growth hormone, aldosterone, and vitamin D) affect Mg metabolism, but there is no evidence of a specific Mg-regulating hormone. Mg homeostasis (especially Mg plasma concentration) results from a balance between gastrointestinal uptake and renal filtration-reabsorption and excretion processes.

The Mg pools in the body are in balance when absorption equates with losses. However and despite extensive research on Mg absorption, the exact site and mechanism of Mg absorption are still largely unknown.[70] The major site of absorption is

the distal small intestine (jejunum and ileum) but colonic absorption is also significant. Two main absorption processes exist, namely an active and a passive (or simple diffusion) absorption process, but the second process is probably much more important than the former. The active transport operates essentially at low Mg concentration. The passive absorption involves paracellular moves between the mucosal cells and accounts for most Mg absorption when Mg intake is adequate or high.[70] It is essentially controlled by osmolarity in the gut lumen and in the extracellular fluids, but also by the intraluminal pH, by the presence of specific compounds (e.g., SCFAs), and by the concentration of Mg in the gut lumen. It is independent of age or vitamin D intake. Usually, approximately 30–50% of Mg in food is absorbed but it can be higher or lower, depending on food composition, especially its daily intake (i.e., fractional absorption is ±70% and ±12% when intake is ±25 and ±1000 mg/d, respectively). In particular, it has been shown that increasing the amount of dietary proteins raises the apparent Mg absorption.

The fecal Mg pool is composed of the nonabsorbed dietary Mg. Indeed, once Mg has reached the bloodstream, the main excretion route is the kidney. But, as the filtration–reabsorption process functions close to saturation, the Mg that is absorbed in excess is easily excreted in the urine.

10.3.2 Magnesium Requirements and Recommendations

The main dietary sources of Mg are cereals and chocolate, but it is widely distributed in most foods. Estimations of the daily requirements vary from 100 to 1000 mg[71,72] and the daily intake in adults varies between 250 and 500 mg.[73] In France, the recommended daily intake is 6 mg/kg/d, but some 18% of men and 23% of women have an intake lower than 4 mg/kg/d. However, such an apparently low value is still in agreement with the recommendation of the European Community, i.e., 3.4 mg/kg/d for adults.[74] The recommendations of the National Research Council in the U.S. are 280–350 mg/d or ±5 mg/kg/d for young women and men.[4]

10.4 METHODOLOGIES FOR THE STUDY OF MINERAL ABSORPTION AND BONE HEALTH

10.4.1 Methodologies for the Study of Ca and Mg Absorption

10.4.1.1 Metabolic Balance Studies

Metabolic balance studies can be performed both in experimental animals and in humans. Metabolic balance is based on the calculation of net Ca or Mg retention as

$$\text{intake (IN)} - \text{excretion (OUT)}$$

over a specific period of time. It is used to determine apparent net absorption but not true absorption. Indeed, disappearance of a mineral during mouth-to-anus transit time does not equate true absorption because minerals, including Ca or Mg, can be

retained in the intestine (due to, e.g., adsorption on insoluble particles, adsorption, or chelation in mucins). Moreover, endogenous mineral ions can be secreted in the gastrointestinal lumen and subsequently excreted in feces via the bile, the pancreatic, or mucosal secretions, or because of mucosal cell loss.

Most studies on Ca/Mg absorption in animals (essentially rats) used the metabolic balance methodology. Animals are kept in metabolic cages for a few days (usually 3–5), food intake is strictly controlled or food is restricted (±90% of daily intake) so as to force the rats to eat all the food and urine plus feces are quantitatively collected. Ca/Mg in food (IN) and excreta (OUT) is measured by atomic absorption spectrometry or inductive coupled plasma emission spectrometry (ICPS). A nonabsorbable marker (e.g., chromium-mordanted cellulose[75]) can be added in food to calculate apparent absorption. In one protocol Ca pool was labeled with a mixture of the radioactive isotopes ^{47}Ca and ^{47}Sc, the latter being used as a nonabsorbable marker, and the radioactivity is measured in feces and urine.[63,76] In order to distinguish between absorption in the whole gastrointestinal tract and in the large bowel, some authors calculated Ca/Mg absorption after oral administration or infusion in the stomach and infusion directly in the cecum of $CaCO_3$, MgO, or $MgCl_2$.[76,77] In the last condition, Ca/Mg absorption taking place in the large bowel only can be calculated. Using a more sophisticated protocol that includes the measurement of cecal blood flow and arteriovenous differences across the cecum, together with the cecal concentration and cecal pool of Ca/Mg, it is also possible to assess the kinetic of Ca/Mg absorption expressed as mmol/min.[78,79]

In humans, even rigorously controlled balance studies will only distinguish large differences (e.g., 200 mg retention per day) unless large numbers of volunteers are included in the protocol. The greatest difficulties when performing such studies are in assessing, with precision and compliance, consumption of the diet and precise Ca or Mg intake, in collecting the totality of urine and feces, and in failing to measure other losses including dermal losses that might account for ±60 mg/d. The best balance studies are those that are conducted in metabolic wards where subjects' activities are monitored, especially food consumption and excreta collection. In experimental studies, the animals are kept in metabolic cages during the whole period of test usually after a pretest adaptation.

10.4.1.2 Tracer Studies

Tracer studies have rarely been performed in experimental animals. One such study was aimed at quantifying the effect of oligofructose on true Ca absorption in rats.[80] It combined the Ca balance approach with a ^{45}Ca kinetics method. Using that method it is possible to analyze the movement of Ca in the intestine, the kidneys, and the bone with the aim to better understand the mechanism of changes in Ca absorption.[81] The kinetics method involves intravenous injection of $^{45}CaCl_2$ solution followed by blood samplings and urine plus feces collection at regular time intervals over a 3-d period and liquid scintillation counting of the radioactivity. Various parameters are then calculated, i.e., endogenous net Ca excretion, urinary Ca, bone resorption, and bone formation.

In human subjects, tracer study is the method of choice to study Ca or Mg bioavailability, i.e., the fraction of ingested ion that is absorbed and utilized for normal physiological functions and storage.[82] Isotope tracer data are less variable than balance data and thus more subtle analysis can be made and considerably more information can be obtained. Tracers are also applicable for kinetic studies that allow study of the movement of Ca or Mg from one compartment to the other, rates of transfer, and body pool size. However, short time tracer studies are not suitable to measure total Ca body pool size because the rate of Ca exchange in bone is too slow. These studies can be used to measure bone turnover based on kinetic analysis that allow quantifying bone formation and bone resorption expressed in mg Ca or Mg/d.[54]

One key step in tracer studies is the mixing up of the isotopes. The method used to incorporate the isotope in food to be tested for bioavailability deserves thoughtful consideration. Two techniques are available:

1. Intrinsic labeling that incorporates a particular isotope during plant or animal growth or during chemical synthesis of a particular product.
2. Extrinsic labeling that involves mixing a soluble salt of the Ca or Mg isotope with the food to be tested. This assumes that the tracer has adequately exchanged with endogenous minerals. It is simple and frequently used, and it allows a fairly good approximation of Ca or Mg absorption.

Tracers can be radioactive or stable isotopes, and a series of isotopes are used to study Ca or Mg bioavailability. With one exception (i.e., the radioactive ^{41}Ca) and for obvious safety reasons, stable isotopes (^{42}Ca, ^{43}Ca or ^{44}Ca, and ^{25}Mg or ^{26}Mg) are preferred for human studies.

In the tracer studies, isotopes are administered orally (po) and intravenously (iv) in doses equivalent to ±10% of the circulating Ca or Mg in order not to disturb the normal movement of the minerals. The best method to assess changes in Ca or Mg bioavailability is the dual isotope procedure in which two different isotopes are administered po and iv, respectively, and isotope ratio measurements are made on urine and fecal samples collected as for a balance study as well as in plasma or serum samples.[83,84] The oral isotope labels the dietary Ca or Mg and is used to measure their absorption whereas the intravenous isotope allows to measures the removal of Ca or Mg from the blood. The analytical methods of choice currently used in such studies are thermal ionization magnetic sector mass spectrometry (TIMS)[85] and high resolution, inductively coupled plasma mass spectrometry (HR-ICPMS).[86]

Another interesting method uses the long-lived radioisotope ^{41}Ca that can be used in such small doses (<100 nCi) that it can be considered safe. Use of just a single dose of this rare isotope of Ca opens the possibility of determining types of diet changes that might suppress bone resorption in individuals followed longitudinally. Indeed, approximately 2 months after dosing the appearance of ^{41}Ca in the urine directly reflects bone resorption. Urinary ^{41}Ca is measured by the highly sensitive accelerator mass spectrometry that allows the tracer to be followed for years, thereby allowing assessments of the effect of changes in diets and other lifestyle factors.[54]

10.4.1.3 Kinetics of Urinary Ca Excretion

Some groups have used a methodology whereby Ca absorption was indirectly assessed by following the kinetics of urinary Ca excretion after a controlled Ca intake.[87–90] In three such studies, the authors measured total urinary Ca [87,88,90] whereas in another study they added ^{44}Ca to the test food and measured total Ca and the ratio $^{44}Ca/^{43}Ca$ to assess more precisely if the urinary Ca had indeed originated from the Ca in food.[89] In such studies, the fasted volunteers were given a breakfast containing a dose of Ca combined with either oligofructose (3 g/d or a ratio Ca/oligofructose of 10:1),[87,89] inulin (15 g/d),[88] or inulin (5 g/8 oz) as part of a complex beverage,[90] and urine was collected at 2 h intervals for up to 8, 12, or even 72 h.

10.4.2 Methodologies for the Study of Bone Health

10.4.2.1 Biochemical Markers of Bone Turnover[3,91]

Besides the tracer methodologies discussed above to assess bone turnover based on quantitative measurements of bone formation and bone resorption, biochemical markers also exist that can be used to determine qualitative changes in bone turnover. Changes in these markers can be used as an early and sensitive sign to monitor an effect on bone. Because of the time required for the skeleton to achieve a new steady state, long-term studies to assess the sustainability of the effect must still be performed before drawing any conclusion. Even though methodologies to measure these markers are often easy and reproducible, they remain poorly specific, and their connections with the functional outcome has not yet been validated. The biomarkers of bone formation assess the synthetic activity of the osteoblasts and especially the metabolism of procollagen, the main anabolic product of these cells. The most commonly used markers of bone formation are serum osteocalcin, bone alkaline phosphatase activity, and procollagen propeptides. Osteocalcin is an indicator of oteoblastic activity but the peptide is unstable and immunologically heterogeneous. Its fully functioning form requires a metabolic vitamin-K-dependent gamma-carboxylation. No internationally validated assay exists today. The activity of bone alkaline phosphatase can easily be measured with the recently developed capture immunoassay that is robust and sensitive to changes in bone metabolism. Procollagen is a complex mixture of propeptides, and there remains some controversy about which molecular forms should be measured. The biomarkers of bone resorption assess the activity of the osteoclasts and especially their capacity to catabolize collagen which represents over 90% of bone matrix proteins. These markers are serum tartrate resistant acid phosphatase, an enzyme synthesized by the osteoclasts to catalyze the first step in matrix resorption, and a series of analytes derived from collagen type I. These include the pyridinium collagen cross links (deoxypyridinoline DPD and pyrilidoline PYD) and the telopeptides associated with cross linking at either the N-terminal (NTX) or C-terminal (CTX) which are usually measured in the urine. The results of these assays are expressed relative to creatinine but they are not in units of bone or Ca and they are highly variable. It is difficult to detect subtle effects of food components unless large cohorts of volunteers are used.

The possible use of the biomarkers of bone turnover in the substantiation of health claims for functional foods or functional food ingredients has been evaluated recently by one of the Individual Theme Groups (i.e., ITGB) in the EU-funded concerted action "Process for the Assessment of Scientific Support for Claims on Foods" (PASSCLAIM — QLK1-2000-00086). The conclusions of that evaluation are:[3]

1. *The value of the biochemical markers of bone turnover is that they can be obtained inexpensively and quickly.*
2. *Such indices are useful in providing supporting evidence in evaluations of the effects of foods and food components on bone.*
3. *At the current state of knowledge, these markers cannot be considered as primary indicators of bone health or surrogates of change in fracture risk.*

10.4.2.2 Bone Mineral Mass and Density[3,54, 92]

Bone mineral density (BMD) is considered a strong biomarker for fracture risk. It is not a true density measurement but rather a mathematical construct obtained by dividing the bone mineral content (BMC) by the area of the scanned bone envelope (BA), and it is expressed as g/cm^2:

$$BMD\ (g/cm^2) = BMC/BA$$

BMC is measured *in vivo* by absorptiometry, based on the attenuation of energy from a beam of penetrating photons (X-ray) during a scan across the skeletal region of interest. Single-energy absorptiometry (SXA) are used for measuring the BMC of the bones of the arms and legs, but dual energy (DXA) instruments are required for axial (hip and spine) and whole-body measurements that need correction for the overlying soft tissue of variable composition.[94] The effective dose of radiation per measurement is low and remains generally within natural background radiation levels.[95] The method is precise with coefficients of variation usually less than 1–2% for repeated scans of phantoms and between 2–5% for repeated scans of the same person. In addition long-term reproducibility is good. The accuracy of absorptiometry is more problematical, being affected by choice of calibrating materials, assumptions built up in the computer algorithms, the depths of tissues in the scan path, the nonuniformity of soft tissues overlying bone, and differences in clothing and bedding. The result of absorptiometry is not limited to osseous tissue *per se.* Indeed, it represents an integration of absorption over all elements within the bone envelope. Moreover, it cannot differentiate between cancellous and cortical bone, nor exclude abnormalities that interfere with the measurement of bone mineral content (e.g., crush fractures or calcifications).

Absorptiometry can be used both in humans and in laboratory animals. In each case specific computer software is required. Especially whole-body bone mineral content (WBBMC) and whole-body bone area (WBBA) can be measured in anaesthetized rats using DXA to allow calculation of whole body bone mineral density (WBBMD). The coefficient of variation (CV ± sem) is 1.74 ± 0.15, [96] and the

method is highly accurate as demonstrated by a correlation coefficient of 0.99 between total body Ca measured by DXA and atomic absorption spectrometry of bone ash, respectively.[97]

When performing absorptiometry studies in humans seeking to evaluate the impact of an (e.g., dietary) intervention on bone mineral status, 6–12 and 24–36 months are a minimum to evaluate short- and long-term effect, respectively.[3] The intervention periods need thus to be much longer than for evaluating parameters of Ca metabolism (e.g., bioavailability). Ideally, it would have the duration of 3–4 complete cycles of bone turnover (i.e., ±4 months in man).[54]

More modern technologies are being used increasingly for bone mineral assessment, but there are no prospective data yet that link these measurements to fracture incidence, and their value in longitudinal studies need further evaluation. These technologies include quantitative computed tomography and quantitative bone ultrasound.[3]

10.5 INULIN-TYPE FRUCTANS: MINERAL ABSORPTION AND BONE HEALTH

10.5.1 Inulin-Type Fructans and Ca Absorption[98]

10.5.1.1 *In Vitro* Data

The effect of oligofructose was studied *in vitro* using the isolated ileum, jejunum, cecum, and colon after removal of their serosa and muscle layers.[99] The stripped preparations containing the mucosa and submucosa were then mounted onto diffusion chambers to expose the intestinal segments to Ca solutions (1.25 and 10 m*M* for the serosal and the mucosal sides, respectively). Increase in Ca concentration in the serosal medium was used to calculate transepithelial Ca transport and changes thereof in the presence of inreasing concentrations of oligofructose on the mucosal side of the preparations. Segments of the jejunum, ileum, and cecum transported Ca at a rate of ±10 nmol/min cm^2, but the transport through the colon, even though significant, was slower (±5 nmol/min cm^2). In the presence of oligofructose, the absorption of Ca increased dose-dependently in all tested intestinal segments. At an oligofructose concentration of 100 m*M*, the percentage increase in transepithelial Ca transport was approximately +560, +225, +350, and 500% in the jejunum, the ileum, the cecum, and the colon, respectively. If a 10 m*M* concentration of Ca is indeed observed in the cecum of a rat fed a standard diet, it must, however, be underlined that, in inulin or oligofructose fed rats, the concentration of Ca in the cecum increases in a dose-dependent manner to reach 31 and 55 m*M* when inulin concentrations in the diet are 5 and 10%, respectively.[79] Because, in addition, the authors recognize that "Ca transport in the different segments of the intestine increased linearly in relation to the Ca concentration in the mucosal medium," the real relevance of these *in vitro* data to *in vivo* conditions remain limited unless they become confirmed by *in vivo* data showing the effect of inulin-type fructans on Ca absorption.

10.5.1.2 Animal Data

Even thought the main site of Ca (as well as Mg) absorption in the rats is in the small intestine, experimental data show that this physiological process also occurs in the large bowel, namely the cecum and the colon.[100–102] The absorption of cations in the large intestine is associated with the consumption of dietary fiber or poorly digestible carbohydrate which cause a decrease in the intraluminal pH due to fermentation and production of lactic and short-chain fatty acids (SCFAs).[102–105] The carbohydrates for which such an effect has been reported first are lactose, lactulose, xylitol, arabinose, raffinose, sorbitol, and resistant starch.[63,106]

Inulin-type fructans similarly influence Ca absorption. Rémésy et al.[107] were the first to report that rats fed a diet supplemented with inulin (15% w/w) absorb Ca more efficiently than rats fed a fiber-free diet. Since that first report, a large number of studies using different protocols have confirmed that effect for both inulin and oligofructose. These studies are summarized in Table 10.1.

The vast majority of these studies used the metabolic balance method to measure fractional or apparent absorption (FA%) calculated as:

Intake (IN) – Excretion (OUT, feces and urine)/Intake (IN) ¥ 100

One study, however, combined the metabolic balance with a kinetic approach using intravenously injected ^{45}Ca to measure true absorption.[80]

In the metabolic balance studies, the concentration of Ca in diet, feces, or urine was measured using atomic absorption, plasma atomic absorption, or inductive coupled plasma emission spectrometry (ICPS). During the metabolic balance study, rats were kept in metabolic cages and dietary intake, fecal, and urinary excretions were quantified for a period of 4 d. The concentration of Ca in diet was ±0.5% (the recommended intake for rats) in most studies, but in some cases it was higher (0.7 or 1%). Rat strains used were Fisher 344, Sprague–Dawley, or Wistar, and all animals were males. Usually the rats were rather young (i.e., 4–6 weeks old) at the start of experiments that lasted for a few weeks (10–31 d). The concentration of inulin-type fructans in the diet was 5 and 10% (w/w) in two and one third of the protocols, respectively. In addition, some experiments did compare the effect of different concentrations, e.g., 1, 2.5, 3, or even 15%, but the database is too limited to draw any conclusion concerning a dose-effect relationship. Moreover, the relative increase (in%) in apparent absorption, calculated as:

FA in the rats fed inulin - FA in the control rats/FA in the control rats

was highly significant but inversely correlates ($r = .788$ $p < 0.01$) with the absorption in the control rats, i.e., the lower the absorption of Ca in the control group, the higher the increase caused by inulin or oligofructose (Figure 10.2).

As it is known that in the growing rats the basal absorption of Ca rapidly declines with age, being ±65%, ±25% and ±17% at week 4, 16, and 24 of age, respectively,[63] it is not surprising that the highest relative increases in apparent absorption are observed after the longest periods of inulin feeding, i.e., in the oldest rats. In one

TABLE 10.1
Effects of Inulin-Type Fructans and Ca Balance in Rats

Product Tested Rats	Dose (%) Duration	Method	Results	References/ Comments
Oligofructose Fisher 344 males, Age: 38 weeks, N = 8	**5** (Ca = 0.5) 1 d	Ratio $^{47}Ca/^{47}Sc$	**FA[1] (%)** 12.8 ➠ 21 (+67%)	Miller[66]
Oligofructose Sprague-Dawley males, Age: 4 weeks, N = 7	**1–15** (Ca = 0.5) 10–31 d	Balance Ion plasma spectral analysis	**FA (%)** 1% NS 3% 56.5 ➠ 61 (+8%) 5% 56.5 ➠ 65 (+15%) 15% 56.5 ➠ 82 (+45%) **Day 3–7:** 58.6 ➠ 75 (+28%) **Day 14–18:** 55.3 ➠ 64 (+15.7%) **Day 27–31 :** 38.3 ➠ 51.4 (+34.2%)	Delzenne et al.[112]
Oligofructose Sprague-Dawley males, Age: 4 weeks, N = 7	**5** (Ca = 0.5) 28 d	Balance In days: 4, 10, 17, 24, Atomic absorption spectrometry	**FA (%)** **Day 4–8:** 64.5 ➠ 80.5 (+24%) **Day 10–14:** 64.5 ➠ 76 (+18%) **Day 17–21:** 59.5 ➠ 71 (+19%) **Day 24–28:** 53.5 ➠ 60 NS	Ohta et al.[111] The increase in Ca absorption seems to disappear after 3 weeks of oligofructose feeding The average increase in absolute daily Ca retention is ±13 mg The effect takes place mainly in cecum
Oligofructose Sprague-Dawley males, Age: 5 weeks, N = 6	**1 and 5** (Ca = 1) (Mg= 0.025) 25 d	Balance (ICPS)[2]	**FA (%)** 1% NS 5% NS	Younes et al. [114] In a low Mg (0.025%), high Ca (1%) diet, oligofructose has no effect on Ca absorption
Oligofructose *Inulin* Wistar males, Age: 4 weeks, N = 10	**10** (Ca= 0.7) 24 d	Balance Plasma atomic absorption spectrometry	**FA (%)** Oligofructose: 25.4 ➠ 43.5 (+71%) Inulin: 25.4 ➠ 40.3 (+59%)	Beynen et al.[115]

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TABLE 10.1 (continued)
Effects of Inulin-Type Fructans and Ca Balance in Rats

Product Tested Rats	Dose (%) Duration	Method	Results	References/ Comments
Oligofructose Sprague-Dawley males, Age: 6 weeks, N = 14	**5** (Ca = 0.5) 7 d	Balance (ICPS)	**FA (%)** 37.5➠57.7 (+54%)	McCredie et al.[78] The increase in Ca absorption takes place both in cecum and colon (50/50)
Oligofructose Sprague-Dawley males, Age: 6 weeks, N = 7	**5** (Ca = 0.5) (Mg = 0.05) 14 d	Balance (ICPS)	**FA (%)** **Day 3–6:** 66.8➠75.2 (+12.6%) 63.2➠75 (+11.9%) **Day 10–13:** 58➠65 (+12%) 57.9➠69.5 (+20%)	Coudray et al.[122]
Oligofructose Sprague-Dawley males, Age: 5 weeks, N = 11	**5** (Ca = 0.5) (Mg = 0.05) 15 d	Balance (ICPS)	**FA (%)** **Day 3–7:** 45.4 ➠ 60.9 (+34.1%) **Day 10–14:** 47.7 ➠ 52.9 (+11%) **Day 3–7:** 47.9 ➠ 59.6 (+25%) **Day 10–14:** 44 ➠ 66.8 (+51.8%)	Lopez et al.[113] Protocol designed to test for the effect of coprophagy Data showed no effect of coprophagy on Ca absorption
Oligofructose Sprague-Dawley males, Age: 4 weeks, N = 7	5 (Ca-free diet) $CaCO_3$ infused in stomach/cecum (Ca = 0.5) 10 d	Balance (ICPS)	**FA (%)** Infusion in Stomach: 34.5 ➠ 47.1 (+37%) Infusion in Cecum: 30.2 ➠ 34.4 NS	Baba et al.[80] No effect on Ca in serum No effect on Ca in femur Increase in FA after infusion in stomach but not in cecum
Oligofructose Wistar males, Age: 6 weeks, N = 8	**5** (Ca = 0.5) 14 d	True absorption ^{48}Ca kinetic. Balance Atomic absorption spectrometry	True absorption**:** 50.3 ➠ 59.5 (+18%) Balance: +7.5 (mg/d) (+19%)	Jackson[84] No effect on endogenous Ca excretion in intestine No effect on bone formation or resorption
Oligofructose Sprague-Dawley males, Age: 5 weeks, N = 9	**5 and 10** (Ca = 0.6) 10 d	Balance Plasma atomic absorption spectrometry	**FA (%)** 5% 63 ➠ 78 (+24%) 10% 63➠89 (+41%)	Buts et al.[167] Short term treatment With 5%, increase is identical to that in 108

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TABLE 10.1 (continued)
Effects of Inulin-Type Fructans and Ca Balance in Rats

Product Tested Rats	Dose (%) Duration	Method	Results	References/ Comments
Oligofructose Sprague-Dawley males, Age: 5 weeks, N = 9	**5 and 10** (Ca = 0.6) 10 d	Balance Plasma atomic absorption spectrometry	**FA (%)** 5% 62.5 ➠ 78 (+25%) 10% 62.5 ➠ 89 (+45%)	Zafar et al.[120] Short term treatment. With 5%, increases is identical to that in 108 and 136
Oligofructose Sprague-Dawley males, Age: 5 weeks, N = 10	**1, 3, 5** (Ca = 0.6) 27 d	Balance (ICPS)	**FA (%)** 1% NS 3% NS 5% NS	Hillman et al.[144]
Inulin Wistar males, Age: 6 weeks, N = 8	**10** (Ca = 0.5) 21 d	Balance Atomic absorption spectrometry	**FA (%)** 33 ➠ 42 (+27%) **Balance** +15.2 (mg/d) (+47%)	Ohta et al.[116]
Oligofructose Wistar males, Age: 6 weeks, N = 8	**5** (Ca = 0.5) 18 d	Balance Atomic absorption spectrometry	**FA (%)** 43.7 ➠ 53.2 (+22%) **Balance** +10 (mg/d) (+24%)	Richardson et al.[136]
Inulin Wistar males, Age: 8 weeks, N = 8	**10** (Ca = 0.7) 21 d	Balance Atomic absorption spectrometry	**FA (%)** 23 ➠ 37 (+61%) **Balance** +18 (mg/d) (+55%)	Pallarés et al.[117]
Oligofructose Wistar males, Age: 8 weeks, N = 8	**5** (Ca = 0.76) 28 d	Balance Atomic absorption spectrometry	**FA (%)** 52.9 ➠ 41 (- 23%)	Baba et al.[143] Oligofructose feeding reduces Ca absorption
Oligofructose **Inulin HP** Synergy®-1 Wistar males, Body weight 170 g N = 10	**2.5 (1 week)** **5 (1 week)** **10 (2 weeks)** (Ca = 0.5) 28 d	Balance Atomic absorption spectrometry	**FA (%)** Oligofructose +10% (NS) Inulin HP + 13% (NS) Synergy® 1 47.9 ➠ 58.1 (+21%) **Balance** Oligofructose + 7.4 mg/d (NS) Inulin HP + 4.7 mg/d (NS) Synergy® 1 + 11.7 mg/d	Briet et al.[149] Only Synergy® 1 has a statistically significant effect on Ca absorption

[1]FA = Fractional absorption.
[2]ICPS = Inductive coupled plasma emission spectrometry.

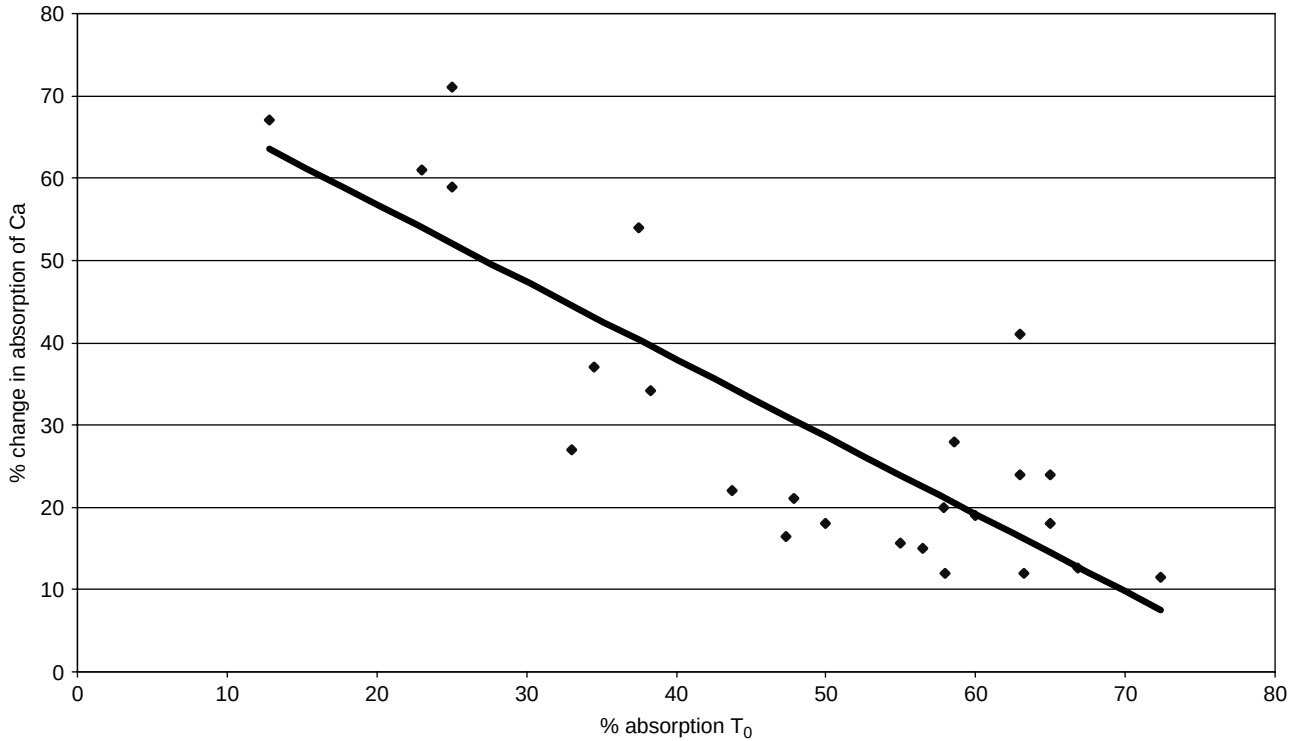

FIGURE 10.2 Correlation between the percentage of change in Ca absorption induced by inulin-type fructans and the basal Ca absorption (% absorption T_0).

study that measured the FA percentage four times during the oligofructose feeding at one week intervals, data seem to indicate that, in rats, the increase in Ca absorption progressively diminishes and finally levels off to become statistically nonsignificant after 4 weeks of feeding.[108] This observation is not confirmed in another study of the same group.[109] Finally, it has been reported that preventing coprophagy, a common behavior in many rodent species, modified neither the basal absorption of Ca nor the increase induced by oligofructose.[110]

However, increasing gastrointestinal Ca absorption by inulin-type fructans is not accompanied by an increase in Ca serum concentration.[76,80,111–114] In a high Ca (1.2% w/w)-high P (1.1% w/w) diet, adding oligofructose (5% w/w) has been reported even to lower serum Ca concentration (6%, $p < 0.05$) after 25 d.[111]

In a protocol comparing the absorption of Mg (0.025% w/w in diet) from different sources (i.e., MgO, cocoa, defatted rice bran, or acid extract of defatted rice bran), it was shown that adding oligofructose (5% w/w in diet for 21 d) significantly increased the absorption ratio of Ca in all cases.[77]

One crossover protocol applied to Beagle dogs (n = 5; age 3–11 years) confirmed the enhancing effect of an oligofructose-supplemented (1% w/w) diet fed for 3 weeks on Ca absorption (8.6% to 16% or +86%).[115]

Using a more complex protocol to measure the arteriovenous differences across the cecum in anaesthetized rats, Levrat et al. have demonstrated a dose-dependent increase in Ca concentration in the cecum and in the cecal pool as well as in cecal absorption of Ca by inulin (0, 5, 10, and 20% w/w in diet).[79] In that protocol, the 3 doses of inulin increased cecal blood flow (1.5¥, 1.8¥, and 3¥, respectively) similarly to the increase in cecal wall weight (1.3¥, 1.6¥, 2.6¥, respectively). However, and at each dose, the stimulation of Ca absorption was higher (1.9¥, 4.3¥, and 8¥, respectively), indicating that the effect of inulin was not only due to an increased blood flow or cecal absorptive surface.[79] The same group has confirmed that effect by showing that, at two different concentrations of Ca in the diet (0.3 and 0.8% w/w), inulin (15% w/w in the diet) increased Ca cecal absorption 4–6 fold.[107] Using the same protocol, Lopez et al. similarly showed a significant increase in cecal absorption of Ca (+40%) by inulin (10% w/w in diet) even in the presence of phytic acid (0.7% w/w in diet).[113] In that experiment the addition of phytic acid in the diet did not modify basal cecal Ca absorption but in the presence of inulin Ca absorption was more than twice as high as in the absence of phytates (+100%).[113]

Additional studies were performed that used cecectomized, gastrectomized, or iron-deficient rats. In cecectomized rats, Ohta et al. have reported that oligofructose had no effect on Ca absorption, concluding that the cecum was the site of action of oligofructose, even though its fermentation was still observed in the colon.[108] However, using a different protocol to search for the site of the enhancing effect of oligofructose on Ca absorption, the same authors compared FA(%) after administration of $CaCO_3$ by gastric intubation or cecal infusion. Results showed that oligofructose increased Ca absorption in the former but not in the later condition. According to these data, the cecum and the colon do not appear to be the major sites of action of oligofructose.[76]

The gastrectomized rats (4-week-old male Sprague–Dawley) were used to test for the preventing effect of oligofructose (10% w/w in diet for 10 d) on osteopenia know to occur in gastrectomized patients. In that protocol, oligofructose highly significantly increased Ca absorption when comparing the gastrectomized rats fed the standard diet and the oligofructose-containing diet (17% and 62%, respectively, or a 3.65-fold increase). In that case the absorption of Ca was similar to that in the control sham-operated rats (61%).[116]

Iron-deficient rats have a lower Ca absorption capacity than normal rats.[117] That experimental model was thus used to test the effect of oligofructose and results showed that, like in normal rats, Ca absorption was significantly increased (from 66.8 to 75.2% or +12%) in oligofructose-fed iron-deficient rats.[118] The positive effect on mineral availability was demonstrated by prevention of anemia, i.e., haematological parameters or by prevention of mineral deficiency.

Aged virgin ovariectomized female rats is an accepted experimental model for human postmenopausal osteoporosis.[119] Experiments have thus been performed using that protocol (5 month-old ovariectomized virgin female rats) to test for the effect (at weeks 4, 8, and 16) of low (2.5%), medium (5%), and high (10%) doses of inulin-type fructans in the presence of the recommended dietary intake of Ca (0.5%) and P (0.5%), and of medium dose (5%) of oligofructose in the presence of high-dietary Ca (1%) on Ca absorption and bone health (see Section 10.5.3).[120] In the group of rats receiving the recommended Ca intake (0.5% w/w in the diet), Ca apparent absorption was not increased by oligofructose feeding except at week 8 in rats fed the 10% oligofructose-containing diet. But in the group of rats fed the diet containing the high Ca concentration (1% w/w in the diet), supplementing the diet with 5% oligofructose significantly increased Ca absorption by 45, 30, and 21% at week 4, 8, and 16, respectively ($p < 0.05$) due to persistently reduced fecal calcium loss. Increased absorption was associated with increased urinary calcium, reflecting metabolic reaction on higher calcium influx from the gut and not calcium mobilization from the skeleton because bone mineral content was higher on oligofructose.[120] In a similar experiment in which 6-month-old ovariectomized virgin female rats were fed a diet containing 0.5% Ca and 5% oligofructose-enriched inulin Synergy 1, Ca absorption was increased 4-fold ($p < 0.005$).[121]

10.5.1.3 Human Data

In their study with ileostomy volunteers, Ellegärd et al. have shown that feeding inulin (17 g/d) or oligofructose (17 g/d) for 3 d had no significant effect on Ca absorption in the upper part of the gastrointestinal tract.[122]

Early studies examining the effect of inulin-type fructans on calcium absorption in healthy human subjects have been somehow contradictory. Two studies have shown a beneficial effect of inulin [123] or oligofructose[67] on calcium absorption in young men and adolescent boys on modest calcium intakes, but a third study found no effect of oligofructose in a similar population.[68] These discrepant findings may be due to methodological differences (see Section 10.7.1 and Section 10.7.2),[124] to differences in the product tested (inulin vs. oligofructose), or to a type II error, as all the studies included a relatively small number of volunteers. Moreover, calcium

intakes (approximately 800 mg/d) were relatively low, a situation where calcium retention is significantly dependent on calcium intake. Especially in children at or near puberty, calcium retention appears to reach a maximum (or even a plateau) at an intake of about 1200 mg/d.[125]

Further, previous studies have examined the effect of relatively large doses (15 and 40 g/d) of inulin-type fructans on calcium absorption. Coudray et al. fed nine healthy young men a control diet or the same diet supplemented with inulin (40 g/d) for 26 d (2 d of control diet followed by 14 d of progressive increase in inulin amount and then 12 d at the maximum consumption, i.e., 40 g/d). Using the metabolic balance methodology, they determined apparent Ca absorption and found that, upon inulin ingestion, that parameter increased significantly from 21.3 to 33.7% (or +58%; $p < 0.01$).[123]

In a randomized, double blind, cross-over design study, Van den Heuvel et al. fed 12 healthy male adolescents (aged 14–16 years) either orange juice supplemented with oligofructose (5 g three times/d) or sucrose (control treatment) for 9 d, after which time, they measured true fractional Ca absorption by the dual stable isotope technique. The true fractional Ca absorption increased significantly (from 47.8% in the placebo group to 60.1% in the oligofructose-fed volunteers).[67] In an earlier study by the same group, a daily supplement of 15 g of oligofructose failed to show an effect on Ca absorption in healthy adult men.[66] However, in that study, unlike the previous one, the colonic component of Ca absorption (a putative target for enhancement by inulin-type fructans) was not included because the urine collection period was limited to 24 h after isotope administration.

In a more recent, randomized, double-blind, crossover design study aimed at examining the effect of relatively modest intakes of different inulin-type fructans on Ca absorption, 29 young adolescent girls (11–14 years, consuming a relatively high Ca intake (1500 mg/d) received either 8 g/d of oligofructose, oligofructose-enriched inulin Synergy 1 or sucrose (as placebo) in a Ca-fortified orange juice for 3 weeks. True Ca absorption was measured using the dual stable isotope method. In order to detect any modulatory effect of the inulin mixture on the colonic component of Ca absorption, urine was collected over the 48-h period following the administration of the isotopes. Consumption of oligofructose had no significant effect, whereas consumption of oligofructose-enriched inulin Synergy 1 resulted in a significant ($p = 0.004$) increase (+18%) in true fractional Ca absorption and in a significant ($p = 0.004$) absolute increase (+90 mg/d) in Ca absorption.[126] By combining these data with those of an additional 25 newly recruited subjects with similar age (11–13.9 years old) and ethnic characteristics, the beneficial effect of Synergy was confirmed by showing, in an identical protocol, a significant ($p = 0.027$) increase (+9%) in true fractional Ca absorption in girls at or near menarche with calcium intakes approximating the currently recommended dietary intake (±1550 mg/d).[127]

The effect of oligofructose on Ca absorption in human volunteers has also been evaluated by measuring acute short-term changes in urinary Ca (total Ca or $^{44}Ca/^{43}Ca$ ratio) excretion in two Japanese studies (randomized, double-blind, placebo-controlled protocols).[87,89] The authors concluded that after feeding tablets or a malt drink containing Ca (total intake 0.3 g) and oligofructose (total intake 3 g) with a ratio oligofructose/Ca of 10:1, urinary Ca excretion was significantly

increased, compared to feeding the same food products containing sucrose in place of oligofructose. The calcium outputs listed for the volumes of urine in these papers are unusually low. By adding a tracer dose of ^{44}Ca and measuring the ratio $^{44}Ca/^{43}Ca$, they concluded that the effect was indeed due to an increase in the urinary excretion of Ca originating from the diet. These data are surprising because they would imply that oligofructose acted directly (i.e., without previous adaptation) on gastrointestinal Ca absorption, and that Ca consumed as part of a meal was almost immediately recovered in the urine. In a similar protocol including young healthy women, the addition of inulin (15 g/d) to breakfast in a cheese containing 210 mg of Ca, did not acutely (within 8 h after ingestion) affect serum ionized Ca and parathyroid hormone concentrations. Nor did it affect Ca urinary excretion over the 8 h period of test.[88] However in adults (N = 50 and age range 45–75 years) given either a $CaCO_3$ tablet or a formulated beverage containing Ca, Mg, Zn, P, vitamins C, D, and K, soy isoflavones, and inulin (5 g/8 oz) for 4 weeks, cumulative urinary excretion of Ca and Mg, measured over a 72-h period, was significantly increased (+29 and +22%, respectively).[90] Because of the complexity of the beverage and the difference in the chemical nature of the Ca salts used either in the tablet ($CaCO_3$) or in the beverage (Ca-lactate), it cannot be concluded if inulin was the only factor responsible for the enhanced Ca/Mg urinary excretion. Still, and compared to the last three previous studies, the beverage was given to the volunteers for a period of 4 weeks before the test thus allowing, eventually, for the gastrointestinal effect of inulin to take place.

The effect of inulin-type fructans on Ca absorption has also been investigated in postmenopausal women. In a randomized, double-blind, crossover protocol, oligofructose (10 g/d for 5 weeks) failed to show any effect on Ca absorption in 12 healthy postmenopausal women. Ca absorption was measured by quantifying the fecal excretion, over a 5–7 d period, of the orally administered stable isotope ^{44}Ca and radio opaque markers. In 6 women, out of a total of 12, who had been going through menopause for more than 6 years, there was a tendency (but no statistical significance) for a higher Ca absorption during the oligofructose feeding period.[128] In addition, plasma concentrations of total Ca, parathyroid hormone, and 1,25-dihydroxyvitamin D, as well as ^{44}Ca plasma enrichment, remained identical during both the placebo and the oligofructose periods.[128]

In a more recent study (randomized, double blind, placebo controlled, crossover design) oligofructose-enriched inulin Synergy 1 (equivalent to 10 g/d of inulin-type fructans) was shown to significantly ($p < 0.05$) increase Ca absorption (+7% as measured by the dual stable isotope method using the ICP – mass spectrometry) in 15 postmenopausal women after 6 weeks of treatment. The volunteers included in that study were a minimum of 10 years past the onset of menopause, and they had taken no hormone replacement therapy for the past year. Ca intake and absorption rate at baseline were 1–1.1 g/d and 20–24%, respectively. During the oligofructose-enriched inulin Synergy 1 feeding period Ca, absorption rate increased in 10–15 volunteers and decreased slightly but nonsignificantly in one volunteer but difference in response was not a function of initial absorption rate at baseline.[129]

10.5.2 Inulin-Type Fructans and Mg Absorption

10.5.2.1 Animal Data

In the rat, Mg absorption occurs in the large bowel, namely the cecum and the colon, as well as in the small intestine.[68,101–106] The absorption of Mg in the large intestine is associated with a low intraluminal pH due to the presence of lactic acid and SCFAs, the products of fermentation of poorly or nondigestible carbohydrates.[102–105] Inulin-type fructans increase Mg absorption as shown by a large number of studies with different protocols using both inulin and oligofructose. These studies are summarized in Table 10.2.

The vast majority of these studies used the metabolic balance method to measure fractional or apparent absorption (FA%) calculated as:

Intake (IN) - Excretion (OUT, feces and urine)/Intake (IN) ¥ 100

One study, however, used a more sophisticated kinetic approach to measure true absorption.[80] The concentration of Mg in diet, feces, or urine was measured using atomic absorption spectrometry, plasma atomic absorption spectrometry, or inductive coupled plasma emission spectrometry (ICPS). During the metabolic balance study, rats were kept in metabolic cages and dietary intake, fecal, and urinary excretions were quantified for periods of 4 d. The concentration of Mg in diet was ±0.05% (the recommended value for rats) in most studies, but in some cases it was lower (0.02%) or higher (0.09 or 0.1%). Rat strains used were Sprague–Dawley or Wistar, and all animals were males. Usually the rats were rather young (i.e., 4–6 weeks old) at the start of experiments that lasted for a few weeks (10 to 31 d). The concentration of inulin-type fructans in the diet was 5 and 10% (w/w) in 2 of the protocols, respectively. In addition, some experiments did compare the effect of different concentrations e.g., 1, 2.5, 3, or even 15% but the database is too limited to draw any conclusion concerning a dose–effect relationship. As with Ca absorption, the relative increase (in %) in apparent Mg absorption, calculated as:

FA in the rats fed inulin – FA in the control rats/FA in the control rats

correlates ($r = 0.859$ $p < 0.01$) with the absorption in the control rats (Figure 10.3).

Similar to what has been observed with Ca, in two studies that measured the FA percentage four times during the oligofructose feeding at 1 week intervals, data seem to indicate that, in rats, the increase in Mg absorption progressively diminishes but it is still statistically significant after 4 weeks of feeding.[107,106] In a protocol comparing the absorption of Mg (0.025% w/w in diet) from different sources (i.e., MgO, cocoa, defatted rice bran, or acid extract of defatted rice bran), it was shown that oligofructose (5% w/w in diet for 21 d) significantly increased the absorption ratio of Mg in all cases.[130] Finally, it has been reported that preventing coprophagy, a common behavior in many rodent species, did not modify the basal absorption of Mg, but it did enhance the absorption of Mg in the rats fed with oligofructose.[110]

TABLE 10.2
Effects of Inulin-Type Fructans and Mg Balance in Rats

Product Tested Rats	Dose% Duration	Method	Results FA[1] (%)	References/ Comments
Oligofructose Sprague-Dawley males Age: 4 weeks, N = 7	**1–15** (Ca = 0.5) (Mg = 0.05) 10–31 d	Balance Ion plasma spectral analysis	1% NS 3% 69.2 ➠ 75 (+**.7%)** 5% 69.2 ➠ 83 (+**20.5%)** 15% 69.2 ➠ 89 (+**28.3%)** **Day 3–7:** 61 ➠ 83 **(+36.2%)** **Day 14–18 :** 55.3 ➠ 78.3 (+41.6%) **Day 27–31:** 49.8 ➠ 73.3 (+**47.2%)**	Delzenne et al.[112]
Oligofructose Sprague-Dawley males Age: 4 weeks, N = 7	**5** (Ca = 0.5) (Mg= 0.05) 28 d	Balance in days: 4,10,17,24 Atomic absorption spectrometry	**Day 4–8:** 62.8 ➠ 87.7 (+41%) **Day 10–14:** 62.8 ➠ 87.6 (+41%) **Day 17–21:** 62.8 ➠ 87.4 (+40.5%) **Day 24–28:** 62.8 ➠ 82.8 (+33%)	Ohta et al.[111]
Oligofructose Sprague-Dawley males Age: 5 weeks, *N = 6*	**1 and 5** (Ca = 1) (Mg = 0.025) 25 d	Balance **(**ICPS)[2]	1% **Day 7–11: NS** **Day 21–25:** 34.1 ➠ 51.6 (+51.5%) 5% **Day 7–11:** 54.4 ➠ 71.8 (+32%) **Day 21–25:** (34.1 ➠ 62.5) (+83%)	Younes et al.[114] In a low Mg (0.025%), high Ca (1%) diet, oligofructose has no effect on Ca absorption but it increases Mg absorption. The effect is equivalent to increasing dietary Mg intake by 2 fold
Oligofructose **Inulin** Wistar males Age: 4 weeks, N = 10	**10** (Ca = 0.7) (Mg = 0.1) 24 d	Balance Plasma atomic absorption spectrometry	Oligofructose: 27 ➠ 64.7 (+140%) Inulin: 27 ➠ 64.7 (+140%)	Beynen et al.[115]

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TABLE 10.2 (continued)
Effects of Inulin-Type Fructans and Mg Balance in Rats

Product Tested Rats	Dose% Duration	Method	Results FA[1] (%)	References/ Comments
Oligofructose Sprague-Dawley males Age: 6 weeks, N = 14	**5** (Ca = 0.5) (Mg = 0.04) 7 d	Balance (ICPS)	52.4 ➠ 83.1 (+58.5%)	McCredie et al.[78] The increase in Mg absorption takes place both in cecum and colon (50/50)
Oligofructose Sprague-Dawley males Age: 6 weeks, N = 7	**5** (Ca = 0.5) (Mg = 0.05) 14 d	Balance (ICPS)	**Day 3–6:** 58.9 ➠ 76.1 (+29.2%) 54.5 ➠ 79.5 (+45.9%) **Day 10–13:** 43.5 ➠ 74.9 (+72.2%) 49.3 ➠ 76.1 (+54.4%)	Coudray et al.[122]
Oligofructose Sprague-Dawley males Age: 5 weeks, N = 11	**5** (Ca = 0.5) Mg = 0.05) 15 d	Balance (ICPS)	**Day 3–7:** 58.8 ➠ 76.3 (+30%) **Day 10–14:** 52 ➠ 68.1 (+31%) **Day 3–7:** 55.9 ➠ 87.9 (+57.2%) **Day 10–14:** 52 ➠ 83.6 (+60.8%)	Lopez et al.[113] protocol designed to test for the effect of coprophagy preventing coprophagy enhanced the effect of oligofructose on Mg absorption
Oligofructose *Cecal canulated* Sprague-Dawley males Age:4 weeks, N = 7	**5** (Ca = 0.5) (Mg = 0.02) 15 d	Balance (ICPS)	**Day 3–7:** 50 ➠ 88 (+76%) **Day 11–15:** 60 ➠ 78 (+30%)	Demigné et al.[81]
Oligofructose Sprague-Dawley males Age: 4 weeks, N = 7	**5** (Ca-free diet). $CaCO_3$ or MgO infused in stomach or cecum (Ca = 0.5) (Mg = 0.04) 10 d	Balance (ICPS)	Infusion in Stomach: 53 ➠ 70 (+32%) Infusion in Cecum: 53 ➠ 66 (+24.5%)	Baba et al.[80] No effect on Mg in serum
Oligofructose Sprague-Dawley males Age: 5 weeks, N = 10	**1, 3, 5** (Ca = 0.6) (Mg = 0.06) 27 d	Balance (ICPS)	1% 70 ➠ 72.9 (+4.1%) 3% 70 ➠ 73.8 (+5.4%) 5% 70 ➠ 77.4 (+10.5%)	Hillman et al.[144]

-- continued

TABLE 10.2 (continued)
Effects of Inulin-Type Fructans and Mg Balance in Rats

Product Tested Rats	Dose% Duration	Method	Results FA[1] (%)	References/ Comments
Inulin Wistar males Age: 6 weeks, N = 8	**10** (Ca = 0.5) (Mg = 0.05) 21 d	Balance Atomic absorption spectrometry	35 ➠ 69 (+97%)	Ohta et al.[116]
Inulin Wistar males Age: 8 weeks, N = 8	**10** (Ca = 0.7) (Mg = 0.09) 21 d	Balance Atomic absorption spectrometry	35 ➠ 57 (+**63%**)	Pallarés et al.[117]
Oligofructose Wistar males Age: 8 weeks, N = 8	**5** (Ca = 0.75) (Mg = 0.025) 28 d	Balance Atomic absorption spectrometry	84.6 ➠ 90.5 **(+7%)**	Baba et al.[143]
Oligofructose **Inulin HP** **Synergy® 1** Wistar males Body weight 170 g N = 10	**2.5 (1 week)** **5 (1 week)** **10 (2 weeks)** (Ca = 0.5) (Mg = 0.05) 28 d	Balance Atomic absorption spectrometry	*Oligofructose:* 48.8 ➠ 71.3 (+**46%)** *Inulin HP:* 48.8 ➠ 76.4 (+**56.5%)** *Synergy® 1:* 48.8 ➠ 76.7 (+**57%)**	Moshfegh et al.[148]

[1]FA = Fractional absorption.
[2]ICPS = Inductive coupled plasma emission spectrometry.

Increasing gastrointestinal Mg absorption by inulin-type fructans is, however, not accompanied by an increase in Mg serum concentration.[76,110,111,114]

Using a more complex protocol to measure the arteriovenous differences across the cecum in anaesthetized rats, Levrat et al. have demonstrated an increase in Mg concentration in the cecum and in the cecal pool, as well as in cecal absorption of Mg by inulin (0, 5, 10, and 20% w/w in diet).[79] Only the effect on Mg transport was dose dependent, whereas the effect on Mg concentration or cecal pool was not. Like for Ca (see Section 10.5.1.1) and at each dose of inulin, the stimulation of Mg absorption was higher (2.1¥, 305¥, and 4.9¥, respectively) than the increase in cecal blood flow (1.5¥, 1.8¥, and 3¥, respectively) and cecal wall weight (1.3¥, 1.6¥, and 2.6¥, respectively) indicating that the effect of inulin on Mg absorption also cannot simply be explained by an increased blood flow or enlargement of cecal absorptive surface.[79] The same group has confirmed that effect by showing that, at two different concentrations of Ca in the diet (0.3 and 0.8% w/w), inulin (15% w/w in the diet) increased Mg cecal absorption 3–4-fold.[107] Using the same protocol, Lopez et al. similarly showed a highly significant increase in cecal absorption of Mg (+100%) by inulin (10% w/w in diet) even in the presence of phytic acid. In that last

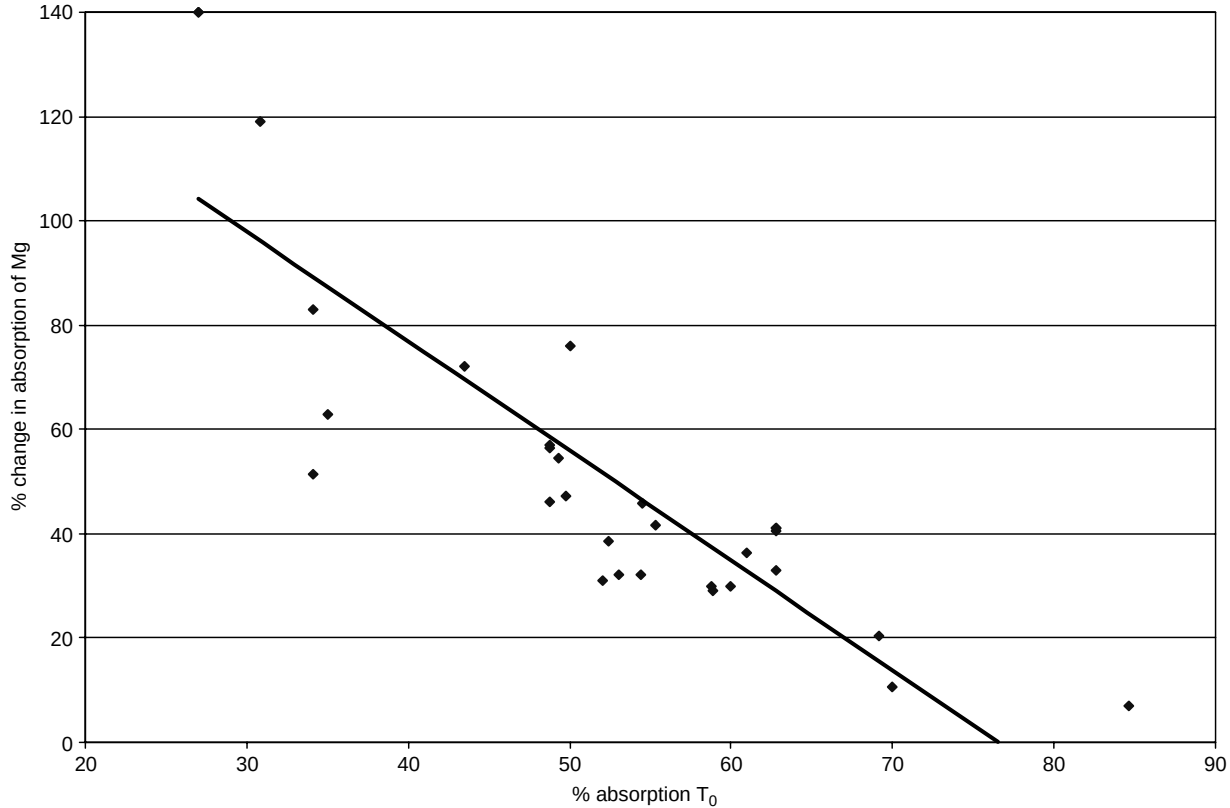

FIGURE 10.3 Correlation between the percentage of change in Mg absorption induced by inulin-type fructans and the basal Mg absorption (% absorption T0).

experimental condition, the effect of inulin was even stronger (+150%).[113] One crossover protocol applied to Beagles dogs (n = 5; age 3–11 years) confirmed the enhancing effect of an oligofructose-supplemented (1% w/w) diet fed for 3 weeks on Mg absorption (14–23.4% or +67%).[115]

Additional studies were performed that used cecectomized, gastrectomized, magnesium-or iron-deficient rats. In cecectomized rats, Ohta et al. have reported that, even though it did not affect Ca absorption, oligofructose significantly enhanced Mg absorption, and they concluded that the cecum was not the only the site of action.[108] Using a different protocol to search for the site of the enhancing effect of oligofructose on Mg absorption, the same authors compared FA(%) after administration of MgO by gastric intubation or cecal infusion. Results showed that oligofructose increased Mg absorption in both conditions confirming that the lower intestine was likely not to be the only site of action of oligofructose.[76]

The gastrectomized rats (4 weeks old male Sprague–Dawley) were used to test for the preventing effect of oligofructose (10% w/w in diet for 10 d) on osteopenia known to occur in gastrectomized patients. In that protocol, oligofructose slightly but significantly increased Mg absorption when compared to the gastrectomized rats fed the standard diet and the oligofructose containing diet (82 and 90%, respectively, or a 10% increase). In that last condition, the absorption of Mg was significantly higher (+20%) than that in the control sham-operated rats (75%).[131]

In rats fed a low-Mg, high-Ca, and high-P to induce Mg-deficiency, Ohta et al. have shown that with oligofructose (1 and 5% w/w in diet), apparent Mg absorption was significantly increased.[111] In a protocol comparing rats fed a Mg-containing diet or a Mg-deficient diet plus cecal infusion of Mg ($MgCl_2$), Baba et al. demonstrated that oligofructose increased Mg absorption in both conditions concluding that the site of action of the inulin-type fructan was the large bowel.[77] In addition the symptoms associated with Mg deficiency, i.e., skin inflammation (auricular and facial hyperemia) and hemorrahage were prevented by oligofructose feeding confirming that Mg was well absorbed in both protocols.[77,111]

Iron-deficient rats have a lower Mg absorption capacity than normal rats.[117] That experimental model was thus used to test the effect of oligofructose and results showed that, like in normal rats, Mg absorption was significantly increased (from 58.9–76.1% or +29%) in oligofructose-fed iron-deficient rats.[118]

10.5.2.2 Human Data

In their study with ileostomy volunteers, Ellegärd et al have shown that feeding inulin (17 g/d) or oligofructose (17 g/d) for 3 d had no significant effect on Mg absorption in the upper part of the gastrointestinal tract.[122] In healthy adult volunteers, Coudray et al. similarly reported no effect of inulin on Mg absorption.[123]

Postmenopausal women with no hormone replacement therapy (n = 11) were used to test for the effect of a diet supplemented with oligofructose (10 g) as compared to sucrose (placebo) for 5 weeks on net intestinal Mg absorption. A single stable isotope (^{25}Mg) tracer methodology was used and the protocol was designed as randomized, double blind, and crossover (with a washout period of 3 weeks). During the last two weeks of the protocol, the volunteers received a controlled diet providing 250 mg Mg/d. One week before the end, they were given a lunch

containing the isotope dose (87.5 mg ^{25}Mg) and 40 radio opaque pellets as fecal markers. For analysis of Mg content (atomic absorption spectrometry), feces, and urines were collected for 5–7 and 2 consecutive days, respectively. Mg absorption increased in 6–11 volunteers and, on average, it increased by 12.3% (30.2 ± 5 to 33.9 ± 7.2 — $p < 0.02$). In addition, both plasma and urine ^{25}Mg enrichment were significantly ($p < 0.01$ and $p = 0.02$, respectively) increased by oligofructose feeding thus confirming the enhancing effect on intestinal Mg absorption.[132]

In a more recent study (randomized, double blind, placebo controlled, crossover design) oligofructose-enriched inulin Synergy 1 (equivalent to 10 g/d of inulin-type fructans) was shown to significantly ($p < 0.05$) increase Mg absorption (+10% as measured by the dual stable isotope method using the ICP–mass spectrometry) in 15 postmenopausal women after 6 weeks of treatment. The volunteers included in that study were a minimum of 10 years past the onset of menopause and they had taken no hormone replacement therapy for the past year. Mg intake and absorption rate at baseline were 0.25–0.3 g/d and 52–56%, respectively. During the oligofructose-enriched inulin Synergy 1 feeding period, Mg absorption rate increased in 10 out of the 15 volunteers and decreased slightly but nonsignificantly in two volunteers but difference in response was not a function of initial absorption rate at baseline.[129]

10.5.3 Inulin-Type Fructans and Bone Health

10.5.3.1 Bone Structure and Bone Quality

In healthy rats, feeding oligofructose (5% w/w in diet) influenced local bone structure as shown by an enhanced ($p < 0.05$) bone volume in the neck and in the secondary spongiosa in the metaphysis of the femur, suggesting that oligofructose feeding might have an inhibitory effect on bone resorption.[133]

The effect of oligofructose on bone structure was studied in a long-term protocol using adult ovariectomized female rats and a computer-supported image analysis of contact microradiographs[134] was used to assess bone trabecular structure.[120] Ovariectomy-induced loss of tibia trabecular bone, especially the trabecular area (Tb.Ar), and the trabecular perimeter (Tb.Pm) were reduced, whereas tissue area (T.Ar) was not affected. To prevent ovariectomy-induced bone loss, rats were fed either a diet high in calcium (1.0% vs. 0.5%,) or a diet containing 5% oligofructose or by combining both treatments for 8 and 16 weeks. Trabecular structure was affected differently by these treatments: Feeding a high Ca or an oligofructose-containing diet preserved the trabecular area almost to the same extent. Higher dietary calcium, however, conserved fewer but thicker trabecules, while oligofructose conserved more trabecules and induced higher values for trabecular perimeter. The combined intervention, i.e., including 5% oligofructose into a diet containing 1% calcium, preserved a larger Tb.Ar/T.Ar ratio at almost the same number but larger trabecular perimeter, thus indicating longer trabecules. Moreover, cortical thickness was significantly higher. Based on the bone structure analysis the authors concluded that different diets differently affected bone quality. Using the same experimental model of ovariectomy, Zafar et al. concluded that feeding Synergy 1 had no significant effect on femoral weight, femoral length, or resistance of the femur to breaking forces.[121]

10.5.3.2 Bone Mineralization

The effect of inulin-type fructans on bone mineral content (Ca, Mg, P, and Zn) was investigated not only in rats fed a standard diet[76,109,111,133,135] or a diet supplemented with phytic acid[112] but also in ovariectomized female rats.[120,121,136] Results are somehow contradictory.

In their early study to investigate the effect of oligofructose (5% w/w in diet for 31 d) on mineral absorption, Ohta et al. have reported no effect on femur dry weight but a small significant increase in ash (+2.8%, $p < 0.001$), Ca (+7.8% $p < 0.001$), and Mg (+5% $p < 0.05$) contents.[109] In a study aimed primarily at investigating if coprophagy significantly influenced Ca and Mg absorption in rats, the same research group has shown that oligofructose (5% w/w in diet for 10 d) significantly increased Ca (+20% $p < 0.001$) but not Mg content in the femur.[111] These data were not confirmed in a later publication that reported no effect of oligofructose (5% w/w in diet) on Ca and Mg concentrations in the femur after direct infusion of these minerals into the stomach.[76] However, after feeding oligofructose (5% w/w in diet) for a longer period (8 weeks), Takahara et al. have reported a significant increase in the concentration not only of Ca but also of Mg and P in three regions of the rat femur bone surface, i.e., distal metaphysis, the middle of diaphysis, and neck.[133] Moreover, Ca concentration in bone correlated with absorbed Ca ($r = 0.72$; $p < 0.001$). Similarly, and after ash analysis, Richardson et al. concluded that feeding (for 12 weeks) male Fisher 344 rats a diet containing either oligofructose or inulin significantly increased Ca concentration in the femur (+14 and +8%, respectively).[136] Using whole body dual x-ray absorptiometry, Roberfroid et al. have also reported that dietary inulin (5 and 10% w/w in diet) increased bone mineral content ($p = 0.02$) in rats fed diets with low (0.2%), recommended (0.5%) or high (1%) Ca content.[135] The effect was similar at 5 or 10% (w/w) inulin in the diet and it was significant at all time points between 9 and 22 weeks.

Adding phytic acid (7% w/w) to the diet had no effect on Ca or Mg content but reduced Zn content (- 25%) of rat tibia after 3 weeks. Feeding inulin (10% w/w) increased Mg femur concentration (+13%) both in rats fed the standard and the phytate-containing diet. It restored the Zn concentration back to the control value. But it had no significant effect on Ca content.[113]

Ovariectomy (OVX) in the adult or aged rat is an accepted method to simulate human postmenopausal state.[119] In adult ovariectomized female rats, oligofructose effectively prevented loss of bone mineral content. The preventive effect in the femur was more prominent after 8 weeks than after 16 weeks while in the lumbar vertebra it occurred persistently over 16 weeks but with less magnitude. The addition of 5% oligofructose prevented bone loss significantly in the femur and lumbar vertebra in the presence of high dietary calcium (1%) but not at 0.5%. At 0.5% calcium 10% oligofructose were needed to significantly increase bone mineralization, and this was only observed in the femur.[120] In the same experimental model, Zafar et al. reported that feeding (3 weeks) the inulin mixture oligofructose-enriched inulin Synergy 1 (5% w/w in diet) signifcantly increased femoral Ca content (+7%; $p < 0.05$).[120]

10.5.3.3 Bone Density

The effect of inulin-type fructans on bone density was investigated in two experiments only. Ohta et al. were the first to report a slight but significant increase (+5%) in femur bone mineral density. However, there was no effect on the same parameter in the tibia.[136,137] After gastrectomy, a surgery that is known to reduce Ca absorption capacity, bone mineral density was reduced both in the femur and the tibia and oligofructose feeding prevented these losses. Indeed, in gastrectomized rats fed oligofructose, femoral and tibia bone mineral density was not different compared to control sham-operated rats.

In normal healthy rats, feeding inulin (5 and 10% w/w in diet) significantly increased whole body bone mineral density ($p < 0.001$) but the effect was already maximun in the group of rats fed the lowest inulin concentration (5% w/w). That effect was due to an increase in whole body Ca mineral content (see Section 10.5.3.1) since whole mineral bone area was not affected.[135]

In ovariectomized female rats, Zafar et al. reported that feeding oligofructose-enriched inulin Synergy 1 (5% w/w in diet) significantly improved Ca content and mineral density but not breaking strength of the femur.[121]

10.5.3.4 Bone Turnover

In ovariectomized female rats, Zafar et al. have demonstrated that feeding oligofructose-enriched inulin Synergy 1 (5% w/w in diet) significantly improved Ca balance ($p < 0.05$) and decreased bone formation and bone resorption rates ($p < 0.001$), thus suggesting a slower turnover rate.[121]

In postmenopausal women, in two separate studies in which Mg absorption was increased and Ca absorption was not increased, respectively, oligofructose did not modify bone turnover as assessed by measuring plasma osteocalcin and urinary deoxypyrinolidine.[128,132]

But in a more recent study (randomized, double blind, placebo-controlled, crossover design), oligofructose-enriched inulin Synergy 1 (equivalent to 10 g/d of inulin-type fructans) was shown to significantly ($p < 0.05$) increase both urinary excretion of deoxypyridinoline cross-links (+1.1 nmol/mmol creatinine) and serum concentration of osteocalcin (+4.7 ng/ml) in 15 postmenopausal women after 6 weeks of treatment. These effects were even more pronounced (+1.7 nmol/mmol creatinine for deoxypyridinoline cross links and +6 ng/ml for osteocalcin) if analysis was limited to the volunteers who had an increased Ca and Mg absorption rate following inulin intake. The volunteers included in that study were a minimum of 10 years past the onset of menopause and they had taken no hormone replacement therapy for the past year. Moreover, there was a strong positive correlation ($p < 0.001$) between bone resorption at baseline as measured by deoxypyridinoline cross links and increase in Mg (but not Ca) absorption rate.[129]

10.6 INULIN-TYPE FRUCTANS AND GASTROINTESTINAL ABSORPTION OF IRON, COPPER, ZINC, AND PHOSPHATE

10.6.1 Inulin-Type Fructans and Absorption of Iron

10.6.1.1 Animal Data

Worldwide, iron deficiency is among the most common human nutrition deficiencies. In most cases, it is due more to poor Fe bioavailability than inadequate intake.[138,139] In line with the observation of an enhanced Ca and Mg absorption (see Section 10.5.1 and Section 10.5.2), different research groups have thus looked at the effect of inulin-type fructans on Fe balance.[112-113,118,139,140] In addition, the potential of oligofructose to overcome biochemical markers of iron deficiency has also been investigated[118,137,142] (Table 10.3).

In healthy growing male rats (body weight 100–210 g) fed standard diet supplemented with oligofructose or inulin (1, 3, 5, or 10% w/w), results are contradictory (Table 10.3), some groups reporting a significant increase in Fe absorption[112,113] whereas other groups reported an increase or a slight decrease, depending on the composition of the dietary fiber fraction of the diet (i.e., cellulose 5%, oligofructose 5%, or cellulose 2.5% + oligofructose 2.5%)[140] or no effect.[141] In addition to Fe absorption, Delzenne et al. and Lopez et al. have also investigated the effect of inulin-type fructans (10% w/w in diet) on iron concentration in plasma (m*M*) and showed no significant changes.[112,113] Lopez et al. have also analysed markers of iron status, i.e., liver iron concentration (mg/g dry tissue), and transferin saturation (%) in control rats and in rats fed a diet containing phytic acid (7% w/w) and reported no effect of inulin. In control rats fed a standard diet, inulin had no effect on these parameters. Although adding phytic acid had significantly reduced the three markers (12, 35, and 32%, respectively), adding inulin to the phytic containing diet restored the normal values.[113] In rats (male Sprague Dawley, body weight 175 g) fed a iron-deficient diet for 3 weeks followed by a low (15 mg/kg) or a high (30 mg/kg) iron diet for an additional 3 or 10 d, adding oligofructose (5% w/w in diet) significantly ($p < 0.001$) increased Fe absorption.[118] In their experiments, Lopez et al. have also tested the effect of inulin (10% in diet) on iron absorption in rats fed a phytate-containing diet (7% w/w). Results show that phytates indeed reduced Fe absorption by ±50%, but that adding inulin not only restored but even significantly improved (1.5 fold) that parameter as compared to rats receiving the standard diet.[113]

Rats fed an iron-deficient diet show signs of anemia, i.e., lower hematocrit, lower blood concentration of haemoglobin, and lower haemoglobin regeneration efficiency, as well as changes in biochemical markers of iron status. Similar symptoms of anemia are induced by gastrectomy due to impaired Fe absorption. In both protocols, feeding rats a diet supplemented with oligofructose (5 or 7.5% w/w in diet) improved hematocrit ratio, haemoglobin concentration, and haemoglobin regeneration efficiency thus showing signs of improved recovery from anemia. In the iron-deficient protocol, oligofructose (5% w/w in diet) had no effect on biochemical markers of anemia (i.e., serum iron concentration, unsaturated, or total iron-binding capacity)[118]

whereas in the gastrectomy protocol, oligofructose (7.5% w/w in diet) partly suppressed the changes in these parameters induced by gastrectomy.[136] In a study comparing the effect of oligofructose and inulin (7.5% w/w in diet), Sakai et al. have concluded that the former, but not the latter, promoted recovery from postgastrectomy anemia in rats.[142] Indeed, as compared to the sham-operated control animals, the biochemical indicators of anemia were close to normal in rats fed oligofructose but not inulin.

10.6.1.2 Human Data

In their study with ileostomy volunteers, Ellegärd et al. have shown that feeding inulin (17 g/d) or oligofructose (17 g/d) for 3 d had no significant effect on Fe absorption in the upper part of the gastrointestinal tract.[122] In healthy adult volunteers, Coudray et al.[123] and van den Heuvel et al.[67] similarly reported no effect of inulin on Fe absorption. Moreover, serum parameters of iron status (concentration of hemoglobin and ferritin, transferring saturation and total iron-binding capacity) were not significantly modified by the inulin-type fructans, with the exception of a small but significant ($p < 0.05$) decrease (3.3%) in total iron-binding capacity after oligofructose feeding.[67]

10.6.2 Inulin-Type Fructans and the Absorption of Copper and Zinc

10.6.2.1 Animal Data

Only animal data are available so far on the effect of inulin-type fructans on the absorption of Cu and Zn (Table 10.3). Again, data are somehow confusing, and more research is certainly needed. Delzenne et al. and Lopez et al. report no effect of inulin on Cu absorption but a significant ($p < 0.01$) increase (1.8-fold) in Zn absorption.[112,113] Wolf et al. report no effect on Zn but a significant ($p < 0.01$) dose-dependent decrease in Cu absorption by oligofructose (–23, –43, and –40% for diets containing 1, 3, or 5% w/w, respectively).[141] As for iron absorption, Gudiel-Urbano and Goni report a decrease in Zn absorption when oligofructose is added (5% w/w) to a fiber-free diet but a significant increase (1.2-fold $p < 0.05$) when both cellulose and oligofructose are added (2.5% w/w each) to the diet.[140] Addition of phytic acid (7% w/w in diet) reduced both Cu and Zn absorption (40 and 60%, respectively), but, in the presence of phytic acid and inulin (10% w/w), the absorption of Zn was almost completely restored (19%), whereas the absorption of Cu was 50% higher (24%) as compared to control rats (21 and 16%, respectively). In that protocol adding phytic acid (7% w/w), inulin (10% w/w), or both did not significantly affect plasma and liver concentration of Cu and Zn. The only statistically significant effect was a reduction of the hepatic concentration of Zn (25%) that was only partly (7%) restored by inulin, but that effect remained statistically not significant.[113] These data confirm the observation made by Delzenne et al. that inulin-type fructans had no effect on Zn or Cu serum concentrations.[112]

TABLE 10.3
Effects of Inulin-Type Fructans and Fe, Cu, and Zn Balance in Rats

Product Tested (Dose) Rats Duration	Method	FA[1] (%) Fe	FA (%) Cu	FA (%) Zn	Refs.
Oligofructose *(10%)* **Inulin (10%)** Wistar males Age: 4–10 weeks, N = 10, **24 d**	**Balance** Plasma atomic absorption spectrometry	Oligofructose: 16.7➡33.2 (100%) Inulin: 16.7➡30.8 (+80%)	Oligofructose: NS Inulin: NS	Oligofructose: 43.6➡55.3 (+30% Inulin: 43.6➡53.8 or +20%	(115)
Oligofructose (1, 3, 5%) Sprague-Dawley males Age: 5–9 weeks, N = 10, **27 d**	**Balance** (ICPS)[2]	1% NS 3% NS 5% NS	1%: 29.7➡22.8 (–23%) 3%: 29.7➡17.1 (–43%) 5%: 29.7➡17.6 (–41%)	1% NS 3% NS 5% NS	(144)
Inulin (10%) Wistar males Age: 6–9 weeks, N = 8, **21 d**	**Balance** Atomic absorption spectrometry	31➡40 (+30%)	16➡29 (+80%)	NS	(116)
Oligofructose (5%) Wistar males Age: 8–12 weeks N = 8, **28 d**	**Balance** Atomic absorption spectrometry	Cellulose 5% 81.1% Oligofructose 5% 71.3% (12%) Cellulose 2.5% + Oligofructose 2.5% 85.4% (+ 5%)	Cellulose 5% 61.8% Oligofructose 5% 56.5% (9%) Cellulose 2.5% + Oligofructose 2.5% 71.6% (+ 16%)		(143)

[1]FA = Fractional absorption.
[2]ICPS = Inductive coupled plasma emission spectrometry.

10.6.2.2 Human Data

In their study with ileostomy volunteers, Ellegärd et al. have shown that feeding inulin (17 g/d) or oligofructose (17 g/d) for 3 d had no significant effect on Zn absorption in the upper part of the gastrointestinal tract.[122] In healthy adult volunteers, Coudray et al. similarly reported no effect of inulin on Zn absorption.[123]

10.6.3 Inulin-Type Fructans and Phosphate Absorption

In most studies so far reported, phosphate absorption and P serum concentration were not affected by inulin-type fructans neither in rats[77,79,109,111,141–143] nor in dogs.[115] However, in their publication of 1993, Ohta et al. had reported a slight (+13.5%) but significant increase ($p < 0.05$) in that parameter in rats fed a diet containing 15% (w/w) oligofructose.[109] But, and in the same publication, these authors reported either no change or an increased phosphate absorption as well as an increased P content of femur (+19%) at a dietary oligofructose concentration of 5%. The increse in Ca absorption declined progressively with time (+13.5, +6, and +5% at day 7, 18, and 31, respectively).[109]

In gastrectomized rats (4-week-old male Sprague-Dawley) that were used to test for the preventing effect of oligofructose (10% w/w in diet for 10 d) on osteopenia known to occur in gastrectomized patients, a significant increase in P absorption was reported when comparing the gastrectomized rats fed the oligofructose-containing diet the control sham-operated rats (89 and 76.5%, respectively or a 16% increase). But in the two groups of gastrectomized rats the absorption of P was similar (89 and 92%).[131]

10.7 INULIN-TYPE FRUCTANS — MINERAL ABSORPTION AND BONE HEALTH: DISCUSSION, PERSPECTIVES, AND CONCLUSION

10.7.1 Protocols and Methodologies

In experimental animals, almost all studies performed to test for the effects of inulin-type fructans on mineral absorption used protocols that included healthy young growing male rats (Fisher 344, Sprague–Dawley, or Wistar) or adult virgin female ovariectomized rats (Fisher 344 or Sprague–Dawley). In some protocols, cecectomy or gastrectomy was performed before starting the inulin-feeding period. One study was also performed in adult Beagles dogs. In some of the protocols, rats were also fed a diet containing different concentrations of Ca, Mg, or Fe to induce either deficiency or excess intake. In others, Ca and/or Mg were infused directly into the stomach or the cecum with the aim to identify the site of action of inulin-type fructans. One study also included phytic acid in the diet, a food component known to interfere with the gastrointestinal absorption of minerals. The length of the treatment with the inulin-type fructans varied from a few days till a few weeks.

To quantify the effects of inulin-type fructans on mineral absorption, the metabolic balance methodology (see Section 10.4.1.1) was used in most experimental studies. The amount of the minerals (essentially Ca and Mg but also sometimes Cu, Fe, P, and Zn) in diet, feces, and urine plus eventually serum and cecal content was quantified using either atomic absorption spectrometry or, and in most cases, inductively coupled plasma–mass spectrometry (ICP–MS). Only a few groups utilized the tracer methodology (see Section 10.4.1.2).

In human volunteers, one study utilized the metabolic balance approach but the others utilized the dual stable isotope method (Section 10.4.1.2). That methodology

has been discussed in detail.[83,84,143] It is based on the assumption that the orally and intravenously administered isotopes (minimal quantities to avoid perturbation in mineral metabolism) are metabolized at the same rates once Ca pools have reached equilibrium.[124] The main advantage over the metabolic balance methodology is that it measures true absorption by allowing a separate distinction between exogenously unabsorbed and endogenously secreted minerals. Because it is hypothesized that the effects of inulin-type fructans on mineral absorption take place in the large bowel where they are fermented, it is essential that urine is adequately collected to allow accurate measurement of all components of minerals absorption.[84] Indeed, if, in normal circumstances, very little calcium absorption occurs in the colon,[144] it is the case in the presence of nondigestible carbohydrates that shift part of the absorption process in the lower part of the intestine. Thus, it has been argued that a longer urine collection is required to capture this late colonic phase of absorption.[10,66] For this reason, it has been recommended to collect the urines for 48 h after administration of the intravenous tracer or for at least 36 h after administration of the second dose of oral Ca tracer. To study changes in Mg absorption, a urine collection time of 72 h is recommended. Using too short of a collection period will lead to erroneous results as it will underestimate the changes. Moreover, it has been reported that measuring Ca absorption based on analysis of urine pools collected during hours 0–8, 9–16, or even 16–24 h was not suitable, leading to underestimates.[84] Four studies have investigated, in adults, the effect of inulin-type fructans by measuring changes in the kinetics of Ca urinary excretion (see Section 10.4.1.3) over a given period of time (i.e., 8 to 12 h or 72 h) after a single meal (especially breakfast) or a beverage containing a dose of Ca. In three of these experiments, oligofructose or inulin was given as an acute dose without any previous treatment that could have modified the composition and activities of the colonic microflora. This method has not really been validated and certainly not for quantifying changes in Ca absorption induced by differences in food composition that are expected to influence Ca absorption by acting in the large bowel. Urinary Ca excretion represents only a small percent of the dietary Ca, actually 2–5% in rats.[80] Moreover, Ca intake explains only 6% of the variance in urinary Ca.[144] In studies aimed at investigating the effect of increasing Ca intake on bone health, it is considered that urinary Ca is not sufficiently sensitive to evaluate compliance in a randomized controlled trial of 1 g Ca/d, compared to a placebo (C. Weaver, personal communication). For evaluating the effects of inulin-type fructans on mineral absorption, an adaptation period of at least a few days would be required.

10.7.2 Effects of Inulin-Type Fructans on Absorption of Minerals

In experimental animals (mostly rats), a large number of publications demonstrate that inulin-type fructans significantly increase mineral absorption, essentially Ca and Mg, but also Fe and Zn. The effects on Cu and P absorption are less documented, and the available data are less convincing. Except for one study that reported that oligofructose was more efficient than inulin in promoting recovery from postgastrectomy anemia in rats,[142] all inulin-type fructans (native inulin, oligofructose, inulin

HP, or oligofructose-enriched inulin Synergy 1) were equally effective in modulating mineral absorption even though some qualitative differences might exist when comparing the effects of different types of inulin. In a study aimed at comparing oligofructose, inulin HP, and oligofructose-enriched inulin Synergy 1, Coudray et al. have shown that the last product, composed of a mixture of both oligofructose (short oligomers with a DP_{av} = 3.6) and inulin HP (polymers with a DP_{av} = 25), was more active than oligofructose or inulin HP alone in enhancing Ca and Mg absorption.[145]

The effects of oligofructose have been investigated in a wide variety of particular experimental protocols besides the normal healthy rats. Cecectomized rats as well as rats receiving Ca and Mg directly by stomach gavage or by cecal intubation have been used to test for the hypothesis that the effect of inulin-type fructans might be mediated through large bowel fermentation. Protocols in which rats were fed a Mg or a Fe-deficient diet were also used demonstrating that improving mineral absorption was an effective way to reduce the incidence of symptoms known to be associated with such deficiencies. With the same objective, gastrectomized rats, known to be at high risk of developing anemia were also used. Finally, adult virgin ovariectomized female rats were also used because this well-recognized protocol mimics the physiological conditions prevailing in postmenopausal women. The conclusions of all these studies are that inulin-type fructans:

1. Significantly increase mineral, especially Ca and Mg, absorption
2. Protect rats from developing symptoms known to be associated with some mineral deficiencies (especially Mg and Fe)
3. Help rats overcome symptoms of anemia and osteopenia
4. Restore a close to normal Ca and Mg balance in adult virgin female ovariectomized rats

Because of their high fermentation rate in the large bowel, due to the presence of an anaerobic microflora, the effects of inulin-type fructans on mineral are likely to take place primarily in the lower part of the intestine. But that is probably more so for Mg than for Ca, the effects on the former being exclusively mediated in the large bowel, whereas for the latter it might involve both the upper and the lower gut on a 50/50 basis.

For both Ca and Mg the relative increase in intestinal absorption has been shown to inversely correlate at high significance with the basal absorption capacity, being higher when basal absorption is lower. Because Ca absorption capacity decreases with age, the relative increase in absorption induced by inulin-type fructans increases as animals become older. Since it has been shown that the beneficial effects of inulin-type fructans on mineral absorption still persist after ovariectomy, it can be hypothesized that in females such effects are hormone independent.

In young growing rats, two studies have shown that the effects of oligofructose might decrease with the length of the treatment, indicating a possible adaptation and/or a possible feedback down-regulation of the active absorption (see Section 10.7.3). These observations need, however, more specific investigations.

In humans, inulin-type fructans have no effect on mineral absorption in the small intestine and their effects on Ca and Mg absorption are likely to be mediated via

changes in the lower part of the intestine that are mediated by the activity of the microflora. The most convincing data have been obtained in adolescents and in postmenopausal women, but one study confirmed these effects in adult men.

In their first study on mineral absorption, van den Heuvel et al. found no effect of 15 g/d inulin-type fructans on calcium absorption in young men, using a dual-isotope tracer method.[66] One criticism of this study was that urine was only collected for 24 h, potentially missing the late colonic phase of absorption.[124] Indeed, a subsequent study by the same group, using a 36 h urine collection, showed that 15 g/d oligofructose significantly increased calcium absorption.[67] Further data have shown a significant increase in calcium absorption in adolescent girls in response to the consumption of 8 g/d of oligofructose-enriched inulin Synergy 1 but no beneficial effect to the same dose of oligofructose.[126,127] This suggests that this mixture may be a more potent promoter of calcium absorption than oligofructose, thus confirming the experimental data reported by Coudray et al.[145] The studies by Griffin et al. differed from previous studies in a number of important aspects.[126,127] All previous studies were smaller in scale, involving only 9–12 male subjects, and they used higher intakes of oligosaccharides (15 g/d and 40 g/d for van den Heuvel et al.[66,67] and Coudray et al.,[123] respectively). The dose used in Griffin's studies was only 8 g/d. This compares to a typical dietary intake of 2.6 g/d of inulin and 2.5 g/d of oligofructose in the Western diet,[146] and well below the amount of oligosaccharides that may cause abdominal symptoms.[147] Moreover, the earlier studies have evaluated subjects with calcium intakes on the order of 800 mg/d, well below the RDA for this age group of 1300 mg/d (see Section 10.2.3).[7,125] Indeed, upon approaching and attaining puberty, it has been shown that net calcium balance increases with increasing calcium intake to a maximum of about 1200 mg/d, a value beyond which further increases in calcium intake do not improve calcium balance. Despite the fact that adolescent girls, enrolled in the most recent studies, averaged total daily calcium intakes that achieved and even surpassed this value, oligofructose-enriched inulin Synergy 1 significantly increased their absorption of calcium. The absolute increase in calcium absorption due to consumption of oligofructose-enriched inulin Synergy 1 was approximately 90 mg/d, which might be, physiologically, of significance, because even if only part of this additional calcium was utilized for bone mineral production, it could lead to a significant increase in peak bone mineral density during this critical period. Another interesting conclusion of the studies in adolescents is the inverse correlation between the relative increase in absorption caused by inulin-type fructans and the basal absorption capacity as measured before the intervention. The same correlation was demonstrated when analyzing the animal data (see Section 10.5.1.2 and Section 10.5.2.1). This would indicate that, with regard to mineral absorption, consuming inulin-type fructans would benefit more to the adolescents who have a low level of such an important physiological activity. As genetic polymorphisms are known to account for differences in Ca absorption,[148] it has been speculated that some genotypes could be more likely to benefit from consumption of inulin-type fructans and especially oligofructose-enriched inulin Synergy 1.[127]

Even if Ca intake and Ca urinary excretion possibly correlate in adults when bone formation is expected to equal bone resorption, it is not the case in adolescent ages when bone formation is expected to be greater than bone resorption to allow

the regular growth of bones.[149] Indeed, and confirming previous results,[150,151] van den Heuvel et al. have reported that, in adolescent girls and boys, Ca absorption did not correlate with urinary Ca excretion.[67] Moreover, the oligofructose-induced enhancement of Ca absorption reported by these authors was not reflected by an increase in urinary Ca excretion nor by an increase in the excretion of the intravenously injected ^{48}Ca isotope.

Even if the data available do not allow the conclusion that the additional amount of absorbed calcium was utilized for bone mineral production, no increase in urinary calcium excretion was observed that would have negated the increase in calcium absorption. Compared to these data, the two Japanese studies that claim for an acute enhancing effect on Ca absorption of a food supplement containing both oligofructose (3g) and Ca (300 mg as sole source during the trial period) in a 10:1 ratio are difficult to understand, and it remains to be demonstrated that the small increase in urinary Ca excretion was actually coming from the newly absorbed Ca pool.[88,89] Uenishi et al.[89] did attempt to answer that question by adding a tracer dose of ^{44}Ca and measuring the $^{44}Ca/^{43}Ca$ ratio in the urine samples. But because that parameter increased significantly only at two time points, i.e., 8 and 10 h after oligofructose feeding, no firm conclusion can be made. Moreover, using a similar protocol, Teuri et al. reported no difference in Ca urinary excretion after ingestion of a single dose of inulin.[88]

A recent nutrition intervention study in postmenopausal women has confirmed the effects of oligofructose-enriched inulin Synergy 1 on Ca and Mg absorption. However, given the relatively small number of volunteers, no correlation could be demonstrated between basal absorption capacity and increased absorption. These data again support the hypothesis that oligofructose-enriched inulin Synergy 1 is more active than other inulin-type fructans in enhancing mineral (especially Ca) absorption. Indeed, Tahiri et al. have shown that, in postmenopausal women, oligofructose (10 g/d) had no effect on Ca absorption, [128] even though it significantly increased Mg absorption.[132] Still, in that case, the net increases in Mg absorption and urinary excretion were almost identical (±11 mg/d) so that the net benefit of increasing Mg colonic absorption for the overall Mg balance was virtually nil.[132]

To explain the absence of effect of oligofructose on Ca absorption in adults and in postmenopausal women[128] the argument has been used that a high rate of passive Ca absorption in the large intestine could trigger a feedback mechanism involving inhibition of duodenal active absorption as a consequence of a change in endocrine factors.[152,153] Moreover, Tahiri et al. argued that 5 weeks of treatment with oligofructose could, particularly, be effective in triggering such a feedback mechanism.[128] The data obtained in other studies by van den Heuvel et al.[67] using a more suitable protocol for urine collection and by Holloway et al.[129] after an even longer treatment period (6 vs. 5 weeks) do rule out that argument. However, and as mentioned above, data in rats showing a progressive decline in Ca absorption as well as a decrease in small intestinal Ca transporter after a few weeks of oligofructose feeding could be used to support the hypothesis of a feedback down-regulation. Because such observations were done only in protocols that used oligofructose, it remains to be demonstrated that such an adaptation mechanism, if it exists, is not a property of the

shortest oligomers of inulin that are known not be very rapidly fermented in the most upper segment of the bacteria-containing part of the gastrointestinal tract.

Some studies have also measured the serum concentration of the minerals. With regard to Ca, the serum concentration is so strongly regulated (because of its key physiological roles) that it does not sensitively reflect Ca absorption or changes therein. Measuring the concentration of Ca in serum is, thus, not a suitable methodology to assess the effects inulin-type fructans. The control of the concentration of other minerals in the serum is less well understood.

All these findings show that regular intake of even modest amounts of inulin-type fructans might significantly increase Ca and Mg absorption in girls at, or near, menarche and in postmenopausal women, with adequate or high calcium intakes, without any compensatory increase in urinary excretion. Investigation of the effects on more sophisticated measures of calcium metabolism and on bone mineral accretion, bone resorption, and bone turnover rates are required. Still, the findings of the above studies strongly suggest that addition of inulin-type fructans to food represents an opportunity to increase the uptake of Ca present in the diet, even if further studies are necessary to prove that the benefits of these ingredients to Ca absorption persist in the longer term and, importantly, that they can be translated into benefits to bone health.

Experimental data do already support the hypothesis that the beneficial effects of inulin-type fructans target not only the mineral absorption phase but also other aspects of bone health, especially bone mineralization, bone density, bone accretion, and resorption, i.e., bone turnover. However, to what extent observations on mineral balance allow assumptions on bone mineralization or bone quality requires information on the persistence of the stimulating effect of inulin-type fructans on mineral absorption, and on the relevance of improved calcium absorption with respect to bone mineralization, bone density, and bone structure.

10.7.3 Mechanisms

Even though inulin-type fructans might also affect the gastrointestinal absorption of other minerals like Fe or Zn (see Section 10.6), the present discussion on mechanisms will focus on Ca and Mg absorption, the two, up to now, most extensively studied processes.

In the gastrointestinal tract, Ca absorption is either active, being vitamin D dependent, or passive, being vitamin D-independent. The first mechanism is already saturated by low Ca intake whereas the second, which may involve both a para- and a transcellular mechanism, is not saturable. This implies that all of the increment in Ca absorption that occurs when diet is supplemented with extra Ca or when it provides a food component that increases Ca absorption is likely to be mediated by the vitamin D-independent mechanism. The absorption of Mg is considered to be exclusively passive (mostly paracellular) since no active mechanism has yet been described.

To explain the increased absorption of Ca and Mg after inulin-type fructans feeding, several hypotheses have been proposed (Figure 10.4):

1. Higher water content in the large bowel due to osmotic effect
2. Lower pH due to higher production of SCFAs by fermentation
3. Increased solubility in the intestinal lumen due to formation of SCFA salts
4. Increased absorptive surface due to hypertrophy of the mucosa
5. Increased production of calbindin, a Ca transport protein, especially in the colonic epithelium

These hypotheses are supported by data showing a selective stimulation of bacterial (mainly bifidobacteria and lactobacilli) growth in the intestinal lumen, (see Chapter 5, Section 5.4.1 and Chapter 6, Section 6.2.4) as a result of fermentation of inulin-type fructans by the microflora with, as a consequence, a reduction of pH due to increased production of SCFAs, mainly propionate and acetate and, at a lower level but at a higher rate, butyrate and lactic acid. At this more acidic pH, more Ca and Mg ions are solubilized in the gut lumen and, thus, are more readily absorbed.[75,79,110] Apart from stimulating the passive absorption indirectly by increasing solubility via lowering the pH, SCFAs might also directly stimulate mineral disappearance across the colon, propionate being more effective than acetate. The higher efficacy of propionate compared to acetate might be due to a greater lipid-solubility, which is associated to chain length.[154] Butyrate, the third major SCFA produced by fermentation of inulin-type fructans, is also a potent candidate for enhancing mineral absorption because it is a substrate for colonic epithelial cell growth and proliferation, leading to an enlargement of the gut's absorptive area,

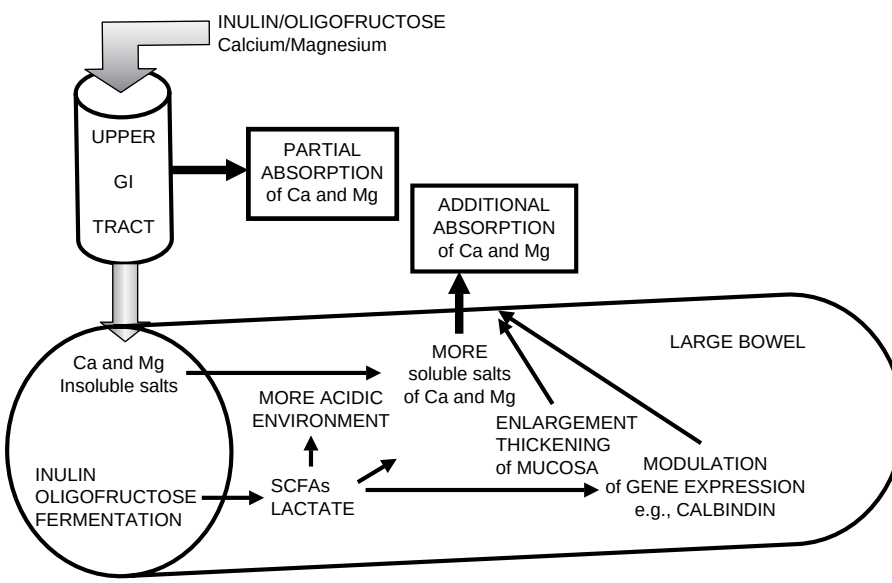

FIGURE 10.4 Inulin-type fructans and enhanced Ca and Mg absorption: hypothetical mechanisms.

another way to contribute to the enhanced mineral absorption.[155] Moreover, increased butyrate production might stimulate the active transcellular transport of Ca via an increased concentration of calbindin and the activity of the $1,25(OH)_2$ vitamin D3 receptor, as shown in chick primary cell culture.[156] In rats, calbindin D9k is an intestine-specific, high-affinity, Ca-binding cytosolic protein, and its expression is regulated by $1,25(OH)_2$ vitamin D3 and Ca intake (up-regulation if low Ca diet and down-regulated if high Ca diet).[157] It has been hypothesized that calbindin contributes directly to transcellular and transepithelial Ca transport,[152] and a correlation has been observed between calbindin D9k expression and intestinal Ca absorption.[158,159] But calbindin also contributes indirectly to miscellaneous essential enzyme and protein-dependent cellular and metabolic processes that are controlled by the "free" intracellular Ca concentration, a key parameter in eukaryotic cells.[160] The expression of calbindin is highest in the duodenum but it exists also in other parts of the intestine and especially in the large bowel, even though at much lower basal level.[161,162] The concentration of calbindin D9k is low in suckling and adult rats, and it is maximum (3-fold higher) during the 3–4 weeks following weaning.

In looking for a mechanism of the observed increase in Ca absorption, Ohta et al. have reported a higher relative expression of the calbindin D9k in the large intestine over that in the small intestine in rats fed oligofructose.[163] At the lowest dose (5% w/w in the diet), the increase in calbindin expression was higher (2.5-fold) in the colon than in the cecum (1.25-fold), and the increase in Ca absorption positively correlated with the increase in calbindin expression. Similarly, in gastrectomized rats, an experimental model of reduced Ca absorption and osteopenia (see Section 10.5.1.2), in which Ohta et al. had previously demonstrated a significant increase in Ca absorption, adding oligofructose to the diet (10% w/w) at the age of 4 weeks, stimulated calbindin expression both in the cecum (3.7-fold) and in the colon (8.7-fold), far above the increase induced by gastrectomy alone (2-fold).[131,136,137] It should be kept in mind that the experiments performed so far have used young weaned rats (4–5 weeks of age), i.e., the age at which the basal level of calbindin D9k in the intestine is maximum.[131,163] Thus, the increase in calbindin might be one of the mechanisms explaining the effect of inulin-type fructans in adolescents (see Section 10.5.2.2) but, at the present state of knowledge, that cannot be used to explain the improved Ca absorption seen in adults, postmenopausal women, or the elderly.

Other candidates for Ca and Mg absorption enhancers are the polyamines, e.g., spermine, spermidine, and putrescine, that are generated by several strains of microbes as well as by higher organisms[163] and that are potent agents to stimulate proliferation and induce enlargement of the intestinal tissues because they are readily taken up by enterocytes or can pass into the circulation to stimulate gene expression of calcium binding proteins in the gut.[155,165–167] Inulin-type fructans have been shown to stimulate ornithine decarboxylase activity, the rate-limiting step in polyamines synthesis, and when rats were fed oligofructose for 4 weeks, putrescine concentration was higher and spermidine concentration lower, whereas total polyamine concentration was unchanged in the cecal contents compared to a standard diet.[107] In addition and in the cecal tissue putrescine, spermidine and total polyamine concentrations were increased.[168]

To further test whether the oligofructose-induced stimulation of Ca absorption was mediated by polyamines, a study with adult female ovariectomized Fisher 344 rats did compare the effect on femur weight and ash, an indirect measure of Ca absorption, of feeding either oligofructose or polyamines. A significant loss of femur weight occurred following ovariectomy but only oligofructose and not exogenous polyamines alone did prevent loss of femur weight and femur ash. Thus, the potential of oligofructose to prevent ovariectomy-induced loss of bone weight and bone mineral content may not be mediated via polyamines, since exogenous polyamines were not protective, possibly because endogenous polyamine synthesis compensated for it.

10.7.4 Conclusion

Adequate and appropriate nutrition is important for all individuals, but not all follow a diet that is optimal for bone health. Ca and Mg are specific nutrients most important for attaining peak bone mass, for reducing the risk of osteoporosis, and possibly for other major physiological functions. However, a significant proportion of some population groups fail to achieve the recommended intakes of these minerals in a number of western countries. The challenge remaining for interested groups (including nutritionists, health professionals, and the food industry) is to encourage such individuals to meet their Ca and Mg requirements. This task is not an easy one when so few high-Ca or even more so high Mg foods, except for dairy products, are readily available. Ca and/or Mg supplements or Ca and/or Mg-fortified foods may be needed by individuals who do not, or will not, consume Ca and Mg-rich foods as recommended in the dietary guidelines of many western countries. Consumption of a functional food, which contains inulin-type fructans that may positively influence absorption of these minerals, will ensure that the bioavailability from foods can be optimized.

Increased bioavailability of an essential nutrient and especially of an essential mineral is recognized as a valid enhanced function claim (see Chapter 1). Supported by the results of a large number of animal studies and human nutrition intervention trials, the claim **"inulin-type fructans enhance Ca absorption"** is scientifically substantiated.[169] Even though a majority of trials have involved adolescents, confirming evidence already exists in adults as well as in postmenopausal women. However, quantitative (in terms of effective daily dose) differences may exist between different inulins, the most active product being a mixture of oligofructose and long-chain inulin (inulin HP), the so-called oligofructose-enriched inulin Synergy 1 that is effective already at a daily dose of 8 g.

Regarding Mg absorption, the human trials performed so far have demonstrated a beneficial effect of inulin-type fructans. Thus, and similarly the claim **"inulin-type fructans enhance Mg absorption"** is similarly scientifically substantiated.

However, the concept of enhanced absorption is likely not to be limited to Ca and Mg, but it might also holds true for other minerals like Fe, Zn, and eventually Cu when further trials are completed. The mechanisms of action of inulin-type fructans still remain hypothetical and further research is needed to fully elucidate them. Similarly, the reason for the higher efficacy of oligofructose-enriched inulin

Synergy 1 compared to inulin and oligofructose alone needs to be further investigated. One hypothesis is that the mixture of oligofructose and long-chain inulin feeds the colonic microflora up to the most distal part of the large bowel and thus causes changes all along the different segments that favor mineral absorption, not only in the proximal-ascending but also in the transverse and eventually even in the descending colon.

Even though some animal data already show that, inside the body, the increased Ca and eventually Mg pool can be used to improve bone structure, bone mineral content, and bone mineral density, data are still not sufficient to substantiate such claims and certainly not to substantiate a claim of reduction of risk of osteoporosis. Still data are sufficient to justify such hypotheses to be tested in human nutrition intervention trials.

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11 Inulin-Type Fructans and the Homeostasis of Lipids

11.1 INTRODUCTION

From a metabolic point of view, the term *lipid* covers two chemical, rather complex, entities, namely triglycerides or triacylglycerols and cholesterol, both free and esterified, that are synthesized and catabolized in various tissues of the organism. Nutrients and food components can both positively and negatively modulate these metabolic processes and, consequently, they can influence the blood concentration of these lipids and the different forms of lipoproteins that serve to transport these water-insoluble compounds. These parameters are indirect, often sensitive, markers of lipid homeostasis. Some of these parameters have also been validated as markers of risk for body-fat-related diseases like atherosclerosis, cardiovascular diseases, obesity, and type II diabetes.

Among the major nutrients (some of the carbohydrates that resist digestion in the upper digestive tract and that are fermented in the large bowel, the so-called nondigestible carbohydrates), some which classify as dietary fiber (see Chapter 4 and Chapter 6) have been shown to positively modulate either the digestion and absorption or the metabolism of triglycerides-triacylglycerols or cholesterol. Indeed, when the dietary intake of such carbohydrates (e.g., resistant starch, b-glucans, pectins, gums, and inulin-type fructans) increases, triglyceridemia (the concentration of triglycerides-triacylglycerols in blood) and/or cholesterolaemia (the concentration of total or lipoprotein-bound cholesterol in blood) may decrease. Moreover, the distribution of the lipids between the different lipoproteins may also change in favor of a more beneficial pattern for health.

The mechanism of action of these nondigestible carbohydrates/dietary fibers on lipid homeostasis involves either a decreased gastrointestinal bioavailability or more specific changes at the metabolic level by decreasing biosynthesis, by increasing catabolism and/or by modifying the interconnections between the cellular metabolic processes that take place in the different tissues.

11.2 BIOCHEMISTRY OF LIPID METABOLISM

11.2.1 Metabolism of Triacylglycerols

Triglycerides/triacylglycerols (TAGs) are long-chain carboxylic acyl (fatty acyl) esters of glycerol.

Their biosynthesis (Figure 11.1) involves the production of glycerol-3-phosphate as a by-product of glucose oxidation via the triose-phosphates, followed by the esterification in the endoplasmic reticulum with long-chain acyl-SCoA (fatty acyl-SCoA), the products either of a repeated sequential biosynthetic pathway using acetyl-S-CoA as substrate or of a thioesterification of free long-chain carboxylic acids (free fatty acids or FFA) with coenzyme A (CoA-SH) in the presence of adenosine triphosphate (ATP). The ester phosphate bound of the intermediate diacylglycerol-3-phosphate (a phospholipid) is then hydrolyzed and the diacylglycerol is further esterified to give the final TAG. The TAGs then either become associated, in the Golgi apparatus, with apoproteins to form very low-density lipoproteins (VLDL) that are secreted in the blood circulation, or they are stored as cytosolic fat particles (especially but not exclusively in the cells of the adipose tissue). The main organ of TAG's biosynthesis is the liver. During their circulation in the blood stream, VLDLs progressively loosen the TAGs that become hydrolyzed to glycerol + FFAs by lipoprotein lipase, an enzyme that is covalently bound to the blood capillaries. The residual VLDLs (i.e., IDLs or intermediate density lipoproteins) are further metabolized by hepatic lipase to eliminate most of the remaining TAGs and become the low-density lipoproteins or LDLs. The hydrolysis of the TAGs released from the lipoproteins produces glycerol and FFAs that serve as metabolic fuel for peripheral tissues (e.g., muscle, liver, etc.), become stored in the adipose tissue or are used to synthesize lipid constituents of the cell membranes or lipid-based signaling molecules.

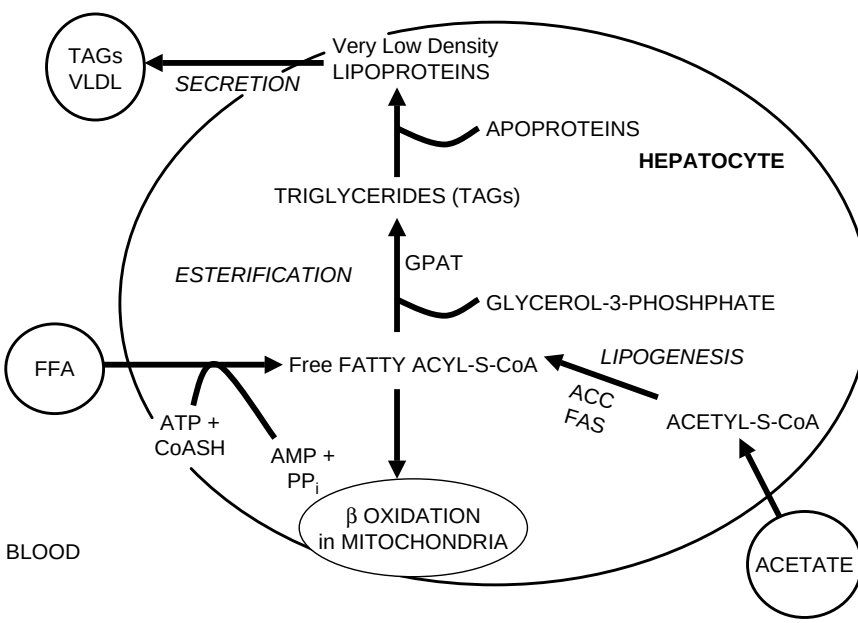

FIGURE 11.1 Biochemical pathways of liver lipid metabolism. FAS = fatty acyl synthase; ACC = acetyl-S-CoA carboxylase; GPAT = glycerol-3-phosphate acyl transferase.

When the metabolic homeostasis is disrupted, TAGs and the VLDLs tend to accumulate and triglyceridemia increases. Hypertriglyceridemia is a situation where the blood concentration of TAGs is higher than 2.3 m*M* (or 2 g/l) in adults. Such a situation, especially when it remains high in a postprandial phase, is more and more recognized as a risk factor for cardiovascular diseases and atherosclerosis.[1] Indeed, both epidemiologic and case control studies have shown that the levels as well as the duration of hypertriglyceridemia are key risk factors in the pathogenesis of atherosclerosis. Even though TAG-rich lipoproteins are probably not atherogenic by themselves, when they remain postprandially elevated, they could promote:

- The appearance and the persistence of chylomicrons that are recognized as atherogenic factors
- A high level of subspecies of LDLs, the so-called small dense LDLs
- A low concentration of HDL-cholesterol (see Section 11.2.2)
- An increased risk of blood coagulation due to activation of the coagulation factor VI

11.2.2 Metabolism of Cholesterol and Lipoproteins[2]

Cholesterol is an essential constituent of all eucaryotic cells especially the peri- and intracellular membranes. It is also the precursor of the steroid hormones, and its catabolism produces the bile acids. In the body, cholesterol is either endogenous, being biosynthesized (in the liver and the intestines), or exogenous, coming from foods (Figure 11.2).

The biosynthetic process is down-regulated by the concentration of exogenous cholesterol in lipoproteins, the proteins that transport this water-insoluble molecule. The dietary cholesterol is absorbed in the small intestine and reaches the general circulation as part of the chylomicrons and chylomicron remnants in which it is present as fatty acyl-esters or esterified cholesterol. It is then rapidly transferred in VLDLs originating in the liver. These are transformed into LDL and the esterified cholesterol is transferred to HDL which also serves to extract the cholesterol stored in peripheral cells, especially cells of the vascular endothelium. With regard to cardiovascular disease, the fasted level of cholesterol in HDL is associated with a reduced risk whereas the same parameter in LDL is associated with an increased risk, and thus the ratio HDL-cholesterol over LDL cholesterol is used as an index for that risk.[3]

Both the HDL and LDL particles bind to specific receptors in the liver and deliver both their esterified and free cholesterol into the hepatic cells that oxidize the cholesterol to produce the primary bile acids that become excreted via the bile into the small intestine and finally the large bowel where they are deconjugated and ultimately transformed into the secondary bile acids. Part of the colonic bile acids pool is then reabsorbed to close the enterohepatic circulation, whereas the rest is excreted in the feces.

The targets for functional food development are thus the concentration of total/esterified cholesterol in blood but more specifically in the lipoproteins with the aim to reduce the LDL while increasing the HDL fractions and thus increasing the HDL-cholesterol over LDL-cholesterol ratio.

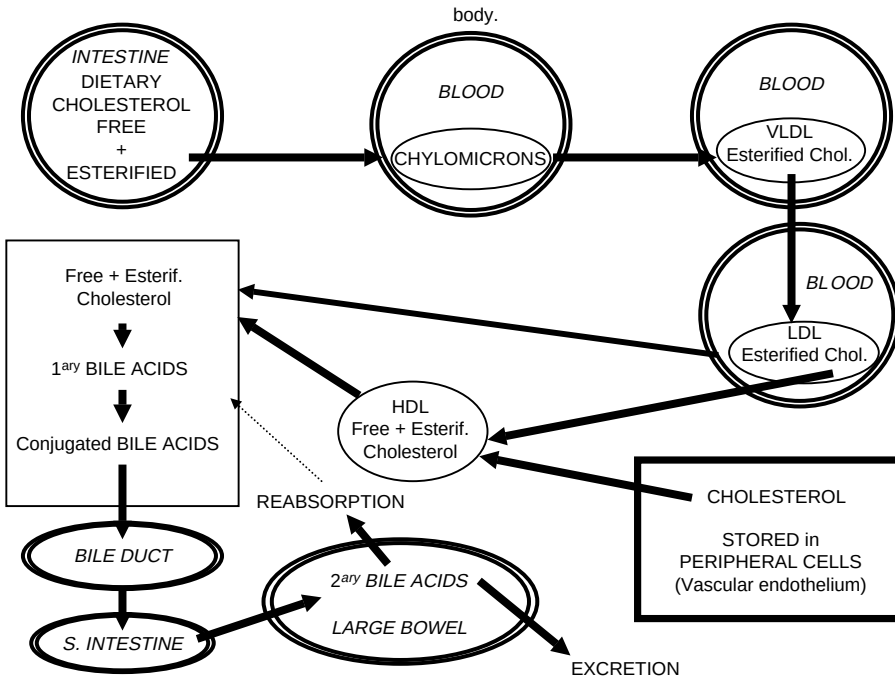

FIGURE 11.2 Schematic representation of the pathways of cholesterol transport in the human body.

11.2.3 Methodologies to Study Lipid Metabolism and Lipid Homeostasis

11.2.3.1 *In Vivo* Experiments

The methodologies used to study the effects of functional foods and especially inulin-type fructans on lipid metabolism measure the parameters classically used as biochemical markers of lipid homeostasis, i.e., blood or liver concentration of TAGs, cholesterol (total, free, esterified) and eventually phospholipids (PLPs), composition (in terms of lipids or apoproteins) and eventually the ratio of their blood carriers, the lipoproteins (very low-density lipoproteins or VLDL, intermediate-density lipoproteins or IDL, low-density lipoproteins or LDL, high-density lipoproteins or HDL). Except for a few studies that used a chemical method, the studies aimed at testing the effect of inulin-type fructans have applied the enzymatic assays classically used in human clinical biochemistry for lipid analysis. The analyses were performed either directly on plasma or serum samples or after extraction of an aliquot of homogenized liver tissue.[4] These assays are based on the enzymatic oxidation of the various lipid molecules to produce H_2O_2 that reacts with 4-aminoantipyrine to form colored quinoneimine that absorbs light at a wavelength between 492 and 550 nm. Absorbance is directly proportional to the amount of lipid expressed either as mg/dl, g/l or mmol/l (m*M*).

To quantify and/or to analyze the composition of the various lipoproteins, an aliquot of serum is ultracentrifugated on a density gradient according to the method described by Chapman et al.[5] The VLDL, LDL, and HDL fractions have a density that is <1.006; between 1.019 and 1.063, and between 1.125 and 1.21, respectively. The total mass of each lipoprotein fraction is then calculated by adding all constituents, i.e., TAGs + cholesterol + PLPs + proteins. The size of the VLDL particles is estimated by calculating the ratio of the concentrations of lipids at the surface (essentially free-cholesterol and PLPs) over that of lipids localized in the core (essentially TAGs and esterified cholesterol).[6] In most of these studies, the lipid parameters have been measured after overnight fasting but in a few protocols they have also been analyzed postprandially.

To assess the capacity of the liver to metabolize TAGs, the activity of the enzymes catalyzing the key steps in either lipid biosynthesis or lipid catabolism are measured in an homogenate of liver tissue collected after the sacrifice of either control or oligofructose-treated rats. If required the liver homogenate is fractionated by ultracentrifugation to isolate the mitochondria-rich,[7] the microsomal (or endoplasmic reticulum-rich),[8] or the cytosolic[9] fractions. The various enzyme activities are measured according to the *ad hoc* methodologies as described in the various publications.

11.2.3.2 *Ex Vivo* Protocols

To study the capacity of the liver to metabolize the lipids (essentially TAGs) hepatocytes from control or oligofructose-fed rats have been isolated using the enzymatic method described by Krack et al.[10] That method involves perfusing the liver *in situ* with a physiologic solution of a hydrolytic enzyme, i.e., collagenase followed by a mechanical dissociation of the cells and a series of washing and filtering steps to eliminate the endothelial and the Küpfer cells that remain at the surface of the filter (100 mm). These isolated hepatocytes are then incubated in suspension under continuous shaking in a Dulbecco's Modified Eagle Medium at a final concentration of $0.5.10^6$ cells/ml for up to 3 h. To test for the metabolism of TAGs, either [1^{14}C] palmitate or [1^{14}C] acetate is added to the suspension medium and the kinetic of incorporation of ^{14}C in the TAGs is measured as a function of time using a liquid scintillation counter.

11.3 INULIN-TYPE FRUCTANS AND LIPID HOMEOSTASIS

11.3.1 Animal Data

The hypothesis that inulin-type fructans might modulate the metabolism of lipids originates in an experiment protocol to evaluate their caloric value (see Chapter 7). Indeed, at the end of the treatment period, analysis of total body composition of oligofructose-fed rats (20% w/w in diet for 6 weeks) revealed a reduction in total body fat mass as compared to age-paired rats fed a control diet (Roberfroid unpublished results).

These data have since been confirmed in a variety of models (Table 11.1) designed to study the effects of inulin-type fructans on blood and/or liver lipid parameters.

11.3.1.1 Effects of Inulin-Type Fructans on Lipid Parameters in Healthy Experimental Animals Fed a Standard Diet

Except for one study that was performed in male beagle dogs, all studies so far reported to test for the effect of inulin-type fructans on healthy animals fed a standard diet have used fasted growing male Wistar rats. In these rat studies,[11–16] both inulin and oligofructose (10% w/w in diet) have consistently been reported to lower the concentration of serum TAGs by an averaged 30% (range 17 to 60%) (Table 11.1).

Only two publications reported no statistically significant effect on serum TAGs in rats fed a diet containing 5/6 or 10% inulin[17–18] even though the former study still reported a significant decrease (31%) in the serum TAGs in rats fed a 20% inulin diet.[17] In the same rats, a statistically significant reduction in serum total cholesterol is reported in 4 studies (18, 11, 15, and 21% in, respectively,[13–14,17–18]) but not in the others.[11,12,15,16] Moreover, in Levrat et al. the effect on serum cholesterol is dose-dependent at 11, 18, and 26% for doses of 5, 10, and 20% inulin, respectively.[11] In the studies in which such parameters have been quantified, the hypocholesterolemic effect concerns esterified cholesterol only[14] or both esterified and free cholesterol.[18] Moreover, one publication[12] reports an increase (1.9-fold) in the ratio HDL cholesterol over LDL cholesterol and two publications report a decrease in serum PLPs (- 15 and - 17% in, respectively,[14] and[15]). Liver TAGs have only been analyzed in three studies, one showing a 24% reduction[15] while the others did not report any significant effect.[16,18] In terms of serum lipoproteins, Fiordaliso et al.[14] report no change in LDL and HDL fractions but a statistically significant reduction in the number, but not the lipid composition nor the size of the VLD particles, thus explaining the reduction in plasma TAGs, esterifed cholesterol, and PLPs. In control male Wistar rats fed a standard diet, oligofructose has no effect on the concentration of plasma free fatty acids.[15,19]

When comparing the timing of the effect of oligofructose (10% w/w in diet) on the concentration of lipids in the serum, Kok et al. reported a progressive decrease in serum TAGs in fasted rats which became statistically significant at week 9, whereas in the postprandial state, the decrease of that parameter was already significant after 1 week of treatment and remained lower for up to at least 12 weeks.[20] In the postprandial state and after 4 weeks of treatment (oligofructose 10% w/w in diet), the reduction in plasma TAGs was 40%. The plasma concentration of PLPs (16%) but not that of total cholesterol was also significantly reduced. Similarly, in the liver of these rats, the concentrations of both TAGs and PLPs were significantly reduced (26 and 12%, respectively) but not that of cholesterol. In the postprandial state, oligofructose feeding had no effect on plasma-free fatty acids.[20]

In beagle dogs, Diez et al. similarly showed that supplementing the diet with mixtures of oligofructose (5 and 10% w/w) and sugar beet fiber for 6 weeks reduced the serum TAGs both in starved and postprandial states. Serum total cholesterol was significantly reduced only in dogs fed the mixture containing the highest dose of oligofructose.[21]

TABLE 11.1
Effects of Inulin-Type Fructans on Lipid Parameters in Experimental Animals

Animal Species bw[1] N	Diet Duration (weeks)	Inulin (%)	Blood Cholesterol (m*M*)	Blood TAGs	Blood Lipids[2]	Liver TAGs nmol/ mg proteins	Miscellaneous	Refs
MW[3] rats 40–50 g N = 6	Fiber-free std. diet 6–8 weeks	OFS 0 10 20	63[4] NS[5] NS	209.2[6] ¥0.72[7] ¥0.69				11
MW rats 170 g N = 12	Fiber-free std. diet 3 weeks	INU 0 5 10 20	1.76 ¥0.89 ¥0.82 ¥0.74	1.19 NS NS ¥0.63				17
MW rats 160 g N = 15	Std. diet 4 weeks	INU 0 10 20	0.80 NS NS	0.67 ¥0.40 ¥0.40	HDL/ LDL 0.94 ¥1.90			12
MW rats 160 g N = 15	Fiber-free std. diet 4 weeks	INU 0 10	1.40 ¥0.89	0.79 NS				13
MW rats 230 g N = 8	Hyperchol- esterol Diet[8] 2 weeks	INU 0 6[9] 6[10]	1.89 ¥0.79 ¥0.79	0.65 NS NS			Bread added to diet	18
MW rats 160 g N = 12	Std. diet 12 weeks	OFS 0 10	1.70 ¥0.85[11]	1.14 ¥0.76	Reduced number of VLDL		Decreased PLPs in serum ¥0.85	14
MW rats 120 g N = 10	Std. diet 4 weeks	OFS 0 10	1.77 NS	1.94 ¥0.61		58.3 ¥0.76	Decreased PLPs in serum ¥0.83	15
MW rats 120 g N = 5	High-fat diet 3 weeks	OFS 0 10	2.03 NS	1.45 ¥0.43		138.6 NS	Decreased PLPs in serum ¥0.80	20
MSD[12] rats N = 7	High- sucrose diet 3 weeks	OFS 0 10	2.60 NS	3.00 ¥0.70			No change in serum PLPs	19
MW rats 150 g N = 5	Std. diet High- fructose diet 4 weeks	OFS 0 10 OFS 0 10		3.70 ¥0.67 1.47 NS		173.8 NS 303.6 ¥0.72		16

-- continued

TABLE 11.1 (continued)
Effects of Inulin-Type Fructans on Lipid Parameters in Experimental Animals

Animal Species bw[1] N	Diet Duration (weeks)	Inulin (%)	Blood Cholesterol (m*M*)	Blood TAGs	Blood Lipids[2]	Liver TAGs nmol/ mg proteins	Miscellaneous	Refs
Hamsters	Std. diet	INU 0	7.45	6.10	VLDL/TAGs			27
60 g	Cholesterol	8	¥0.80	¥0.66	¥0.63			
N = 10	0.1%	12	¥0.83	¥0.60	¥0.57			
	5 weeks	16	¥0.60	¥0.36	¥0.34			
Mice	Std. diet	INU 0	9.10	1.14	HDL/LDL		Feeding inulin	31
LDL-	16 weeks	10	¥0.70	NS	0.48		prevents the rise	
N = 20					¥1.30		in cholesterolemia	
Beagles	Std. diet	OFS 0	160[4]	50.6[4]				21
dogs	6 weeks	4	NS	¥0.94				
N = 8		8	¥0.91	¥0.89				

[1] b.w = body weight at start of the experiment.
[2] Lps = lipoproteins.
[3] MW = male Wistar.
[4] mg/dl.
[5] NS = no statistically significant difference when compared to control value.
[6] mg/dl.
[7] fold decrease or increase in parameter as in control group
[8] Hypercholesterolemic diet containing 1% cholesterol and 0.1% cholic acid.
[9] Inulin baked in bread.
[10] Noncooked inulin added to bread.
[11] Decreased esterified cholesterol, no change in free cholesterol.
[12] MSD = male Sprague-Dawley rats.

11.3.1.2 Effects of Inulin-Type Fructans on Lipid Parameters in Healthy Experimental Animals Fed Hyperlipidemic Diets

Various protocols have been developed to induce hyperlipidemy in experimental animals. These are based on changes in diet composition and include:

- Supplementing the standard diet with cholesterol (0.1–0.2 or 1%) with or without cholic acid (0.1–0.2%)[22–24]
- Using sucrose or fructose as the major (±50–57.5%), if not the only, carbohydrate source in diet[25]

- Increasing fat content in diet from the recommended 3–5% up to 10–15% or even more
- Combining two of these approaches.

Supplementing such diets with inulin-type fructans has been used to test for their effects on lipid parameters in hyperlipidemic animals, a recognized model for humans at an increased risk of atherosclerosis.

Diets supplemented with cholesterol (0.1–0.2% or 1%) with or without cholic acid (0.1%) have been reported to induce mild to severe hypercholesterolemia in experimental animals. In a protocol designed to investigate the effect of consumption of unprocessed inulin and inulin baked in bread on the lipid metabolism in rats (male Wistar) made hypercholesterolemic (±2-fold increase in plasma total cholesterol, $p < 0.05$) by adding 1% cholesterol and 0.1% cholic acid, Vanhoof and Deschrijver reported no effect on plasma triglycerides and plasma cholesterol (total, free, or esterified).[18] In the same study, the hypercholesterolemic diet increased all liver lipid parameters (i.e., triglycerides, 3.25¥; total, 22¥, esterified, 54¥ and free cholesterol, 1.2¥; $p < 0.05$) but inulin (6% w/w in diet) had no effect on any of these parameters.[18] Using male Sprague-Dawley rats fed a diet supplemented with 0.2% cholesterol, Kim and Shin similarly reported that feeding a diet supplemented with inulin (5% w/w) had no effect on serum total cholesterol and triglycerides but significantly reduced LDL-cholesterol (28%) while increasing HDL cholesterol (1.12¥), thus increasing the ratio of HDL cholesterol over LDL cholesterol (1.43-fold).[26] Moreover, the inulin treatment significantly reduced the liver TAGs (47%), but it had no effect on liver PLPs or liver cholesterol. A dried water chicory extract (1 and 5% w/w in diet) had essentially the same effect as inulin.[26] In male Syrian hamsters fed a standard diet supplemented with 1% cholesterol without cholic acid, Trautwein et al. reported that inulin (the high molecular weight HP product) significantly reduced the plasma concentration of TAGs and total cholesterol.[27] These results show a dose-dependent effect of inulin on plasma TAGs (34, 40, 64% at 8, 12, 16% w/w in diet, respectively) but not on plasma total cholesterol (20, 17, 40% at 8, 12, 16% w/w in diet, respectively). Changes in the concentration of TAGs in the VLDL fraction accounted for most of the decrease in plasma concentration. Regarding total cholesterol, its concentration in VLDL was significantly reduced (62%) only in hamsters receiving the highest dose (16% w/w in diet).[27] When the core/surface was calculated (as described above) no apparent difference was found, suggesting no difference in particle size, thus confirming the observation of Fiordaliso et al. in rats.[14]

High sucrose/fructose diets are hyperlipidemic, especially hypertriglyceridemic. In male Wistar rats given access to either drinking water containing 10% fructose for 48 h[15] or a diet in which fructose was the sole source of carbohydrates for 4 weeks[16] an increase in serum triglycerides (1.2¥ and 2.1¥, respectively) has been reported but no effect on total serum cholesterol and PLPs. Adding oligofructose to the diet (10% w/w) to pretreat the rats (30 d) before giving them access to fructose in drinking water did not affect serum PLPs and cholesterol, but it significantly further increased triglyceridemia (1.2¥).[15] In such a protocol, fructose, oligofructose, or a combination of both had no effect on plasma free fatty acids. In the liver of fructose-fed rats, the concentration of triglycerides was increased

(1.25¥) but not that of PLPs or cholesterol as compared to control conditions. Prefeeding rats with oligofructose (10% in diet for 30 d) prevented the rise in the hepatic concentration of triglycerides that remained at the control level.[15] In a protocol in which male Wistar rats were fed a fructose-rich (65% w/w) diet supplemented with oligofructose (10% w/w) for 4 weeks, the concentration of plasma TAGs was still higher than in rats fed a starch-containing diet (1.7¥) but it was lower (33%) than in rats fed a fructose-rich diet without oligofructose. Similar modifications of the concentration of TAGs were observed in the liver, i.e., an increase (1.7¥) due to fructose intake both in the presence and in the absence of oligofructose, but also a smaller increase due to fructose intake in the oligofructose-containing/fructose-rich diet as compared to the oligofructose-free/fructose rich diet.[16]

In Sprague–Dawley rats, a sucrose-rich diet (57.5% w/w) has also been shown to induce hypertriglyceridemia and hyperinsulinemia followed, a few days later, by insulin resistance.[27] In male Sprague–Dawley rats, feeding a high sucrose (57.5% w/w) diet increased the concentration of plasma triglycerides (1.9¥). Adding oligofructose (10% w/w) to the diet significantly reduced plasma TAGs (30%) and plasma free acids (23%) but had no effect on plasma PLPs, and total and free cholesterol.[19]

11.3.1.3 Effects of Inulin-Type Fructans on Lipid Parameters in Genetically Modified Animals Prone to Develop Obesity or Hypercholesterolemia

Homozygous low-density lipoprotein receptor knock out mice ($LDLR^{-/-}$) is a recognized model of atherosclerosis.[28] Indeed, these mice develop spontaneous hypercholesterolemia with elevated levels of LDL and IDL and arteriosclerosis due to a genetic defect-deficiency of functional LDL receptors which is analogous to the genetic defects in humans with familial hypocholesterolemia.[29,30] After 3 weeks of treatment and throughout the study that lasted for 16 weeks, $LDLR^{-/-}$ mice fed inulin (10% w/w of the high molecular weight HP product in diet) had a significantly lower concentration of plasma cholesterol (30%) but the same concentration of plasma TAGs compared to mice fed the standard diet.[31] At the end of the protocol, the cholesterol content of VLDL, IDL, and LDL fractions of inulin-fed mice was lower (33, 40, 28%, respectively) than that of control mice. The concentration of cholesterol in HDL remained the same but the ratio HDL cholesterol over LDL cholesterol increased from 0.48 to 0.61 (1.28¥). Aortic arteriosclerosis expressed as the ratio intima/media tended to be lower (15%) in the inulin-fed than in the control mice but the difference was not statistically significant ($p = 0.17$).[31]

11.3.2 Human Data

The data obtained from animal studies suggest lipid-lowering properties in inulin-type fructans, and they justify investigating the effects in humans.[32] Indeed, in agreement with the strategy for development of functional foods (see Chapter 1, Section 1.3.3), convincing data in human nutrition intervention studies are required to substantiate such a claim. To date, a total of 12 studies that have investigated

effects of inulin or oligofructose on blood lipids in humans have been reported in the peer-reviewed literature. Table 11.2, Table 11.3, and Table 11.4 summarize the key features of these studies that have shown positive (8 studies) and negative (4 studies) outcomes, respectively, for the effect of inulin or oligofructose on blood lipids and other related parameters. For their review, these studies are classified according to the type of subjects that were included, i.e., normolipidemic, (slightly) hyperlipidemic, or noninsulin-dependent diabetic subjects.

11.3.2.1 Effect of Inulin-Type Fructans on Lipid Parameters in Normolipidemic Subjects

1. Luo et al. investigated effects of feeding oligofructose compared to sucrose (20 g/d in cookies for 4 weeks) in a randomized cross over design (12 males).[33] No changes in serum TAGs, cholesterol, or apolipoproteins were observed in either the treatment or placebo periods, although there was a strong trend for the concentration of free fatty acids (FFA) to be reduced at the end of the oligofructose feeding period.[33]
2. In a rigorously designed study with adequate statistical power (double-blind randomized cross over design in 66 young healthy women) reported by Pedersen et al., inulin (14 g/d in a low-fat spread for 4 weeks) had no effect on blood lipids.[34] Although HDL cholesterol and the LDL: HDL

TABLE 11.2
Effect of Inulin-Type Fructans on Lipid Parameters in Normo-Lipidemic Subjects

Subjects (Age+/Sem)	Diet Duration (Weeks)	Inulin g/d	Blood Cholesterol (Total)	Blood Triglycerides (TAGs)	Miscellaneous	Refs
Men (24 ± 1) N = 12	Low fiber 4 weeks	OFS 20 g/d (cookies)	3.91 m*M* NS[1]	0.72 m*M* NS	No effect on plasma glucose or insulin	33
Women (22 ± 2) N = 66	Habitual 4 weeks	INU 14 g/d (spread)	4.25 m*M* NS	0.98 m*M* NS		34
Men (23 ± 1) N = 12	Controlled 3 weeks	INU 15 g/d OFS 15 g/d (orange juice)	4.56 m*M* NS NS	1.40 m*M* NS NS	No effect on glucose absorption	35
Men (23.3 ± 1) N = 12	Habitual 4 weeks	INU 9 g/d	4.24 m*M* ¥ 0.92[2]	0.84 m*M* ¥ 0.73	No effect on cholesterol in lipoproteins	36

[1]NS = not statistically significant

[2] -fold decrease in parameter in control group

cholesterol ratios were lower at the end of both the control (placebo low-fat spread without inulin) and test (low-fat spread with inulin) periods as compared to habitual diet, there were no significant differences in blood lipids between placebo and inulin. However, in that study, the subjects were required to consume 40 g of spread per day corresponding to approximately twice the normal level of spread intake and 50% of total fat intake for young women, and that may have contributed to the negative findings observed in this group.[34]

3. Van Dokkum et al. designed a study (Latin square, randomized, double-blind, diet-controlled) to test for the effect of nondigestible oligosaccharides on large bowel function, blood lipid concentration, and glucose absorption in 12 young healthy male subjects.[35] In that study, both inulin and oligofructose (15 g/d in orange juice for 3 weeks) had no effect on blood lipid parameters, i.e., total, HDL, and LDL cholesterol, TAGs, PLPs, apolipoprotein-A-1, and apolipoprotein-B. The other nondigestible oligosaccharide tested, namely galactooligosaccharides (GOS) were also ineffective in reducing blood lipids. Neither oligofructose nor GOS significantly influenced glucose absorption.[35]
4. Brighenti et al. observed significantly lower plasma TAGs (27%) and cholesterol (8%) concentrations in young male volunteers (n = 12) who consumed 9 g/d of inulin added to a rice breakfast cereal for a period of 4 weeks and levels remained significantly lower (25%) 4 weeks after the end of the inulin phase.[36]

11.3.2.2 Effect of Inulin-Type Fructans on Lipid Parameters in (Slightly) Hyperlipidemic Subjects

1. Hidaka et al. reported reductions in blood total cholesterol (8%) in a group of Japanese subjects with hyperlipidemia (20 males and 17 females) but no effect in hyperliproteinemic (Type IIa) patients (7 men and 1 women). However, in the second group, if only the responders (N = 4) were taken into account, a 11% reduction in plasma total cholesterol was observed.[37]
2. In a randomized crossover trial in subjects with modest hyperlipidemia, Davidson et al. showed significantly lower total (8.7%) and LDL cholesterol (14.4%) concentrations during inulin compared with placebo phases, but the authors reported no effects on HDL cholesterol or serum TAGs concentrations.[38]
3. In a double-blind randomized parallel study conducted in 54 middle-aged subjects with moderately raised blood lipid concentrations over a longer period than any of the previous human studies (i.e., 8 weeks compared to 3–4 weeks), inulin (10 g/d in a powdered form added to beverages, soups, cereal, etc.) had no significant effect on total LDL or HDL cholesterol or apolipoproteins B and A. However, after intervention, serum TAGs levels were significantly lower in the inulin treated group (19%) than in the control group.[39]

TABLE 11.3
Effect of Inulin-Type Fructans on Lipid Parameters in (Slightly) Hyperlipidemic Subjects

Subjects (Age)	Diet Duration (Weeks)	Inulin g/d	Blood Cholesterol (Total)	Blood Triglycerides (TAGs)	Miscellaneous	Refs
Men and women 1. (58) N = 20/17 2. (37–67) N = 7/1 (N = 4)[1]	Habitual 5 weeks	OFS 8 g/d (Confectioneries) OFS 8 g/d (syrup)	241 mg/dl × 0.92[2] 278 mg/dl NS (× 0.89)[1]	206 mg/dl NS[3] mg/dl NS NS	The average basal level of serum TAGs was 337 mg/dl in the oligofructose-fed subject No effect on HDL-cholesterol or glycemia in any of the subjects	37
Men and women (58 ± 3.7) (63 ± 3.3) N = 21	NCEP[4] step 1 diet 6 weeks	INU 18 g/d (chocolate bar, chocolate spread, and coffee sweetener)	6.2 m*M* × 0.91	1.65 m*M* NS	Significant difference in LDL C (14.4%) No effect on TAGs or other lipoproteins	38
Men and women (35–65) N = 54	Habitual 8 weeks	INU 10 g/d (powder)	6.46 m*M* NS	1.59 m*M* × 0.81	No effect on cholesterol in lipoproteins. Significant decrease in insulin (× 0.66)	39
Men (27–49 year) N = 12	Controlled 3 weeks	INU 20 g/d (ice cream)	228 mg/dl NS	283 mg/dl × 0.86	No effect on plasma glucose or insulin after OGTT	40
Men and women (23–32 year) N = 8	Habitual diet 3 weeks Controlled 3 weeks	INU 10 g/d (powder)	4.12 m*M* NS	0.92 m*M* × 0.84	Reduced hepatic lipogenesis but no effect on hepatic cholesterol synthesis	41

[1] These data concern four out of the eight volunteers who responded to the treatment.

[2] -fold decrease of parameter in control group

[3] NS = not statistically significant

[4] NCEP = National Cholesterol Education Program. The step I diet limits total fat to <30% and saturated fat to <10% of energy intake and dietary cholesterol consumption to no more than 300 mg/d.

4. In a randomized, double-blind, diet-controlled crossover study, Causey et al. also observed a significant ($p < 0.05$) reduction in serum TAGs (14%) in adult male subjects (n = 12) with moderate hyperlipidemia, given inulin (18 g/d in ice cream as substitute for sugar for 3 weeks).[40]
5. A double-blind, randomized, placebo-controlled, crossover study has been designed with the aim to determine whether the addition of a moderate dose of inulin (10 g/d high molecular weight inulin HP) to a moderately high-carbohydrate diet would decrease plasma TAGs and/or cholesterol and reduce hepatic lipogenesis and/or cholesterol synthesis as measured by the deuterated water method.[41] During the 6 weeks preceding the deuterated water test, the volunteers (n = 8) consumed either inulin (2 ¥ 5 g/d) or a placebo (2 ¥ 5 g/d maltodextrins) included in their usual diet, but during the last 3 weeks before the test they were given a controlled diet (55% of total energy as carbohydrates, 30% as fats and 15% as proteins) that provided the same dose of either inulin or maltodextrin. Although inulin had no effect on plasma cholesterol and hepatic cholesterol synthesis, it significantly reduced plasma TAGs (17%) and hepatic lipogenesis (28%).[41]

11.3.2.3 Effect of Inulin-Type Fructans on Lipid Parameters in Noninsulin-Dependent Diabetic (NIDDM) Subjects

1. Administration of oligofructose in a packed coffee drink or coffee jelly for 14 d to uncontrolled diabetics (8 men and 10 women) fed a diabetic diet (55% carbohydrates, 25% fat, and 20% proteins) reduced total (8%) and LDL cholesterol (10%), compared with a control group given sucrose in the same food vehicles. No effects on other serum lipids but a decrease in blood glucose concentrations were observed.[42]
2. In a study with the aim to test the effects of oligofructose (15 g/d added as powder in a low-fat yogurt for 3 weeks) in noninsulin-dependent diabetic (NIDDM) subjects (n = 9 men and 11 women), no effects on blood lipids, lipoproteins, or glucose were observed.[43]
3. In NIDDM subjects (n = 12) oligofructose (20 g/d in habitual diet) had no effect on total or lipoprotein-bound cholesterol, plasma TAGs, hepatic glucose production, or insulin resistance.[44]

Data with respect to effects of inulin-type fructans on blood lipids in humans appear to be inconsistent, with reports of both positive and negative outcomes obtained from recent well-designed, double-blind, randomized, placebo-controlled human studies.[45] There appear to be no obvious differences in the sex and ages of the subjects, the dosages employed (average 13.9 ± 4.8, range 8–20 g/d), duration of treatment (average 4 ± 1.6 weeks, range 2–8 weeks), and basal levels (i.e., before the treatment started) of either cholesterol (average 4.8 ± 1, range 3.9, 6.46 m*M*) or TAGs (average 1.16 ± 0.4, range 0.72, 1.65 m*M*) in the blood, between negative and positive studies (Table 11.2, Table 11.3, and Table 11.4). However, positive outcomes

TABLE 11.4
Effect of Inulin-Type Fructans on Lipid Parameters in Noninsulin-Dependent Diabetic (NIDDM) Subjects

Subjects	Diet Duration (Weeks)	Inulin g/d	Blood Cholesterol (Total)	Blood Triglycerides (TAGs)	Miscellaneous	Refs
Men and women (48.5 ± 7.3) N = 8/10	Habitual 2 weeks	OFS 8 g/d (coffee drink, coffee jelly)	242 mg/dl ¥ 0.92[1]	132 mg/dl NS[2]	Uncontrolled diabetics Diabetic diet (55% CHO,[3] 25% fat, 20% proteins) Decreased blood glucose	42
Men and women (586 ± 5.2) (62 ± 4.1) N = 9/11	Habitual 3 weeks	INU 15 g/d (powder to be added to yogurt)	6.01 m*M* NS	2.44 m*M* NS	No effect on HDL or LDL No effect on FFA[4] No effect on glycemia	43
Men and women (57+/2) N = 12	Habitual 4 weeks	OFS 20 g/d (powder to sweeten beverages)	5.15 m*M* NS	1.42 m*M* NS	No effect on cholesterol in lipoproteins or in plasma glucose and insulin	44

[1] -fold decrease of parameter as in control group
[2] NS = not statistically significant
[3] CHO = carbohydrates.
[4] FFA = free fatty acids.

have been observed more frequently in those studies conducted in subjects with moderate hyperlipidemia (4/5 positive outcomes) than in normal lipidemic healthy volunteers (1/5 positive outcome) or in noninsulin-dependent diabetics (1/3 positive outcome). In these three categories of subjects, data (Table 11.5) show that:

- Inulin (5/8 positive outcomes) is more effective than oligofructose (1/5 positive outcome) in reducing blood cholesterol but mostly blood TAGs.
- The effect on triglyceridemia (average 19%) is larger than on cholesterolemia (average 8%).
- Both the hypotriglyceridemic and the hypocholesterolemic effects, when present, are fairly constant (average 19%; range 14–27%, and average 8%; range 8–9%, respectively).

TABLE 11.5
Summary Analysis of the Human Nutrition Intervention Trials Designed to Test the Effect of Inulin-Type Fructans on Blood Lipid Parameters

Condition	Oligofructose	Inulin
Normolipidemy		
No effect (NS)	2	2
Reduction in blood lipids	0	1
Cholesterol		¥0.92[1]
TAGs		¥0.73
Hyperlipidemy		
No effect (NS)	1	0
Reduction in blood lipids	1	4
Cholesterol	¥0.92	
TAGs	(¥0.89)[2]	¥0.91
		¥0.81
		¥0.86
		¥0.84
NIDDM		
No effect (NS)	1	1
Reduction in blood lipids	1	0
Cholesterol	¥0.92	
TAGs		
Total		
No effect (NS)	4	3
Reduction in blood lipids	1	5
Cholesterol average	¥0.92	¥0.915
TAGs average		¥0.81

Note: Each number indicates the number of studies reporting either no effect or a beneficial effect.

[1] -fold decrease of parameter as in control group

[2] These data concern four out of the eight volunteers who responded to the treatment.

- The studies reporting a beneficial effect of inulin included 107 (52%) out of the 205 treated volunteers.
- The study reporting a beneficial effect of oligofructose included a total of 55/87 subjects.

11.3.3 Mechanisms of the Effects of Inulin-Type Fructans on Lipid Homeostasis

That inulin or oligofructose decreases serum triglyceridemia both in fed and fasted lean subjects has consistently been reported in several studies mainly in rats[14,46,47]

but also in hamsters.[27] Especially, feeding male Wistar rats a diet supplemented with oligofructose (10% w/w in diet) significantly lowers the concentration of triacylglycerols, esterified cholesterol, and phospholipids, both in the serum and in the liver.[14] The hypotriglyceridemic effect is mostly due to a decrease in the plasma concentration of the very low-density lipoproteins (VLDLs),[46] resulting from a decrease in the hepatic synthesis of TAGs and not from an increase in the catabolism of TAGs-rich lipoproteins.[48]

In an *ex-vivo protocol*, it has also been shown that hepatocytes isolated from oligofructose-fed rats have a slightly lower capacity to esterify [^{14}C]-palmitate into triglycerides, but, and more importantly, a 40% reduced capacity to synthesize triglycerides from [^{14}C]-acetate (Table 11.6).[14] These data support the hypothesis of a decreased *de-novo* hepatic lipogenesis in oligofructose-fed rats. As a matter of fact, the activity of all enzymes involved in the process of hepatic lipogenesis, i.e., acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), ATP citrate lyase (ATPCL), and glycerol-3-phosphate acyltransferase (GAPT) are decreased by about 50% (Table 11.6). The activity of these enzymes, especially FAS, is regulated only through modifications of their intracellular content. Such a coordinated decrease of their activities thus supports the hypothesis that oligofructose administration could modify lipogenic enzymes gene expression. Indeed, the liver concentration of the messenger RNA of FAS, as measured by northern blot analysis, is reduced (43%) in the liver of oligofructose-fed as compared to control rats.[48]

Oligofructose has also been shown to positively modulate lipid balances induced by dietary manipulation in animals:

- When added to hyperlipidemic high-fat/cholesterol diets fed to rats or hamsters, oligofructose reduced the postprandial triglyceridemia and prevented the increase in serum-free cholesterol level induced by the high-fat diet. These results suggest that oligofructose may also decrease serum TAGs through an extra-hepatic mechanism, namely by enhancing the catabolism of TAGs-rich lipoproteins.[20,27]
- Fructose added in the drinking water of rats for 48 h causes an increase in the liver concentration of TAGs, but chronic prefeeding of the fructose-treated rats with oligofructose protected against liver accumulation of TAGs.[15] The lower lipogenic capacity of the liver might be one of the key events in such a protection because, even after the fructose load, FAS activity remained significantly lower in oligofructose-fed as compared to the fructose-fed control rats. However, despite its protective effect on the liver accumulation of TAGs, oligofructose was unable to prevent the fructose-induced hypertriglyceridemia, suggesting that oligofructose feeding could not counteract the fructose-induced change in the clearance of VLDL-TAGs.
- Dietary oligofructose also reduced both the peripheral fat mass deposition and the hepatic steatosis in the obese Zucker fa/fa rats.[49] But the "hepatoprotective" effect of oligofructose was not accompanied by any significant

TABLE 11.6
Parameters of Hepatic Lipid Metabolism in Rats Fed a Control or Oligofructose-Supplemented (10% w/w) Diet

In vivo

Liver (nmol/mg protein)	TAGs[1]	PLPs[2]	Total Cholesterol	Glycerol 3 Phosphate
Control	51.2 ± 3.9	126.6 ± 3.7	25.3 ± 1.0	0.24 ± 0.01
Oligofructose-fed	**37.9 ± 2.5**[3]	**111.4 ± 3.3**	23.2 ± 0.8	**0.38 ± 0.04**

In vitro

Isolated Hepatocytes (nmol/mg protein)	Palmitate oxidized	Palmitate esterified	TAGs synthesized	TAGs secreted
Control	1.12 ± 0.19	8.12 ± 0.29	10.61 ± 1.75	2.89 ± 1.29
Oligofructose-fed	1.08 ± 0.19	7.91 ± 0.58	**5.01** ± **1.51**	**3.57 ± 1.22**

In vivo

Liver (lipogenic activity) (mU/mg protein)	GPAT[4]	ACC[5]	FAS[6]	ME[7]	ATPCL[8]
Control	3.35 ± 0.07	0.53 ± 0.05	36.70 ± 2.00	31.40 ± 2.20	22.30 ± 1.50
Oligofructose-fed	**2.38** ± **0.06**	**0.32** ± **0.05**	**22.60** ± **2.90**	**15.50** ± **2.40**	**12.30** ± **2.20**

[1] TAGs = triglycerides.

[2] PLPs = phospholipids.

[3] All values in bold are significantly different from control.

[4] GPAT = glycerol-3-phosphate acyltransferase.

[5] ACC = acetyl CoA carboxylase.

[6] FAS = fatty acid synthase.

[7] ME = malic enzyme.

[8] ATPCL = ATP citrate lyase.

Source: Adapted from Kok, N., Roberfroid, M., Robert, A., Delzenne, N., Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. *Br. J. Nutr.*, 76, 881–890, 1996; Delzenne, N., Kok, N., Effect of non-digestible fermentable carbohydrates on hepatic fatty acid metabolism, *Biochem. Soc. Trans.*, 26, 228–230, 1998; Delzenne, N., Kok, N., Biochemical basis of oligofructose-induced hypolipidemia in animal models, *J. Nutr.*, 129, 1467S–1470S, 1999.

difference in postprandial serum TAGs concentration or in the activity of the hepatic lipogenic enzymes.

- In the most recent studies, Zucker rats fed oligofructose-enriched inulin Synergy 1 had a lower body weight gain, a lower visceral and epididymal fat mass, and a less severe liver steatosis than the controls fed a diet containing cellulose (10% w/w in diet) used as a poorly fermented non-digestible carbohydrate.[50]

As a putative mechanism to explain the decrease in body weight and in fat mass in oligofructose-fed obese Zucker rats, it is worth mentioning a transient "satietogenic" effect leading to a decrease in food intake, observed during the first 3 weeks of the treatment.[49] Such an effect of dietary oligofructose on food (and calorie) intake has not been observed in nonobese animals.

11.4 INULIN-TYPE FRUCTANS AND LIPID HOMEOSTASIS: DISCUSSION, CONCLUSION, AND PERSPECTIVES

A series of animal studies demonstrate that inulin-type fructans affect the metabolism of the lipids primarily by decreasing triglyceridemia both in the fasted and the postprandial state. In animals fed a diet supplemented with 10% inulin or oligofructose, triglyceridemia is reduced by 36 ± 3.4% (mean ± sem of 8 different studies). That decrease is likely to be due to a reduction in the number of VLDL particles with the same composition in lipids and the same size. In rats fed various hypertriglyceridemic diets, inulin-type fructans have also demonstrated the capacity to reduce plasma and/or hepatic triacylglycerols. In rats fed a standard diet or hypercholesterolemic diets, the effect of inulin-type fructans on cholesterolemia are less constant, being statistically significant in only part of the studies so far reported. In one study in which hamsters were fed a diet supplemented with 1% cholesterol, both plasma TAGs and plasma cholesterol were significantly reduced, the effect on TAGs being dose dependent. In homozygous $LDL^{-/-}$ mice, inulin significantly reduced cholesterol both in the serum and in the lipoproteins.

The human data largely confirm the animal experiments. They demonstrate mainly a reduction in triglyceridemia and only a relatively slight decrease in cholesterolemia both in normo- and (slightly) hypertriglyceridemic conditions. In human nutrition intervention trials, inulin appeared to be more effective than oligofructose in reducing triglyceridemia whereas in animals (especially in rats) both products were equally active. But the dose used in animal experiments (10% or more w/w in diet or >4 g/kg bw/d) is likely to be far higher than in human trials (±0.2 g/kg bw/d) and that might explain the difference.

With respect to the mechanism, adding inulin-type fructans to rodents' diets reduces liver lipogenesis by reducing the expression of the genes coding for the lipogenic enzymes. In humans a similar mechanism is likely to operate. Indeed, Letexier et al. have demonstrated a reduced hepatic lipogenesis but not cholesterol synthesis in subjects receiving 10 g/d of inulin.[41] However, and especially in situation of more severe dysbalances in lipid homeostasis, other mechanisms might also operate like an enhanced catabolism of TAGs-rich lipoproteins.

Whatever the mechanism is, the question still remains open of the links between the gastrointestinal site of the fermentation (and thus the disappearance) of inulin-type fructans) and their effect on lipid homeostasis inside the body (the so-called systemic effect). Different hypotheses have been tested to tentatively answer that question:

1. Modifications of glucose and/or insulin levels

Dietary modulation of lipogenesis is often linked to changes in the blood concentration of these important physiological players:

- Indeed, glucose increases gene transcription of lipogenic enzymes and its effect is potentiated by insulin.[51]
- Resistant starch that decreases serum TAGs concentration and reduces FAS activity concomitantly lowers postprandial insulinemia.[52,53]

However, the effects of inulin-type fructans on glycemia and insulinemia are not yet fully understood and available data are still conflicting indicating that they may depend on physiological (fasting vs. postprandial state) or disease (diabetes) conditions. The available data show that:

- Oligofructose (10% w/w in diet of male Wistar rats for 30 d) reduces postprandial insulinemia by 26%,[54] but the glycemic response during a glucose-tolerance test after overnight fasting is identical in control and oligofructose-fed rats.[49,54]
- In streptozotocin-treated (diabetic) rats, feeding a diet containing oligofructose (20% w/w in diet for 2 months) decreases postprandial glycemia, despite a lack of effect on the glycemic/insulinemic response to a sucrose or maltose load.[55]
- But in the diabetes-prone BB rats, oligofructose failed to affect the incidence of type I diabetes or plasma levels of glucose, cholesterol, and TAGs.[56]
- Also in humans, the effects of inulin-type fructans on various markers of glucose homeostasis are inconsistent (Table 11.7).

However, and based on the results presently available, the role of glucose and/or insulin in the lowering effect of oligofructose on hepatic lipogenesis cannot be discarded, but additional studies are need to clarify how and when oligofructose supplementation eventually modulates glucose, insulin secretion, and response.

2. Modifications of the absorption of macronutrients

Even though they are not digested in the upper part of the gastrointestinal tract, inulin/oligofructose may, like other dietary fibers, influence the absorption of macronutrients, especially carbohydrates, either by delaying gastric emptying and/or shortening small intestinal transit time. Indeed, it has been reported that feeding rats a diet containing oligofructose (10 and 20% w/w in diet for 6 weeks) shortens mouth-to-anus transit time (–25 and –50%, respectively), a result that suggests a dose-dependent effect.[57] It must be underlined, however, that inulin-type fructans do not have the high viscosity of other nonstarch polysaccharides, a physical property that is usually correlated with their effect on absorption of macronutrients.

3. Increased production of fermentation end products (SCFAs)

Some of the SCFAs (essentially acetate and propionate), the end products of the fermentation in the large bowel, are absorbed via the portal vein. They are thus interesting candidates to explain the effects of nondigestible but fermentable carbohydrates on liver metabolism.[58] The concentration of these 2 major SCFAs is increased by more than twofold in the portal serum of oligofructose-fed rats.[59]

TABLE 11.7
Effect of Inulin-Type Fructans on Various Markers of Glucose Homeostasis in Healthy and Diabetic Human Subjects

Product (g/d)	Conclusion of the Studies	Refs
Oligofructose (20 g/d)	No statistically significant effect on: Insulin suppression of hepatic glucose production Insulin stimulation of glucose uptake (hyperinsulinemic clamp) Erythrocyte insulin binding Decreased basal hepatic glucose production ($p < .02$)	33
Oligofructose (15 g/d)	No statistically significant effect on glucose absorption (glucose tolerance test)	35
Inulin (15 g/d)	No statistically significant effect on glucose absorption (glucose tolerance test)	
Inulin (9 g/d)	No statistically significant effect on glucose absorption (meal glucose tolerance test)	36
Inulin (10 g/d)	Decreased fasting plasma insulin concentration ($p < .05$)	39
Oligofructose (8 g/d)	Decreased fasting blood glucose level in diabetic subjects ($p < .05$)	42
Oligofructose (15 g/d)	No statistically significant effect on blood glucose in diabetic subjects	43
Oligofructose (20 g/d)	No statistically significant effect on: Fasting plasma glucose and insulin concentrations Basal hepatic glucose production Plasma glucose response to insulin bolus Erythrocyte insulin binding	44

However, the exact role of these short chain carboxylic acids is difficult to demonstrate because they have antagonistic effect. Indeed:

- Propionate has been reported to inhibit fatty acid synthesis, but acetate is a lipogenic substrate.[60–63]
- More recently it has been shown that propionate, at concentrations found in the serum of the portal vein of oligofructose-fed rats (0.6 m*M*), inhibits the carrier-mediated acetate uptake in cultured isolated hepatocytes but was unable to modify either palmitate or glucose incorporation into esterified fatty acids, at least after short term incubation (cited in Delzenne et al.,[64] and Declerck, unpublished results).
- But, and at the same concentration (0.6 m*M*), propionate was still able to decrease the concentration of FAS mRNA in cultured hepatocytes, and it may thus be a putative mediator of the antilipogenic effect of dietary oligofructose.

Other molecules are also produced in the large bowel, as a result of inulin-type fructans fermentation. For example, an increased concentration of polyamines, especially putrescine, has been observed in the cecum of oligofructose-fed rats. But their concentration did not change in the portal vein or the liver. Thus, their role as mediators of the effect of oligofructose on lipid metabolism remains equivocal.[65]

4. Changes in the production of gut peptides (see Chapter 2, Section 2.3)

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1-(7-36)amide (GLP-1) are important mediators in the regulation of postprandial insulin release. Both peptides are released from endocrine cells localized in the intestinal mucosa after ingestion of carbohydrates, and they enhance postprandial insulin release from the pancreatic b cells.[66,67] In addition to their insulinotropic effects, GIP and GLP-1 also bind to specific receptors and consequently influence glucose and lipid metabolism, at least in the adipose tissue. Both are able to promote insulin-induced glucose uptake and *de novo* lipogenesis in peripheral adipose tissue. Dependent on its concentration, GIP has been shown to stimulate or inhibit lipolysis in adipocytes and to stimulate lipoprotein lipase in cultured preadipocytes.[66,68] In that context an important observation has been reported that demonstrates that oligofructose supplementation in the diet of rats increases postprandial serum GIP level as well as GLP-1 concentration in the cecal tissue and in the portal serum.[50,54] Even if further studies are needed to clarify the metabolic consequences (on lipid metabolism and on glucose/insulin homeostasis) of the modulation of secretion of these incretins, the hypothesis of their role in mediating the effects of inulin-type fructans on lipid homeostasis remains most interesting and most promising.

According to the European consensus on "Scientific concepts of functional foods," the scientific substantiation of a type A claim (enhanced function claim) must follow a stepwise approach that includes (see Chapter 1, Section 1.3.2 to Chapter 1, Section 1.3.4):[69]

- Identification of a beneficial physiological effect in relevant experimental models
- At least a partial understanding of the mechanism of that effect
- Formulation of a hypothesis
- Test of the hypothesis, demonstration, and confirmation of the effect in (targeted) human nutrition intervention studies

With regard to their effects on triglyceridemia, inulin-type fructans fulfill all these criteria. Their dietary consumption significantly reduces the concentration of blood TAGs. That beneficial effect is well documented in experimental animals in which a series of experiments have led to the formulation of a sound hypothesis regarding the mechanism. In (slightly) hypertriglyceridemic human subjects as well as in subjects prone to develop such lipid dysbalances (e.g., because of consumption of high-carbohydrates diets), inulin has been shown in a majority of studies (3/4) to significantly reduce (–19%) triglyceridemia (see Table 11.5). Moreover, a recent publication has given support to the hypothesis that, as in rats, this effect could originate from a reduced hepatic lipogenic capacity due to inulin feeding.[41] In the

present state of knowledge, human data have demonstrated such effects only for inulin and not for oligofructose, but more work is needed to definitively prove such a qualitative difference between these two fructans.

In conclusion, inulin appears thus eligible for an enhanced function claim mostly related to normalization of blood triglycerides level, even though an effect on cholesterolemia cannot be ruled out. Since hypertriglyceridemia is recognized as an independent risk factor for cardiovascular disease and atherosclerosis,[1] the approval of such a claim and its communication (see Chapter 1, Section 1.3.5) to the consumers might contribute to improving human health and well-being.

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12 Inulin-Type Fructans and the Defense Functions of the Body

12.1 INTRODUCTION: THE DEFENSE FUNCTIONS OF THE BODY

The defense systems, which separate the inside of the body from the outside world, play the roles of "wall," "filter," "barrier," and "safeguard" to protect against chemical and biological "aggressors" that may cause toxic effects or initiate infections (Table 12.1).

These aggressions represent constant and varying challenges to health and well-being. Indeed, when these appear, pathological processes can start and diseases may develop. To cope with these challenges, a wide diversity of defense mechanisms have developed that include physical and functional barriers, and the abilities to recognize, destroy, and eliminate potentially harmful organisms, and to transform toxic and carcinogenic chemicals, while tolerating the host body, beneficial organisms, and substances (see Chapter 2, Section 2.4).[1] The most active "defenders" are found in the mucosal and the lymphoid tissues. The wall or barrier functions require not only integrity of the mucosal tissues, but also, in most cases, production of the right type, the right combination, and the right quantity of specific proteins and peptides, essentially the mucins and the trefoil peptides, that form effective protection layers. The safeguard functions utilize specific molecules and specialized cells that are competent in inactivating foreign (antigens) or potentially toxic molecules and inhibiting the proliferation of (or even neutralizing and destroying) pathogenic microorganisms or self-cells infected by these microorganisms (Figure 12.1).

The immune system, whose activities have been classified as innate (inborn) or acquired (adaptive) components, is an essential player especially in the intestine-associated safeguard functions. The components and cells that comprise these two arms of the immune system are presented in Table 12.2.

12.1.1 Innate Components of the Body's Defense

The innate functions are nonspecific, and they contribute to the early phases of the host defense system that do not require prior exposure to the aggressor (antigens). It is the first line of defense that reacts rapidly within the initial hours following the aggression. These functions consist of physical, cell-mediated barrier processes, as well as soluble mediators. The physical protection is linked not only to the integrity of the mucosa but also, as is the case in the gastrointestinal tract, to the physical,

TABLE 12.1
Major Aggressors of the Body That Are Likely to Enter the Gastrointestinal System

Chemical Aggressors	Biological Aggressors
Toxic Chemicals	**Harmful Microorganisms**
Chemical carcinogens	Pathogens (bacteria, fungi, and virus)
Chemical antigens	Virus-infected self cells
	Cancer cells

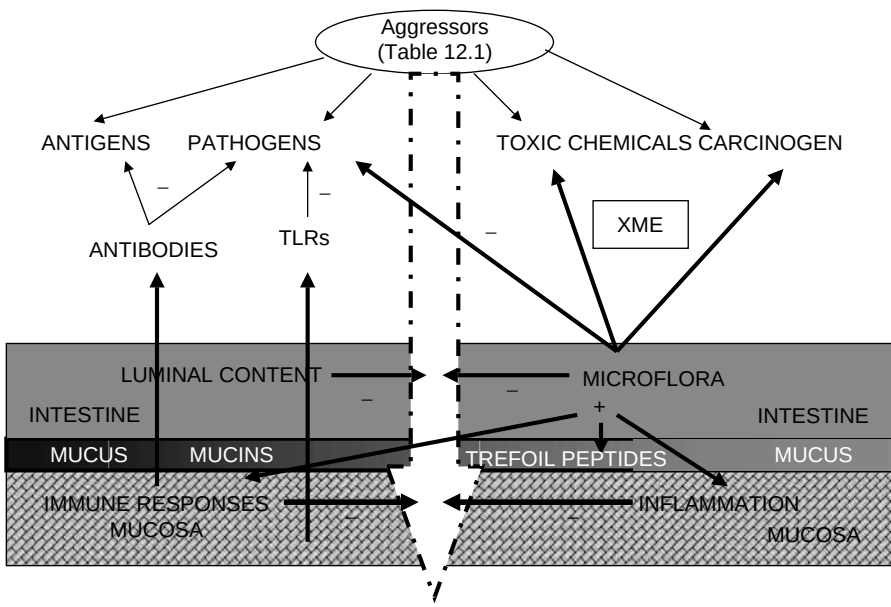

FIGURE 12.1 Schematic representation of the body's defense with special emphasis on the intestinal processes (TLRs = Toll-like receptors; XME = xenobiotic metabolizing enzymes).

chemical, biological, and microbiological status of the luminal content. Inflammation is one of the cell-mediated barrier processes that contribute to eliminating the aggressor by the activities of the complement, phagocytosis, etc. Major cell players are phagocytic cells (macrophages and their precursor monocytes), inflammatory cells (polymorphonuclear leukocytes or neutrophils), dendritic cells, and natural killer (NK) cells. Macrophages are essential not only in directly destroying microorganisms but also in processing and presenting antigens to helper T-cells to initiate the acquired immune functions. Natural killer cells are effective against self-cells that have been transformed, e.g., by DNA damage. The soluble mediators are cytokines, and complement and acute-phase proteins.[2] When the aggressor is a microorganism, the innate immunity acts via the recognition of antigenic structures present

TABLE 12.2
Components and Cells That Comprise the Two Arms of the Immune System, with Special Emphasis on the Intestine

Arm	Components	Defenses
Innate immunity	**Barrier**	**First line of defense**
	Intestinal content (pH, microflora, aggregates, etc.)	Physical adsorption Prevention of entry into the tissue
	Mucosal tissue Mucus	Absorption, and translocation to the systemic circulation, tissues, and organs
	Safeguard	
	Enzymatic (xenobiotic metabolizing enzymes)	Transformation, inactivation, neutralization, repair, inhibition of proliferation, destruction, and elimination
	Molecular (cytokines, complement, acute phase proteins, defensins, TLRs, and NOD proteins)	Relay to acquired immunity, presentation of antigens (macrophages and dendritic cells)
	Cellular (phagocytic, inflammatory, MAIT, NK, and dendritic cells)	
Acquired immunity	**Safeguard**	
	B lymphocytes (plasma cells and antigen presenting cells)	Recognition and presentation of antigens Production of antibodies
	T lymphocytes Helper T cells (CD_4^+)	Lymphocyte activation, and promotion of cell-mediated and humoral response
	Suppressor/cytotoxic T cells (CD_8^+)	Cell destruction and suppression of lymphocyte activity

Source: Adapted from Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Bauer, L. L., Healy, H-P., Dawson, K. A., Merchen, N. R., Fahey, G. C., Supplemental fructooligosaccharides and mannooligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs, *J. Nutr.*, 132, 980–989, 2002.

in a large group of microorganisms (virus, bacteria, fungi, yeast, and so forth). Among these antigenic structures are lipopolysaccharides of Gram-negative bacteria, lipoteichoic acid and peptidoglycans of Gram-positive bacteria, and mannans and mannoproteins of yeasts etc.

In the intestine, the recognition of these antigens is due to the presence or the production of proteins such as Toll-like receptors (e.g., TLR-4 and TLR-2) that recognize lipolysaccharides, lipoteichoic acid, or peptidoglycans;[3,4] the cytoplasmic NOD proteins in Paneth cells capable of identifying the peptidoglycans of Gram-positive bacteria;[5] and mucosal associated invariant T (MAIT) cells, a particular family of T lymphocytes found in the *lamina propria* that recognize only specific antigenic structures.[6] Even though they definitively belong to the innate immune system, both Toll-like receptors and MAIT cells play the role of a relay in acquired immunity through regulation of the intestinal B lymphocytes.[6]

12.1.2 Acquired Components of the Body's Defense

The highly specific acquired (or adaptive) functions develop over an individual's lifetime. Generally, they take control of the innate functions, and they are more efficient upon secondary exposure to an aggressor.[7] Indeed, the acquired immune system includes a component of memory that allows faster and stronger reaction to a second exposure than the initial response. Both B and T lymphocytes are the major players that directly destroy cells infected with intracellular pathogens or modulate the function of other immune cells.

B-cells, abundant in lymph nodes, recognize foreign antigens through membrane-bound antibodies or immunoglobulins. Upon activation, they become antibody-secreting plasma cells.[2] Antibodies are secreted in soluble form and bind foreign aggressors to facilitate their elimination by phagocytes.[8] B cells can also serve as antigen-presenting cells (APCs) and in this respect influence T cell function.[2] T cells express specific receptors that recognize foreign antigens present in complex with a major histocompatibility complex (MHC) molecule on the surface of an APC.[2] Subpopulations of T cells are characterized by the expression of specific membrane glycoproteins, namely CD4 and CD8 for helper T (Th) cells and cytotoxic/suppressor T cells, respectively.[2] $CD4^+$ and $CD8^+$ T cells recognize antigens in complex with MHC class II molecules (found primarily on APCs such as macrophages and dendritic cells) and MHC class I molecules (most nucleated cells in the body express MHC class I molecules), respectively.[2] In addition, $CD4^+$ cells secrete cytokines that activate B and other T cells, as well as cells of the innate immune system. Depending on the types of cytokines they produce, $CD4^+$ cells are classified into a number of Th types (0, 1, 2, or 3).[2,9,10] Th-1 cells, generally, promote cell-mediated inflammatory responses, whereas Th-2 cells support antibody (humoral) responses.[8] Less is known about the function of Th-0 and Th-3 type cells. $CD8^+$ cells are T cells with cytotoxic or suppressor functions. By releasing granules or inducing apoptosis cytotoxic functions, T cells destroy infected cells and tumor cells.[8] Less is known about $CD8^+$ suppressor cells, but they are believed to suppress the activation or activities of other immune cells and may play a role in immunological tolerance, such as the tolerance to foreign antigens encountered in the gut.[11,12]

A communication exists between the innate and the acquired components, as well as within the acquired immune systems. That communication involves direct cell-to-cell contact via adhesion molecules as well as the production of chemical messengers, especially cytokines like tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), and interleukin-6 (IL-6) that are among the inflammatory components of the innate response. These cytokines produced by the cells are involved in the innate response (especially monocytes and macrophages) and regulate this response, but they also act systemically, e.g., in the liver, to promote the production of acute phase proteins and with T lymphocytes to trigger the acquired cell-mediated immune response.[13] As a consequence, more and new cytokines are synthesized that will regulate the activity of the cells involved in the innate functions and also promote the proliferation of B and T lymphocytes, as well as the production of antibodies by B lymphocytes.

12.2 ROLE OF THE GASTROINTESTINAL SYSTEM IN THE BODY'S DEFENSE

12.2.1 Gastrointestinal Mucosa and Defense Functions: Generalities[14,15]

12.2.1.1 Gastrointestinal Mucosa as a Barrier

The various epithelia of the gastrointestinal tract separate the outside intraluminal environment that often contains aggressive microorganisms and bioactive chemicals (e.g., bile salts, enzymes, microbial toxins, microbial metabolites, and toxic chemicals, etc.) from the internal milieu (Figure 12.2). The secretion by goblet cells of specific glycoproteins, trefoil peptides, and phospholipids forms the hydrophobic mucus, a gel-like layer that strengthens the wall-barrier functions of these epithelia.[16] The mucus is not only a physical barrier but also a biological barrier, because the oligosaccharides of these glycoproteins bind with the bacterial lectins.[17] Moreover, it prevents the adherence and subsequent translocation of bacteria across the epithelial wall.[18] The filamentous brush-border glycocalix, composed of membrane-anchored glycoproteins that are present at the top of the intestinal microvilli, also plays the role of a barrier because it is impermeant to macromolecules or microorganisms.[19,20]

The wall-barrier functions of the gastrointestinal tract require not only integrity of the epithelial tissues but also production of the right type, the right combination, and the right quantity of various mucins, trefoil peptides, and phospholipids to form the most effective mucus layer. The hydrophobicity of the mucus layer is higher in the stomach and the colon than in various segments of the small intestine where most of the absorption processes take place.[21]

For most health-beneficial molecules (essentially nutrients, vitamins, minerals, and eventually phytochemicals), the transfer through the gastrointestinal epithelia in the small intestine and (at least partly) in the large bowel involves very specific and active transport processes, but passive diffusion also exists that is usually limited to water, some ions (e.g., Mg^{2+} in the colon, see Chapter 10, Section 10.3.1), small, mostly lipohilic, molecules, and very small oligomers.[22] Such diffusion involves either a free paracellular passage through intercellular spaces or a transcellular process that often includes a metabolic transformation of the crossing molecules. The passage of toxic (mostly lipophilic) molecules is mostly due to passive diffusion, but, in some cases, those that are structurally similar to physiologically active molecules can make use of their specific transport systems. The passage of microorganisms (bacteria, fungi, and amoebae, etc.) requires a process of translocation that is not possible when the intestinal mucosa is normal and becomes possible only in the case of malnutrition or a diseased state.

In the large bowel, but most likely also in the small intestine, such functions are dependent on a balanced composition of the microflora that colonize these parts of the gastrointestinal tract (see Chapter 9, Section 9.1).

12.2.1.2 Gastrointestinal Mucosa as a Safeguard

The epithelia (or at least some of them) of the gastrointestinal tract act as a safeguard because they actively produce specific molecules or specialized cells that are competent in inactivating potentially toxic molecules and inhibiting the proliferation of (even neutralizing and destroying) pathogenic organisms. Such competence exists all along the gastrointestinal system. Moreover, the epithelia are shared by all the organs including associated organs such as the oral cavity, exocrine pancreas, gut-associated lymphoid tissue (GALT), and liver that is directly connected to the gastrointestinal lumen via the vena cava (Figure 12.2).

12.2.2 Intestinal Microflora and the Gastrointestinal System in the Body's Defense

For the intestinal defense functions, the microflora that colonize the intestinal lumen are known or believed to play a key role. They influence both GALT and the systemic immune system of the host considerably by stimulating, modulating, optimizing, or even directly contributing to most processes and activities discussed previously.[23] Of particular importance is the microfloral colonization of the intestine at birth, which is believed to act as a source of antigens and nonspecific immunomodulators.[24] As a source of antigens, the intestinal microflora elicits specific local and systemic responses; as a source of immunomodulators, it influences the number and distribution of the GALT cell populations and contributes to regulating the immune

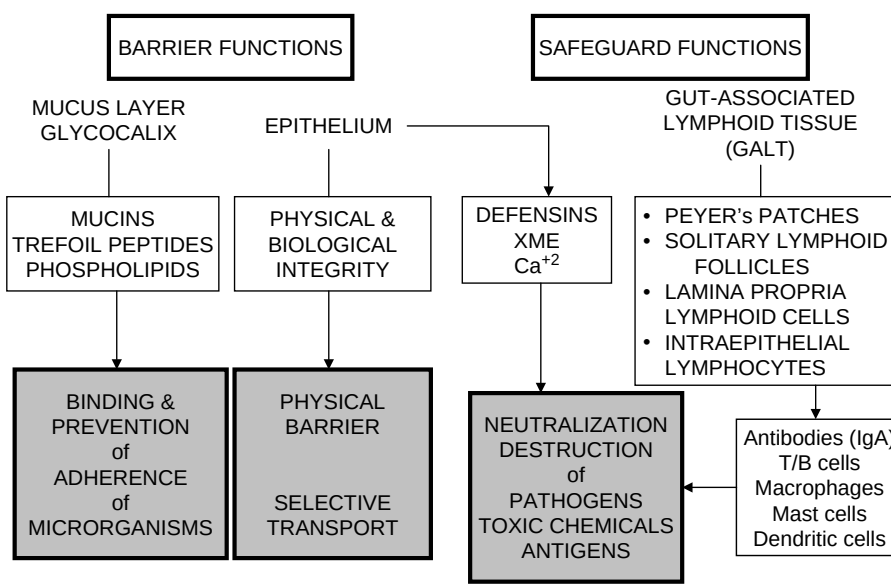

FIGURE 12.2 Intestinal mucosa and the functions of defense (XME = xenobiotic metabolizing enzymes).

responses.[23] The intestinal microflora is also a major antigenic stimulus triggering the migratory pathway and maturation of precursor lymphoid cells in the Peyer's patches, thus contributing to the development and differentiation of IgA plasmocytes, as shown by much lower (10 times) number of such cells in germ-free mice as compared to the control.[23] In an experimental model of germ-free mice infected with a heterologous strain of rotavirus it has been shown that among the major bacterial genera found in intestinal microflora, bifidobacterium was able to enhance antirotavirus IgA antibody response.[25] In gnotobiotic mice harboring a human strain of bifidobacterium or the two bacterial strains from yogurt (*Lactobacillus bulgaricus and Streptococcus thermophilus)*, systemic immunity increased, as shown by the increase in specific serum antibody response and in phagocytic activity of peritoneal phagocytes, respectively.[26] In axenic mice, MAIT cells are absent, an observation that demonstrates the importance of the intestinal microflora for the development of the immune system.[6]

Moreover, the intestinal microflora directly contributes to defense functions by producing strong acids and antibacterial compounds, or by providing enzyme activities that contribute to xenobiotic metabolism. They (at least some of their components) also interact with the enterocytes to modulate the expression of miscellaneous genes.[27,28] But, again, it is likely that it is the general composition of the microflora, rather than the presence of a particular microorganism, that controls these interactions and the modulation processes.

12.2.3 The Gastrointestinal Mucosa and the Body's Defense Functions: Specific Mechanisms

Both the barrier and the safeguard functions differ in different organs of the gastrointestinal system.

12.2.3.1 Defense Mechanisms in the Oral Cavity

In the oral cavity, buccal and gingival epithelial cells produce a series of factors found in saliva that participate in the defense against outside aggressors not only locally but also all along the digestive tract.[29] These are:

- Mucins,[17] the production of which is stimulated by cytokines secreted by the salivary glands (especially, the Epidermal Growth Factor [EGF])[30,31]
- Secretory IgA
- Lysozyme and salivary peroxidase (and its thiocyanate substrate) — both bacteriostatic and bactericidal enzymes[32–36]
- Salivary cystatins, a group of seven proteins with antiproteolytic activity that protect the oral cavity against the deleterious effects of bacterial peptidases[37]
- Salivary histatins, multifunctional proteins with antimicrobial activity especially targeted at fungi (*Candida albicans* and *Cryptococcus neoformans*)[32–39]

12.2.3.2 Defense Mechanisms in the Stomach

In the stomach, the major protecting factor is HCl that has a potent bactericide and fungicide activity not only in the stomach but also in the proximal segments of the small intestine, thus reducing the risk of proliferation of fungi.[40]

12.2.3.3 Defense Mechanisms in the Intestine

The barrier functions in the small intestine and in the large bowel where most absorptive processes operate are more complex, more sophisticated, and probably more important than in the other parts of the gastrointestinal system. Moreover, the intestinal epithelia boasts a number of specialized protective adaptations that are not found in other sites.[41] These defense functions involve:

- Physicochemical processes such as an acidic environment (due to SCFAs or lactic acid production), the presence of solid particles that have the capacity to adsorb chemicals or even microorganisms and to prevent their absorption or translocation, or even the presence of some ions, e.g., Ca^{+2} that form insoluble salts with potentially toxic molecules, e.g., biliary acids.
- Production of antimicrobial substances, especially peptides known as defensins.[42]
- Both innate and adaptive/acquired immune activities of the mucosal-associated lymphoid tissues that protect the intestine. As the intestine is the first line of defense against the environment, and must integrate complex interactions among diet, foreign compounds, external toxic chemicals or pathogens, and local immunological and nonimmunological processes, it is critical that protective immune responses are triggered by potential pathogens, yet it is equally important that hypersensitivity reactions to most dietary antigens are minimized. It has been estimated that approximately 25% of the intestinal mucosa is made up of lymphoid tissue or GALT,[43] and the human intestine represents the largest mass of lymphoid tissue in the body, containing over 10^6 lymphocytes/g of tissue that make about 60% of the total immunoglobulins produced daily.[23] The GALT is composed of:
  - *Aggregated tissue in the form of Peyer's patches.* Peyer's patches or aggregates of lymphoid follicles are found throughout the mucosa and submucosa of the small intestine. These contain both $CD4^+$ and $CD8^+$ T cells, as well as naïve B cells, plasma cells, macrophages, and dendritic cells.[15] Specialized epithelial cells known as M cells overly the Peyer's patches. These M cells lack microvilli; they are able to phagocytose both soluble antigens and microorganisms, and finally to transport and release the antigens from the gut into the Peyer's patches, where these antigens are presented via APCs to T and B lymphocytes.[15,43] Upon activation, B cells undergo class-switching to produce antibodies mainly of the immoglobulin-A type or IgA, a process that

is facilitated by both activated $CD4^+$ and $CD8^+$ cells.[15,43–45] Activated immune cells exit the Peyer's patches via the mesenteric lymph nodes, enter the systemic circulation via the thoracic duct, and then specifically home back to populate the lamina propria and the intraepithelial regions of the intestine.[15,43,46] Thus, Peyer's patches represent a major sampling site for intestinal antigens.

- *Solitary lymphoid follicles.* Solitary lymphoid follicles, together with M cells associated with the overlying epithelia, are the functional equivalent of Peyer's patches that are present throughout the length of the intestinal tract, particularly in the colon and rectum.[47]
- *Nonaggregated cells in the lamina propria.* A diffuse population of T and B cells together with plasma cells, mast cells and macrophages forms the lamina propria[15] where a majority of T cells are $CD4^+$ whereas 90% of the plasma cells (mature B cells) secrete IgA,[47] the best-characterized and the most important component of the gastrointestinal defense.[45] Most of the IgA is secreted into the gut lumen; it is distinct from serum IgA and takes the form of secretory IgA that is resistant to intraluminal proteolysis and does not activate the complement or inflammatory responses, thus making it ideal for protecting mucosa.[23,47,48] The main function of secretory IgA is, in cooperation with innate defense mechanisms,[49] to mediate exclusion of foreign antigens by preventing epithelial adherence and penetration of invasive pathogenic microorganisms and neutralizing toxins.[23,43] Two IgA subclasses have been identified as IgA_1 and IgA_2, respectively; the former predominates in the small intestine, whereas the latter which is resistant to most bacterial proteases, is most frequent in the colonic mucosa.[45]
- *Intraepithelial lymphocytes (IEL).* Intraepithelial lymphocytes (IEL) are located in the interstitial spaces of the mucosal epithelia in a ratio of approximately one lymphocyte for every 6–10 epithelial cells,[15] making IEL the largest immunocompetent cell pool in the body. Although IEL line both the small and large intestine from the crypt base to the villus tip,[50] their exact biological function in the mucosal immune system is not known. They are in continuous contact with luminal antigens through the epithelial layer, and it has been suggested that IEL may be the first compartment of the immune system that responds to gut-derived antigens.[51] Furthermore, as IEL are comprised primarily of $CD8^+$ T cells,[43] they may be functional suppressor cells with a role in oral tolerance.[52]
- *Mesenteric lymph nodes.* Although not situated within the intestinal mucosa, the mesenteric lymph nodes are considered as part of the GALT. Mesenteric lymph nodes are composed of immune cells leaving and entering the gut and which are part of the peripheral circulation. Immune cells drain to the intestinal lymphatics after differentiation in Peyer's patches, pass through mesenteric lymph nodes on route to the thoracic duct and then pass again on route back to the lamina propria regions of the gut.[53]

- Competition and antagonism exist between microorganisms in the intestinal microflora due to the production of strong acids as well as antibacterial compounds that inhibit the growth or even kill other bacteria. The most common strong acids are lactic acid and the SCFAs. Bactericide molecules, essentially proteins and peptides known as bacteriocins, are produced mostly by lactic acid bacteria (*Lactobacillus* spp. and *Bifidobacteria* spp.) but also some strains of the genera bacteroides, bacillus, streptococcus, and lactococcus that are present in the intestinal microflora.[54–56]
- Metabolic activities are carried out in intestinal epithelial cells, liver cells, and various microorganisms in the intestinal microflora. These activities target most of the chemicals (both beneficial and toxic) that enter the gastrointestinal system as part of the diet or as endogenous secretion (e.g., bile salts) to inactivate (i.e., detoxifying and thus facilitating the excretion) but also sometimes to activate (i.e., increasing the chemical reactivity and thus the toxic potential) them. The yield of reactive intermediates in the intestine will depend on the type of compounds and on the balance of metabolizing enzymes therein. One of the most dramatic consequences of the production of such reactive intermediates is DNA damage and mutations.[57] These metabolic activities are part of the complex enzymatic system known as the xenobiotic metabolizing enzymes that include the cytochrome P_{450}-dependent mixed function oxidases, some reductases, various transferases, and miscellaneous hydrolases that catalyze the so-called phase I and phase II reactions (Table 12.3).

  Mixed-function oxidases are present in the intestinal epithelial cells but mainly in the hepatocytes. They both play a major role in protecting the body against the toxic potential of mostly lipophilic chemicals that have been absorbed by or have passed through the intestinal epithelia and have been transferred to the liver via the portal vein. Reductases, transferases, and hydrolases are also present in these cells. But hydrolases and to a lesser extent some reductases are also produced by some bacteria in the intestinal microflora, i.e., glucosidases, glucuronidases, acetylases, methylases, hydrolases of amino-acid conjugates, azo-reductases, and nitro-reductases. These enzymes produced in the intestinal lumen metabolize chemicals mostly in the large bowel. They either contribute to inactivating potentially toxic chemicals, thus preventing their absorption and protecting the body or, on the contrary, they produce more reactive molecular species that become new aggressors in the body (e.g., nitrate that is reduced to nitrite and serves as a substrate for the production of the carcinogenic nitrosamines: polycyclic aromatic amines produced when meat or fish is barbecued, which are hydroxylated to form mutagenic and carcinogenic metabolites). They may also contribute to the production of biologically active, more beneficial molecules that help in improving health. This is the case for phytoestrogens that mainly occur in plants

TABLE 12.3
Enzymology of Xenobiotic Metabolizing Enzymes

Functions of Xenobiotic Metabolizing Enzymes	
Protection via increased water-solubility and decreased fat-solubility to facilitate elimination	
Metabolic activation via production of nucleophiles and radicals with increased reactivity	
Enzymes of Type 1 Reactions	**Enzymes of Type 2 Reactions**
Oxidation	**Group Transfer**
Mixed function oxidases (cytochrome P_{450})	Glucuronyl-transferases
Peroxidases	Sulfonyl-transferases
Amine oxidases	Glucosyl-transferases
Alcohol/aldehyde NAD^+ oxydo-reductases	Glutathione-*S*-transferases
Reduction	Acetyl-transferases
Cytochrome P_{450}-dependent reductases	Methyl-transferases
Glutathione-peroxidases	Acyl-transferases
Hydrolysis	
Epoxyde hydrolase	
Amide hydrolases	

as glycosides and for cycasin present in cycad nuts that have to be hydrolyzed by glucosidases of intestinal bacteria such as lactobacilli, bacteroides, and bifidobacteria to produce the lipohilic aglycone that can be absorbed and exert its physiological (phytoestrogens) or carcinogenic (cycasin) effects. Besides, a greater efficacy of phytoestrogens (e.g., daidzein, mainly found in soy) can be expected if that substrate has been further converted into more potent molecules (i.e., equol) by the intestinal microflora.[58,59]

12.2.4 Biomarkers of Gastrointestinal Defense Functions[13,60]

To study the integrity or the activity as well as to demonstrate modulation, of gastrointestinal defense functions, not just one but a series of parameters must be measured to directly assess either the barrier or the safeguard capacity. Because the responses are dynamic in nature, it must always be kept in mind that absolute response may be different at different time points and that different responses may follow different time courses. Many components of the gastrointestinal defense's system can be measured by studying those following:

12.2.4.1 Biomarkers of Barrier Functions

- Integrity or permeability of mucosa, mucosal morphology or histology
- Composition, integrity, and adherence capacity of the mucus

12.2.4.2 Biomarkers of Safeguard Functions*

- The size and cellularity of lymphoid organs (Peyer's patches, mesenteric lymph nodes)
- The number and percentage contribution of the various immune cells ($CD4^+$ and $CD8^+$ T-cells, as well as naïve B-cells, plasma cells, macrophages, dendritic cells, M-cells, and intraepithelial lymphocytes)
- The concentration of peptides or proteins relevant to defense functions (cytokines, total immunoglobulins, immunoglobulin subclasses, specific immunoglobulins [especially secretory IgA] in the saliva and intestinal lumen, and defensins and bacteriocins in the intestinal lumen)
- *Ex-vivo* activities (e.g., phagocytosis, oxidative burst, chemotactic response, natural killer cell activity, cytotoxic T-lymphocyte activity, lymphocyte proliferation in response to a mitogens, production of cytokines or immunoglobulins) under controlled (i.e., outside the body) conditions (including short- and long-term *in-vitro* cultures) to determine the functional responses of epithelia but especially specific immune cell types isolated from the blood and the saliva, but also (essentially in experimental animals) the GALT, lymph nodes, peritoneal cavity, and eventually other internal organs like the spleen and the liver
- Coordinated *in-vivo* responses (i.e., resistance) to an aggression (e.g., an immunological or a chemical challenge like a vaccine and a live bacteria or a carcinogen, respectively)
- Composition and activities of intestinal microflora, e.g., production of bacteriocins, SCFAs and lactic acid, interaction with epithelia, and adherence to mucus
- Xenobiotic metabolizing activities in intestine or liver and microflora
- Detection of DNA damage in colon cells with the "Comet assay" to reveal the potential of colon cancer risk compounds to induce genetic damage[61]
- *In-vitro* mutagenicity of fecal water
- Concentration of calcium ions in the small and large intestinal lumen

12.2.4.3 Indirect Measurements of Defense Functions

Evaluating the risk of diseases that are related to a dysfunction of these defenses is an indirect but relevant mean to assess the integrated defense's response and eventually its modulation by nutrient. Just to cite a few examples:

- Resistance to a challenge with live pathogens as measured in experimental animals by the translocation of the bacteria and the numbers of pathogens found in different organs but also by the survival of the infected animals
- Incidence and severity of antibiotic-associated diarrhea

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* Among these biomarkers, those used to study intestinal immune functions have been evaluated by Salminen et al. who described their advantages and disadvantages.[23]

- Incidence and severity of gastroenteritis (e.g., rotavirus diarrhea in infants, traveler's diarrhea) in human studies
- Reduction of the risk and/or improvement of symptoms of inflammatory bowel diseases (ulcerative colitis, Crohn's disease, and pouchitis) that are believed to originate from abnormal host responses to some members of the intestinal flora and/or from a defective mucosal barrier[62]
- Modulation of carcinogenesis (especially colon carcinogenesis) in chemically treated or in genetically modified (e.g., knock-out) experimental animals
- Modulation of growth and/or metastasis of externally implanted tumor cells in experimental animals
- Improved efficacy of cancer therapies

12.3 NUTRITION AND GASTROINTESTINAL DEFENSE FUNCTIONS

As stated in a recent book, "a relationship exists between nutrition, gut flora, immunology, and health."[63] Food components that directly modulate the intestinal mucosal functions (including immunity) or act indirectly by modifying the composition or activity of the intestinal microflora (known to modulate some of the major gastrointestinal defense mechanisms) help by increasing activities that suppress pathogens, inactivate toxic chemicals, or down-regulate the response that will reduce the risk of allergic and inflammatory reactions. These food components belong to the functional foods (see Chapter 1) and their effects are in enhancing functions (i.e., the defense functions) and in reducing the risk of diseases like infectious diseases, inflammation, and inflammatory diseases but also neoplasia and cancer.

The relationship between nutrition and defense mechanisms was first observed in malnutrition (starvation, but also protein deficiency and specific deficiencies of micronutrients especially iron, zinc, copper, selenium, and B vitamins) and cachexia which have an adverse effect on T-cell functions and cell-mediated immunity as well as on secretory immunity and, to a smaller extent, on B-cell functions and humoral immunity.[64]

With regard to gastrointestinal defense functions, it is increasingly becoming known that specific food components have also a beneficial effect on the body's defense mechanisms by positively modulating them. Among these, the best immunomodulating food components so far studied are dietary fiber, probiotics, and prebiotics (see Section 12.5).

12.3.1 Dietary Fiber and Immune Function

As discussed above (see Chapter 6), fermentable dietary fiber alters the structure and miscellaneous functions of the gut. To date, relatively few studies have been conducted on their effects on gastrointestinal defense functions. Thus, it is not possible at this time to draw conclusions on the effects of specific fibers. However, although exploration in this area is still in its infancy, animal studies have demonstrated that dietary fiber content and type have the potential to modulate defense

functions, especially the immune functions of GALT. Regarding the safeguard, but nonimmune-dependent functions, it has been reported that apple pectin, wheat bran, and oat bran suppress fecal b-glucuronidase, b-glucosidase, and nitroreductase activities in humans.[65,66]

12.3.1.1 Effects of Fermentable Dietary Fibers on Immune Functions

The major effects of dietary fiber on immune functions that have been reported so far include:[67–78]

- An increase in IgA-positive cells in small intestine and cecal mucosa
- An increase in IgA secretion in cecal content
- An increase in IgA in spleen and mesenteric lymph nodes
- An increase in $CD4^+$ T-cells in mesenteric lymph nodes
- An increased proportion of $CD8^+$ in intraepithelial lymphocytes in colorectum
- A decrease in $CD4^+/CD8^+$ ratio in spleen
- An increase in cecal and colonic macrophages
- An increased phagocytic function in intraperitoneal macrophages
- An increase in serum, mesenteric lymph node, and mucosal immunoglobulin production
- Altered cytokine production (increased interferon-g and interleukin-4) in mesenteric lymph nodes and in spleen
- Altered leukocyte and lymphocyte numbers in tissues such as the spleen and intestinal mucosa

12.3.1.2 Mechanisms of the Effects of Fermentable Fibers on Immune Functions

Even though the mechanism for the effect of fermentable dietary fibers on immune function in the intestine has not been fully established, interesting hypotheses have been proposed that include:

- *Production of SCFAs from fiber fermentation*. A number of studies support the direct or indirect immunomodulatory properties of SCFAs.[79,80] Indeed, supplementing total parenteral nutrition with SCFAs results in increased natural killer cell activity.[79] Moreover, other studies have demonstrated antiinflammatory properties of SCFAs, especially butyrate that was reported to suppress both constitutive and cytokine-induced expression of the transcription factor NFkB in the colonic cell-line HT-29.[81] But whether these effects occur at concentrations seen after consumption of usual dietary concentrations of fermentable fiber is not known. It has also been suggested that high SCFAs production, particularly butyrate in the colon due to fermentation of dietary fibers, may reduce the requirement of

epithelial cells for glutamine, thereby sparing it for other cells, especially lymphocytes for which glutamine is an essential energy source.[82,83]

- *Modulation of mucin production*. There is some evidence indicating that the addition of fermentable fibers to the diet can stimulate the production of mucins,[84] an effect that might contribute to the lower incidence of bacterial translocation across the gut barrier reported in experimental animals fed dietary-fiber-rich diet.[85–88] The increase in mucin production may occur in response to the low intestinal pH caused by the high production of SCFAs.[89,90]

12.3.2 Probiotics, Immune Functions, and the Risk of Immune-Associated Diseases

In contrast to work on fermentable dietary fibers, many more studies have documented effects of feeding probiotics and especially lactic acid bacteria (i.e., lactobacilli and bifidobacteria) on various parameters of immune function, as well as on risk of miscellaneous diseases that are causally linked to a dysfunctioning of the defenses. Reports on the effects of probiotics on immune functions and on the risk of immune-associated diseases often give conflicting results, probably because different species, genera, or strains of microorganisms have different properties and consequently different effects. In addition, their effects also depend on the degree of contact with the intestinal mucosa and the intestinal content while the microorganisms are transiently colonizing the intestinal lumen. Especially, the capacity of the microorganisms to survive in the gastrointestinal tract strongly influences their efficacy.[91–93]

12.3.2.1 Effects of Probiotics on Immune Functions

The major effects of probiotics on immune functions that have been reported so far include:[91,94–114]

- An increased production of immunoglobulins, especially IgA, in GALT
- Modulation of both the number and activity of Peyer's patch immune cells
- Effects on systemic immune functions, and immune parameters in the lungs, peritoneum, and mesenteric lymph nodes
- An increased response of intestinal, and/or systemic T- and B-cells to mitogens

12.3.2.2 Mechanisms of the Effects of Probiotics on Immune Functions

The mechanisms by which probiotics consumed in the diet affect immune function are still largely speculative. These include:

- Immune stimulation through direct contact of the microorganisms with GALT following the transfer of small numbers of bacteria across the

intestinal epithelial barrier into the Peyer's patches, inducing activation or leading to the activation of other immune cells[94,95,106,115]
- Increased production of nitric oxide, H_2O_2, IL-2, IL-5, IL-6, and TNFa by macrophages and/or stimulated $CD4^+$ cells[116,117]
- Increased proliferation of and antibody production by Peyer's patch B lymphocytes and activated macrophage-like cells[118]

Other authors have suggested that it is not the bacteria itself but microbial substances (e.g., cytoplasmic antigens, cell wall components) that penetrate the intestinal epithelia to activate GALT.[97,106,111,112,119,120] Such mechanisms are supported by experiments showing:

- *In-vitro* stimulation of macrophages by cell-free extracts of both *Bifidobacterium longum* and *Lactobacillus acidophilus*[121]
- *In-vivo* stimulation of phagocytic activity of peritoneal and reticulo-endothelial phagocytes and splenocytes activation by supernatants from cultures of *Lactobacillus acidophilus* and/or *Lactobacillus casei*, similar to that produced by the administration of live bacteria[106]
- Stimulation of IgA production by Peyer's patch cells by cytoplasmic components of bacteria as well as live bacteria[111]

The mechanisms of such effects by probiotics and their cell-wall components (such as peptidoglycans) or cytoplasmic antigens are still not well understood. It has been suggested that:

- Lactic acid bacteria can bind on receptor sites on lymphocytes ($CD4^+$ and $CD8^+$).[122]
- Peptidoglycans can bind to the CD14 cell surface antigen, and stimulate mononuclear phagocytes and endothelial cells to release cytokines.[100]

It has also been speculated that the immune effects of probiotic bacteria may actually be due to immunogenic milk peptides present in the fermented milk products used to deliver the probiotic bacteria.[101,106]

12.3.2.3 Probiotics and Disease Risk Associated with Dysfunctional Gastrointestinal Defenses

Some species and strains of probiotics (especially various strains of lactobacilli and bifidobacteria) have proven or are hypothesized to be able to reduce the risk of gastrointestinal diseases. Both human and experimental data have repeatedly been reviewed and critically evaluated.[23,91,124]

In summary, the following effects have either been demonstrated or are tentatively supported by preliminary, mostly experimental, data:

- Reduction of risk and prevention of recovery of gastrointestinal infections especially rotavirus-induced diarrhea in children, traveler's diarrhea,

intestinal colonization by campylobacter or *Clostridium difficile*, colonization of the stomach by *Helicobacter pylori* and antibiotic-induced disorders
- Prevention of bacterial translocations
- Improvement of disease state in patients with inflammatory bowel disease including ulcerative colitis, pouchitis, and Crohn's disease
- Reduction of risk of large aberrant crypt foci and cancer development in the large bowel
- Reduction of risk of recurrence of superficial urinary bladder tumor

12.4 INULIN-TYPE FRUCTANS AND THE GASTROINTESTINAL SYSTEM'S DEFENSE FUNCTIONS

As reviewed in the previous chapters, inulin-type fructans beneficially affect a series of gastrointestinal functions by modulating both the structure and composition as well as miscellaneous activities of the mucosa and the microflora. In this chapter the review will concentrate on the effects of inulin-type fructans on the defense functions in the gastrointestinal system in general but, more precisely, the intestine (and especially the large bowel) and eventually the liver, the mesenteric lymph nodes and the spleen, etc. To do so, two approaches will be used: one that reviews their direct effects on the functions and a second that indirectly targets the functions by looking at the effects on inulin-type fructans on the risk of diseases known to be causally related (at least partly) to dysfunction of the defense mechanisms.

12.4.1 Effects of Inulin-Type Fructans on Biomarkers of Gastrointestinal Barrier Functions

12.4.1.1 Effects of Inulin-Type Fructans on Intestinal Epithelia

The effects of inulin-type fructans on intestinal epithelia (especially the weight and thickness of the small intestinal, cecal, and colonic mucosa) have been reviewed in Chapter 6 (see in particular Chapter 6, Section 6.2.4.2 and Section 6.2.4.4). In a recent study with germ-free, di-associated (*Bacteroides vulgatus* and *Bifidobacterium longum*), and human-flora-associated Wistar rats, Kleessen et al. have demonstrated that, feeding a mixture (50/50) of oligofructose and long-chain inulin (5% w/w in diet) for four weeks in di-associated and human-flora-associated, but not in germ-free, rats beneficially affected:[124]

- Mucosal morphometry, by increasing the height of villi in the distal jejunum (1.25 times), the depth of crypts both in the distal jejunum (1.3 times) and in the distal colon (1.35 times), and the number of goblet cells both in the distal jejunum (1.4 times) and the distal colon (1.35 times).
- Thickness of the mucus layer that was increased both in the distal jejunum (1.5 times) and in the distal colon (1.4 times).

- Histochemical composition of mucins in distal jejunum (predominance of sialomucins) and distal colon (predominance of sulphomucins in treated rats vs. sialomucins in control animals). A previous study had similarly reported that, even though feeding inulin decreased total mucin in colon mucosa, it increased mucin release into the intestinal lumen as well as sulphomucin production in both germ-free and heteroxenic rats.[125] The increased production of sulphomucin is of interest, since it is known that it contributes to resistance of the mucosa to attacks by bacterial enzymes, and to reduction of the risk of colon cancer development.[126–128]

The finding that inulin-type fructans beneficially affected the morphology of intestinal mucosa in bacteria-associated, but not in germ-free, rats strongly suggests that these effects are mediated by the changes in microflora composition and/or require inulin fermentation. In the study reported by Kleessen et al. inulin-type fructans, indeed, selectively stimulated the growth of bifidobacteria in the colonic lumen of human flora-associated rats but also in the mucosa of both di-associated and human-flora-associated rats.[124]

12.4.1.2 Effects of Inulin-Type Fructans on Colonization Resistance and Translocation of Microorganisms

An important role of the barrier function is to prevent microorganisms present in the gastrointestinal tract from invading systemic organs and tissues where they may proliferate, causing infections and eventually becoming lethal, i.e., the process of bacterial translocation, defined as the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa.[129,130] The major mechanisms that promote bacterial translocation are disruption of the ecologic balance of the gut flora, resulting in overgrowth of potentially pathogenic organisms, physical disruption of the mucosal barrier resulting in passage of these microorganisms across the mucosa, and impaired immune defenses.[131] Measuring colonization resistance and translocation of microorganisms is a classical way to assess the efficacy of the gastrointestinal defense mechanisms including the structural integrity of the mucosa. Studies on the effects of inulin-type fructans on these parameters have been performed both in mice and rats.

In mice (C57BL/6NHsd) challenged with an antibiotic (cefoxitin 100 mg/kg bw orogastrically) to compromise autochthonous intestinal microflora and infected with *Clostridium difficile* ($5 \cdot 10^7$ cfu/mouse), oligofructose (30 g/l in drinking water) reduced the growth and toxin production by *C. difficile,* as well as the severity of diarrhea and rate of death . These effects were at least partly attributed to increased SCFAs production and subsequent decreased pH in the intestinal lumen.[132,133] In a gnobiotic mouse model, Oike et al. have compared the efficacy of a probiotic, i.e., *Bifidobacterium adolescentis* and a synbiotic, i.e., *Bifidobacterium adolescentis* + oligofructose (4.3% w/w in diet), in reducing the lethality induced in germ-free mice (BALB/c nu/+) by the inoculation of *Escherichia coli* O157:H7.[134] The *E.coli* of that serotype is recognized as the agent responsible for fatal outbreaks of hemorrhagic colitis, haemolytic uremic syndrome, and thrombotic thrombocytopenic purpura.[135,136] Almost all (90–100%) of the control infected mice died within 12 d, and

both the probiotic and the synbiotic preparations significantly ($p < 0.01$) but similarly increased the survival times. At the same time, both preparations similarly reduced the fecal excretion (a direct measurement of colonization resistance) as well as the translocation in the liver of the *E. coli.* The only difference between the probiotic and the synbiotic was in the percentage of mice with liver translocation (25 and 62.5%, respectively). These data show that in germ-free mice a health promoting bacteria (i.e., *Bifidobacterium adolescentis*) inoculated alone increases colonization resistance and reduces translocation of a potentially pathogenic *E. coli*. However, in such a model, the addition of oligofructose does not induce any further benefit.

In a more recent study, Buddington et al. have demonstrated a strong enteric protection against a challenge by *Candida albicans* in mice (B6C3F1) prefed with either oligofructose or inulin HP (10% w/w in diet) for 6 weeks.[137] Indeed, 1 week after the challenge, the densities of *Candida albicans* (cfu/g) in the small intestinal content were 5,100 ± 640, 2,730 ± 450, and 2,820 ± 450 in control, oligofructose and inulin HP treated mice, respectively. However, in that experiment, the pathogenic microorganism did not translocate, probably because the basal diet already had a high dietary fiber content (10% cellulose) that was likely to be sufficient to maintain the mucosal barrier.[131] The same group has also reported that adding oligofructose to an oral rehydration solution accelerates recovery of lactic acid species endemic to the large bowel and reduces the percentage increase in the numbers of potentially pathogenic microorganisms.[138]

More surprising are the results reported in two recent publications that show an inhibition of intestinal colonization but a stimulation of translocation in salmonella-challenged rats fed oligofructose.[139,140] In these two studies, specific pathogen-free Wistar rats (8 weeks old; n = 8 per group) were fed a diet low in Ca (±25% of the recommended intake for rats) and high in fat (20%) to mimic the composition of Western human diets, and containing 0, 3, or 6%[139] and 4% oligofructose,[140] respectively. After an adaptation period of 2 weeks, the rats received a single oral dose of $1.7 \cdot 10^{10}$ cfu[139] and 1.10^{9} (experiment 1) or 1.10^{8} (experiment 2) cfu[140] of *Salmonella enteritis*, respectively. Feces were collected for microbiological analysis daily during the last 5 d preceding and following the challenge with salmonella. After sacrifice, at day nine after infection, cecal contents and cecal mucosa were also collected and analyzed for the presence of salmonella. Translocation of salmonella was evaluated either indirectly by using the methodology developed by the authors[141] that quantifies the urinary excretion of NO_x (as the sum of NO_3^- and NO_2^-) excreted in urines collected daily from the last day before until the 9th day after infection[139] or directly (and more classically) by quantifying viable salmonella in the spleen, the mesenteric lymph nodes, and the liver.[140]

In the two papers,[139,140] and as expected, feeding oligofructose during the 2-week adaptation period significantly increased the number of fecal bifidobacteria but only in the rats fed the highest dose (6 and 4% w/w in diet, respectively). In the first paper,[139] and even though the pH of the cecal content of rats fed the oligofructose-containing diet before the infection was significantly and dose-dependently reduced in a manner similar to what has been reported by others (see Chapter 6, Section 6.2.4.3), the cecal concentration and the cecal content of SCFAs and/or lactic acid did not increase significantly after oligofructose intake (but the variability of the

data was unusually high as shown by standard deviations varying from 58 to 78%!). Feeding oligofructose to rats increased the number of salmonella in the cecal content and the effect was dose dependent, whereas in the mucosa, only the highest dose had a significant effect. The highest (but not the lowest) dose of oligofructose significantly increased daily urinary excretion of nitrates and nitrites used as an indirect marker of salmonella translocation. In the second paper,[140] and as compared with the other diet groups (i.e., 5% cellulose, 4% wheat bran, 4% resistant starch, or 4% lactulose) the oligofructose-supplemented rats had the best colonization resistance to *S. enteritidis*, as shown by the reduced fecal shedding ($\pm 10^2$ fold less) of this pathogen over time that correlates with a more acidic pH of fecal water. However and besides such a significant increase in colonization resistance, the rats fed oligofructose (and lactulose) had the highest induced urinary NO_x excretion over time with a peak value twice as high as that of the cellulose, wheat bran, and resistant starch groups, thus suggesting a higher bacterial translocation (experiment 1). In addition, quantification of viable salmonella counts in extra-intestinal organs revealed that in the rats fed oligofructose, these counts were statistically significantly increased in the spleen (1.14 times) and in the liver (1.5 times) but not in the mesenteric lymph nodes as compared to the cellulose fed rats. Similarly supplementing diet with lactulose increased the viable salmonella counts in the liver (1.86 times) but not in the spleen or in the mesenteric lymph nodes (experiment 2). In addition, the cecal and colonic, but not ileal, mucosal myeloperoxidase activities (used as a marker of inflammatory response) were two- to threefold higher in the oligofructose- (and lactulose-) fed infected rats in comparison with rats on a cellulose-supplemented diet. With regard to colonization resistance, these two reports are contradictory. Indeed, the conclusion of the first is that oligofructose dose-dependently increases colonization[139] whereas in the second set of experiments, oligofructose (and lactulose but not cellulose, wheat bran, or resistant starch) improves colonization resistance[140] to this invasive pathogen. With regard to bacterial translocation, both publications conclude that oligofructose (and lactulose but not cellulose, wheat bran, or resistant starch) lowers the resistance of rats to translocation of salmonella.[139,140] To explain that discrepancy, the authors hypothesize that intestinal mechanisms responsible for colonization resistance might be different from those determining translocation.[140] They further suggest that oligofructose might impair the intestine mucosal barrier functions due to the production of "aggressive luminal contents that might led to epitheliosys and impairment of the barrier functions" and they speculate that "enhanced production of organic acids due to rapid fermentation of oligofructose and lactulose" (as opposed to a much slower and prolonged fermentation of resistant starch or to a limited fermentation of cellulose and wheat bran) is responsible for such deleterious effects.[140]

However, these studies are highly criticizable, their results are sometimes contradictory, the protocol itself is inadequate, and the discussion is incomplete, making the conclusions largely irrelevant. Indeed:

- In the experiments reported in the first publication, it is difficult to understand how the highest dose of oligofructose could increase salmonella counts in the mucosa and in the cecal contents, while at the same time it

reduces cecal pH and increases, by almost 30-fold, the amount of lactic acid in the cecum. Based on such data, the conclusion would be that the lower the pH and the higher the concentration of lactic acid in the cecum, the better the growth of *Salmonella enteritidis*. This is difficult to accept and contrasts strongly with previous knowledge on salmonella physiology. Indeed, and as shown by the same group of investigators in a previous publication, a concentration of lactic acid of 40 mmol/ml completely suppresses growth of *S. enteritidis in vitro*.[142]

- Also surprising is the observation that the intake of oligofructose and lactulose, but not resistant starch, increases translocation, even though it is likely that these three nondigestible dietary carbohydrates are fermented in the large bowel and produce acids. The obvious question is why does such an increase in translocation not occur in all three experimental conditions? In fact, one should expect lower effects with oligofructose as its fermentation produces much lactate (indeed, lactate is one of the most important acids produced by bifidobacteria). Moreover, possible weakening of the mucosal barrier by SCFAs has only been shown at pH 4 or below.[143] This effect cannot be claimed in the experiments described in this paper since cecal pH, even at the highest oligofructose dose level, remained well above 4. The argument that, as compared to oligofructose or lactulose, resistant starch is more slowly and more distally fermented is speculative and not supported experimentally.
- Urinary excretion of nitrites or nitrates is not a reliable nor a validated marker actually demonstrating translocation, as it may also reflect endogenous production of nitric oxide, which is a plurifunctional mediator involved in a series of physiological as well as pathological processes, such as inflammatory responses, neurotransmission, modulation of vascular tone, modulation of gastrointestinal motility, and platelet function. For instance, the same group of investigators has proposed that this parameter (urinary excretion of nitrites or nitrates) might be used as a marker of intestinal inflammation in patients with celiac disease, a condition in which bacterial translocation does not occur.[145] The lack of specificity of this parameter makes invalid the interpretation of the data by the authors, assuming increases of its urinary excretion as a sign of increased translocation of salmonella. The data as they are presented might, on the contrary, reflect that an oligofructose-supplemented diet enhanced the activity of macrophages against infection by stimulating the production of nitric oxide, which acts as a bactericidal substance. Such an effect has indeed been reported for inulin that, *in vitro*, stimulates NO synthesis *via* activation of protein kinase C-a and protein tyrosine kinase, resulting in activation of NF-kB by interferon-g-primed RAW 264.7 cells.[145]
- The validity of the protocol design is, in itself, highly questionable. Indeed, and in an attempt to mimic human western diets, rats were fed a diet containing only some 25% of the recommended daily intake of Ca. In view of the expertise of authors who have published a series of papers that demonstrate the key role of dietary Ca intake in modulating

resistance of rats to salmonella infection, this is particularly surprising.[146,147] Indeed, in these papers, these authors have demonstrated that a low Ca diet by itself has deleterious effects on rat gastrointestinal defense functions because a substantial amount of dietary Ca reaches the large bowel where it forms insoluble phosphate salts that strongly absorb and precipitate toxic surfactants, especially bile acids and fatty acids that may have major implications for the resistance to intestinal infections and for the mucosal integrity.[148] That observation confirms previous reports showing that Ca binds bile acids and fatty acids in the enteric lumen to form insoluble nonirritant soaps.[149,150] Indeed, these toxic surfactants are known to damage the intestinal epithelia;[153] and to stimulate epithelial cell proliferation,[153] two deleterious effects that are suppressed by supplemental dietary Ca.[154,155]

- Moreover it has been shown that, *in vitro*, Ca may directly facilitate the adhesion of bacteria to intestinal epithelial cells.[156]
- In addition, Rémésy et al. have demonstrated that the protective effects of dietary Ca on colonic epithelia involve not only bile acids binding by insoluble Ca salts but also the control of luminal pH itself and of the production of SCFAs and lactic acid.[157] In their discussion, these authors report that "In rats fed the inulin diet containing only 2 g Ca/kg (0.2%) depressed weight gain, severe diarrhea, and a significant mortality have been observed."

In conclusion, the low intake of Ca (25% of the daily recommendation) is likely to play a major role and it might, by itself, explain most, if not all, of the effects that are attributed to oligofructose feeding. Therefore, that parameter should have been included in the discussion as it was in a previous paper of the same group that studied the effect of lactulose on resistance of rats to the invasive pathogen *Salmonella enteriditis*.[142] In that earlier publication, the authors included three experimental groups (low Ca, low Ca + lactulose, and high Ca + lactulose, the dietary Ca concentration being 0.1, 0.1, and 0.8%, respectively, and the concentration of lactulose being 10%) whereas in the "oligofructose" experiments, they had only two main experimental designs (low Ca and low Ca + oligofructose, the dietary concentration of Ca being 0.13% and that of oligofructose being 0, 3, 4, or 6%). A comparison of the same parameters in the two publications clearly shows that, in rats fed a low Ca diet, both lactulose and oligofructose similarly affected body weight growth, food intake, fecal excretion of cations and mucin, fecal water cytotoxicity, urinary excretion of NO_x, and salmonella translocation, whereas in rats fed a high Ca diet, lactulose supplementation suppressed these deleterious effects and protected against salmonella infection. If, as suggested, oligofructose impairs the intestine mucosal barrier functions due to the production of "aggressive luminal contents that might lead to epitheliosis and impairment of the barrier functions," and if, as speculated, such an aggressive luminal content is the consequence of an "enhanced production of organic acids due to rapid fermentation of oligofructose (and lactulose)," then the low dietary Ca intake is likely

to have played the major role in such deleterious effects, and the conclusions are not relevant.

To justify their experimental design (especially food composition), the authors might argue that, following earlier recommendations, the rats were fed a low Ca/high fat diet to mimic Western-type diets in order to obtain information relative to human conditions, allowing extrapolation of their conclusions to human situation.[158] Indeed, it has been suggested that, because, on the basis of nutrient density, daily Ca intake is several times higher in laboratory rodents than in human, diets for animals (especially rats) should have a lower (±25%) Ca density. These recommendations were made originally for long-term studies in laboratory animals, especially for chronic toxicity testing including carcinogenesis, but they have not been applied nor were they implemented in the guidelines for toxicity testing that are updated on a regular basis. Indeed, it must always be kept in mind that a rat will never be a human being and that feeding rats a human diet will not make its physiology more human-like. Especially in nutrition research, as well as in functional food science, animal data are only useful to formulate hypotheses and eventually to study mechanisms, but they are not aimed at proving or disproving an effect for humans. Such a proof or a disproof can only be obtained in human nutrition intervention trials (see Chapter 1 for an extensive discussion of that topic). Moreover, why then limit changes in food composition to Ca and fats?

An additional argument is that, as discussed in details in Chapter 10, inulin-type fructans (as well as lactulose) enhance Ca absorption, especially in the large bowel. Indeed, oligofructose stimulates (up to 60%) Ca absorption in rats.[159] One obvious consequence of that effect is likely to be a further reduction in the amount of Ca available both in the cecum and in the colon to chelate and form insoluble salts of bile and fatty acids, thus reinforcing the deleterious consequences of a low Ca intake. It can further be speculated that this is even reinforced by the fact that, in an attempt to use a western human-type diet, the authors have fed rats a high fat (20% w/w) diet that is known to stimulate the secretion of bile acids and is likely to provide more fatty acids in the lower intestine. Furthermore, it is not surprising that oligofructose (as well as lactulose) behaves differently than resistant starch since not all fermentable nondigestible carbohydrates are able to stimulate Ca absorption as shown by the absence of effect of pectin, another soluble dietary fiber.[160] Moreover, in C57BL/6J-*Min*/+ mice that are heterozygous for a nonsense mutation of the *Apc* gene and are prone to develop numerous intestinal adenomas and adenocarcinomas, oligofructose, but not resistant starch or wheat bran, was shown to be effective in reducing the incidence of tumors.[161] After such a long discussion, our conclusion is that the papers by ten Bruggencate et al. and Bovee-Oudenhoven et al. do not demonstrate that oligofructose stimulates salmonella translocation in "normal" rats but might do so in rats fed a low Ca/high fat diet that is likely to disturb their gastrointestinal physiology.[139,140]

12.4.1.3 Effects on Chemical Safeguard Functions

The effects of inulin-type fructans on the production as well as the composition of the pool of SCFAs have been evaluated (see Chapter 6, Section 6.2.4.4), the main

conclusions being that the colonic fermentation of inulin-type fructans produces large amount of total SCFAs and lactic acid with, as a consequence, the acidification of the large-bowel content (see Chapter 6, Section 6.2.4.4). Such an acidic environment prevents the proliferation of potentially pathogenic bacteria. It is one of the major consequences of the prebiotic effect of inulin-type fructans (see Chapter 9 and especially Section 9.4.1 and Section 9.4.2), which balances the competitive interactions between all the genera, species, and even strains of microorganisms.

Such a fermentation of inulin-type fructans also produces a high proportion of butyrate, one of the most physiologically relevant bacterial metabolites.[162] Indeed, it is believed that butyrate protects against colon carcinogenesis by inhibiting colon cell proliferation, by inducing proliferation, and by promoting apoptosis.[163] In Sprague–Dawley rats treated with the chemical carcinogen dimethylhydrazine (DMH), a treatment known to stimulate apoptosis, Hughes and Rowland have reported that both oligofructose and inulin HP (5% w/w in diet as pretreatment for 3 weeks) further stimulate the defense mechanism above the levels induced by DMH alone thus strengthening the defense capacity against tumor formation.[164]

Another important mechanism of defense in a balanced colonic microflora is the production of bacteriocins. In particular, it has been demonstrated that the growing *Bifidobacterium infantis* has an inhibitory effect towards *E. coli* and *Clostridium perfringens*.[165] Further studies showed that eight species of bifidobacteria could variously excrete an antimicrobial substance with a broad spectrum of activity affecting the genera salmonella, listeria, campylobacter, and shigella.[166] By virtue of their prebiotic effect, inulin-type-fructans selectively stimulate the growth of bifidobacteria and possibly other health-promoting bacteria thus reinforcing the capacity of the colonic microflora to prevent the development of potentially pathogenic microorganisms. A recent publication has demonstrated that it is indeed the case by showing that the inhibitory effect of *Lactobacillus plantarum* and *Bifidobacterium bifidum* on *E. coli*, *Campylobacter jejuni*, and *Salmonella enteritis* is increased if the probiotics are grown *in vitro* in the presence of oligofructose, inulin, or a mixture of oligofructose and inulin.[167]

12.4.1.4 Effects on Enzymatic Safeguard Functions

Experiments have been performed with the aim to test for the effect of inulin-type fructans on the activity of the enzymes that metabolize the xenobiotics in the colonic microflora, in intestinal tissue, and in the associated organs especially the liver. Concerning the effects of inulin-type fructans on microflora-associated xenobiotic metabolizing enzyme activities, data are available both in experimental animals and in humans (Table 12.4).

Only rats previously treated with a chemical carcinogen (essentially AOM or azoxymethane) have been used as experimental animals, but data are contradictory. Indeed, one study shows no effect, whereas the other reports a statistically significant reduction in cecal b-glucuronidase but an increase in cecal b-glucosidase after inulin feeding.[164,168,169] In humans all *in-vivo* studies reported so far both in healthy adults and in the constipated elderly, show no statistically significant effect of oligofructose, inulin, or inulin combined with bifidobacteria on fecal b-glucosidase or reductases

TABLE 12.4
Effects of Inulin-Type Fructans on Microflora-Associated Xenobiotic Metabolizing Enzymes

Experimental Model	Treatment	Results			Refs
		b-glucuronidase	b-glucucosidase	Reductases	
AOM[1]-treated F344 rats 15 mg/kg/week for 2 weeks n = 12	Inulin 10% in diet for 8 weeks	NS[2]	NS	NT[3]	168
AOM-treated SD[4] rats 12.5 mg/kg/week for 2 weeks n = 15	High-fat diet (44% energy) for 12 weeks Inulin HP 5% Inulin HP 5% + bifidobacteria	¥0.625 ¥0.55	5 ¥ 7 ¥	NT NT	169
DMH[5]-treated SD rats n = 6	Oligofructose 5% Inulin HP 5%	NS NS	NS NS	NT NT	164
Healthy human adults n = 20	Oligofructose 12.5 g/d for 12 d	NS	NT	NS	172
Healthy human adults n = 12	Inulin 18 g/d + bifidobacteria for 12 d	NS	NT	NT	171
In vitro human gut flora In 3-stage continuous culture system	Inulin	Slight decrease	Slight decrease	Increase followed by decrease	174
Human elderly n = 25 (10/15)	Inulin vs. Lactose for 19 d	NS	NS	NT	173
Healthy human adults n = 12	Oligofructose for 4 weeks	Decrease	NT	NS	183

[1] AOM = azoxymethane.
[2] NS = no statistically significant effect.
[3] NT = not tested.
[4] SD = Sprague–Dawley.
[5] DMH = dimethylhydrazine.

(azo- and/or nitroreductase).[170–173] Only one study out of four shows a statistically significant reduction in both b-glucuronidase and glycocholic hydroxylase after the ingestion of oligofructose.[170] The only *in-vitro* study so far available that reports effects of inulin on the activity of miscellaneous enzyme activities has been performed in human feces used to inoculate the three-stage continuous culture system developed by Macfarlane and his associates (see Chapter 5, Section 5.3.5.2). Besides a slight decrease in b-glucuronidase and b-glucosidase activity, this study shows an increase followed by a decrease in reductases (azo- and nitro-reductases) but a significant increase in arylsulfatase. These patterns of changes are complex and difficult to interpret. Moreover, since only a single fecal sample collected from a single donor has been used, making any conclusion is hazardous.[174] In addition to the microflora-associated xenobiotic metabolizing enzyme activities discussed in the preceding text, other miscellaneous parameters more or less directly related to these activities have also been measured. Data show no effect of either total or individual neutral sterols and bile acids on human fecal excretion;[172] only a decrease (0.7 times) in cecal ammonia concentration in rats.[164,169]

Regarding the effect of inulin-type fructans on intestinal and hepatic xenobiotic metabolizing enzymes, Roland is the only author who has investigated such effects in her Ph.D. thesis, showing that inulin (10% w/w in the diet for 6 weeks) has no effect on the concentration of intestinal cytochrome $P_{450\text{-}1A1}$ but increases the total concentration of cytrochromes P_{450} as well as the activity of glutathione-S-transferase (GST) in the liver of rats (male Fisher F344).[175] Such an induction of the hepatic GST is believed to be beneficial because glutathione is recognized as a detoxifying agent that reacts with the very reactive nucleophiles that covalently bind to DNA and proteins to initiate mutagenic and toxic processes, respectively. To confirm such a beneficial effect, Roland has demonstrated that pretreatment with inulin protects rats against the toxic effect of glucosinolates.[175]

12.4.1.5 Effects on Immune Defense Functions

The experimental studies that have examined the effects of inulin-type fructans on the immune system are reviewed in Table 12.5. The main effects reported so far are on the composition of the GALT but not or less so on peripheral immune cells.

In mice it has been shown that inulin-type fructans increase:

1. Number of lymphoid nodules (Peyer's patches) in the small intestine[161]
2. Number of macrophages in the large bowel[71]
3. Phagocytic activity of peritoneal macrophages (collected after recruitment had been stimulated by thioglycolate to induce a chemical peritonitis) against *Listeria monocytogenes*, a marker of TH1-mediated immunity and increase in NK cell activity of splenocytes towards Cr-labeled YAC-1 tumor cells[176]
4. Mean size of Peyer's patches and *in vitro* up-regulation of IgA, IL-10, and interferon-g production by stimulated cells isolated form Peyer's patches; increase in fecal IgA excretion; *in-vitro* upregulation of interferon-g, but downregulation of IL-5 and IL-6 secretion by stimulated $CD4^+$ T-cells isolated from spleen[177]

TABLE 12.5
Summary Table of Results Showing Effects of Inulin-Type Fructans on Intestinal and Systemic Immune Functions

Experimental Model	Treatment	Results	Refs
C57BL/6NHsd-mice	Oligofructose 3% in drinking water	**Increase in the number of cecal and colonic macrophages[1]**	71
C57BL/6-Apc$^{Min/+}$ mice n = 5 per group	Oligofructose 5.8% w/w in diet for 6 weeks	**Increase in the number of macroscopically detectable lymphoid nodules in the small intestine**	161
Adult mongrel dogs n = 16	Fermentable fibers (0.87%) including oligofructose for 2 weeks	**Increase in the proportion of CD8$^+$ T-cells among the IEL[2], PP[3], and LP[4]** **Increase in the proportion of CD4$^+$ T-cells among the MLN[5] and peripheral blood** **Higher mitogens responses in MLN, IEL (T-cell tissues)** but lower responses in LP and PP (B-cell tissues) **Effects on the composition and function of GALT[6]** but not on peripheral immune cells NS[7] in response to mitogens or NK[8] activity in peripheral blood	180, 181
B6C3F1 mice n = 30	Oligofructose 2.5 or 10%, Inulin 10% w/w in diet for 6 weeks	All three treatments significantly reduced (0.56¥) the total WBC[9] but did not affect the ratios of white blood cell types **Treatment with the highest dose (10%) of both oligofructose- and inulin-enhanced phagocytic capabilities of unactivated macrophages** **Treatment with 2.5 and 10% oligofructose** but not with inulin Decreased phagocytic capabilities of activated macrophages Treatment with the highest dose (10%) of both oligofructose- and inulin-enhanced NK cell activity NS on CD4$^+$/CD8$^+$ T-cells and T/B lymphocyte populations from spleen and thymus	176

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TABLE 12.5 (continued)
Summary Table of Results Showing Effects of Inulin-Type Fructans on Intestinal and Systemic Immune Functions

Experimental Model	Treatment	Results	Refs
F344 male rats, 12–13 weeks old n = 20	Synergy 1 10% w/w or Synergy 1 + LGG and Bb12 ($2 \cdot 5 \cdot 10^{11}$ cfu/kg) in high-fat (23%) diet for 4 weeks	NS on $CD4^+$ and $CD8^+$ T lymphocytes in spleen or MLN, on neutrophil or monocytes phagocytosis, on NK cell activity, on lymphocyte proliferation in blood, spleen, or MLN **Reduction of oxidative burst activity in blood neutrophils by Synergy 1 + LGG and Bb12[1]** but not by **Synergy 1** NS on cytokine production in spleen or MLN, but **Synergy 1** significantly increased IL-10 production in PP in which production of interferon and IL-10 was correlated **Enhancement of secretory immunoglobulin A (S-IgA) by Synergy 1 + LGG and Bb12 in the ileum, and by Synergy 1 in the cecum**	178
Adult female dogs n = 4	Oligofructose 1 g/dog Oligofructose + mannans 1 + 1 g/dog for 2 weeks	**Increased (p = .05) ileal IgA by oligofructose + mannans 1 + 1 g/dog but not by oligofructose** NS on fecal IgA NS on total WBC, neutrophil, and lymphocyte counts, not on serum IgA, IgG, or IgM concentrations	182
BALB/C mice n = 7	Oligofructose 2.5 and 7.5% w/w in diet for 4 or 6 weeks	**Increased fecal IgA (2.5%)** **Increased mean size of PP (7.5%)** NS in number of PP nor in number of cells/PP **In PP cells *in vitro*, upregulation (2.5 and 7.5%) of IgA production by peptidoglycans, of IL-10 and interferon-production by peptidoglycans and antigen-presenting cells** **In spleen cells *in vitro*, upregulation of interferon-production but downregulation of IL-5 and IL-6 secretion in $CD4^+$ T cells by peptidoglycans** NS on levels of serum total IgG_1 and IgG_2 **Suppression of Th2-type antibody response**	177

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TABLE 12.5 (continued)
Summary Table of Results Showing Effects of Inulin-Type Fructans on Intestinal and Systemic Immune Functions

Experimental Model	Treatment	Results	Refs
Female BALB/C mice n = 8 Control and lipopolysaccharide LPS-treated (ip)	Oligofructose 10% w/w in diet for 2 weeks	In PP: **Increased total cell yield, B lymphocytes in control and LPS-treated mice** NS on T lymphocytes, $CD4^+$, $CD8^+$ T-cells in control mice **Increased T lymphocytes, $CD4^+$, $CD8^+$ T-cells, and $CD4^+/CD8^+$ ratio in LPS-treated mice**	177
F344 male rats, 12–13 weeks old n = 15 Control and azoxymethane AOM-treated 2 ¥ 15 mg/kg/week (sc)	Synergy 1 10% w/w or Synergy 1 + LGG and Bb12 ($2 \cdot 5 \cdot 10^{11}$ cfu/kg) in high-fat (23%) diet for 4 weeks	**In control mice:** **Stimulation of NK cell activity in spleen by Synergy 1** **Stimulation of *ex-vivo* production of IL-10 in activated MLN cells by Synergy 1 and Synergy 1 + LGG and Bb12** **Stimulation of *ex vivo* production of interferon by Synergy 1 + LGG and Bb12** NS on *ex vivo* production of IL-10 or interferon- by **Synergy 1** and **Synergy 1 + LGG and Bb12** **Reduction of the $CD4^+/CD8^+$ ratio in spleen by Synergy 1** **In AOM-treated rats:** **Prevention of suppression of NK cell activity in spleen, MLN, and PP by Synergy 1 and Synergy 1 + LGG and Bb12** **Stimulation of *ex-vivo* production of IL-10 in activated PP cells by Synergy 1 and Synergy 1 + LGG and Bb12** NS on lymphocyte proliferation in spleen and MLN by **Synergy 1** and **Synergy 1 + LGG and Bb12** **Reduction of PP lymphocyte proliferation by Synergy 1 + LGG and Bb12**	179

[1] Texts in bold, in the column reporting results, indicate statistically significant effects.
[2] IEL = Intraepithelial lymphocytes.
[3] PP = Peyer's patches.
[4] LP = Lamina propria.
[5] MLN = Mesenteric lymph nodes.
[6] GALT = Gut associated lymphoid tissue.
[7] NS = No statistically significant effect.
[8] NK = Natural killer.
[9] WBC = White blood counts.

5. Number of lymphocytes in Peyer's patches in control and lipopolysaccharides-stimulated (endotoxemic) mice but increase in T-lymphocytes and $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio only in endotoxemic mice[177]

In rats (essentially F 344 male), it has been reported that inulin-type fructans (essentially the mixture of oligofructose and long-chain inulin or oligofructose-enriched inulin Synergy 1):

1. Significantly increase IL-10 production in PP
2. Enhance the production of secretory immunoglobulin A (sIgA) in the cecum
3. Stimulate NK cell activity and reduce the $CD4^+/CD8^+$ ratio in spleen
4. Stimulate *ex-vivo* production of IL-10 in activated mesenteric lymph nodes in rats that have been treated (with azoxymethane) to induce aberrant crypt foci and tumors in the large bowel, Synergy 1[178,179]
5. Prevents the suppression of NK cell activity induced by azoxymethane in spleen, mesenteric lymph nodes, and Peyer's patches
6. Stimulates *ex vivo* production of IL-10 in activated cells from Peyer's patches

In dogs, one study has demonstrated that feeding a mixture of fermentable fibers containing oligofructose beneficially affected the composition and the function of the GALT but not peripheral immune cells.[180,181] A second study did not report really significant effect of oligofructose but the dose was particularly low (1 g/dog or ±0.05 g/kg bw) and the number of animals used was only four.[182]

As discussed in Chapter 1, animal experiments are part of the strategy for functional food development because they serve to formulate hypotheses regarding a potential functional effect (either an enhanced function-type effect or a reduction of risk of disease-type effect) to be tested in human nutrition intervention trials. They are also very useful in helping to understand the mechanisms of such effects.

Data available support the hypothesis that inulin-type fructans indeed modulate the immune functions, especially the intestinal immune functions. Dietary inulin-type fructans primarily affect the immune functions by targeting the GALT and especially the Peyer's patches. But indirectly, they also affect the systemic immunity as shown by the increased resistance of inulin and-oligofructose-treated mice to systemic infections by salmonella and listeria.[183] Globally, that immunomodulation leads to a shift to a greater dependence on TH1 vs. a TH2 cell-mediated immunity.[176,177]

It has been suggested that these effects could be mediated *via* the selective stimulation of growth and/or activity of Gram-positive bacteria, essentially the lactic acid bacteria, i.e., lactobacilli and bifidobacteria that are claimed to be associated with enhanced cell-mediated immune functions, changes in cytokines production, and decreased allergic reactions.[184–186] But and by comparing the effect of inulin-type fructans (prebiotics) with those of probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium lactis*) and synbiotics (prebiotics + probiotics), it has been concluded that prebiotics could have a specific mechanism of immunomodulation.[178]

The only study so far reported on the effects of inulin-type fructans on immune system in human concerns the elderly. It shows that by feeding oligofructose (2 ¥ 4 g/d) to nursing home patients (n = 19, average age 85) for 3 weeks significantly increased the percentage of peripheral T lymphocytes as well as the lymphocyte subsets, $CD4^+$, and $CD8^+$ T cells, but it did not affect the total number of white blood cells, activated T lymphocytes, and NK cells. Oligofructose feeding also reduced the phagocytic activity of granulocytes and monocytes as well as the IL-6 mRNA levels in peripheral blood mononuclear cells, thus suggesting a decrease in the inflammatory process.[187]

12.4.2 Effects of Inulin-Type Fructans on the Risk of Diseases Related to Dysfunction of Gastrointestinal Defense Functions

Quantifying the risk of diseases known or hypothesized to be causally related to dysfunction of the gastrointestinal defense functions is a more global approach to evaluate the effect of inulin-type fructans on these functions. Such indirect measurements include parameters related to:

1. Intestinal infection (see Section 12.4.1.2) and especially traveler's diarrhea
2. Inflammatory bowel disease
3. Necrotizing enterocolitis
4. Colon cancer, including aberrant intestinal crypt foci or adenomas and tumors, essentially adenocarcinomas

12.4.2.1 Effects of Inulin-Type Fructans on the Risk of Traveler's Diarrhea

When traveling not only to Central America, India, East Asia, and Africa but also to many European destinations, healthy individuals are often exposed to the risk of diet-related intestinal infections that may lead to diarrhea, the so-called travelers' diarrhea. The incidence of that disease varies between 30 to 50% depending on the origin and the destination of the travelers as well as the mode of travel.[188–190] The most common infecting organism is enterotoxigenic *E. coli* (±40% of cases); salmonella, shigella, campylobacter, protozoa, and virus (e.g., rotavirus) have also been identified, but in 20–40% of cases, no pathogen is detected.[190–192] A commensal balanced microflora (especially the predominance in number of bifidobacteria and/or lactobacilli) is probably the best defense mechanism against intestinal infections and traveler's diarrhea (see Section 12.4.1.2). It has thus been hypothesized that oral doses of probiotics could be used to reduce the risk of traveler's diarrhea and eventually to treat those suffering an acute attack, but the degree of protection is relatively small at around 10%.[193,194] Inulin-type fructans are prebiotics that stimulate the growth of bifidobacteria in colonic microflora (see Chapter 9). Moreover, bifidobacteria, given oligofructose as substrate, secrete, *in vitro*, a peptide that is inhibitory to most of the common pathogenic organisms capable of causing acute diarrhea.[165] Thus, it has been hypothesized that altering the

environment in the hind by orally ingested inulin-type fructans could lead to a reduced risk of traveler's diarrhea.[195] A total of 244 healthy volunteers, traveling to destinations with high and medium risk of intestinal infections and travelers' diarrhea took part in a randomized double-blind, placebo-controlled study. After a preliminary week of recording bowel habit by diary and a 2-week pre-holiday period with the consumption of oligo-fructose (10 g/d) or placebo and diary, the volunteers traveled for a 2-week holiday period during which they continued to take either oligofructose (10 g/d) or placebo and diary. After returning to their home country, they completed a poststudy questionnaire. Data show a nonsignificant tendency for a reduction in the number of episodes of diarrhea in the oligofructose, as compared to the placebo-fed group, (11% vs. 20% $p = 0.08$) in the poststudy questionnaire but not in the diary (38% vs. 46% $p > 0.1$). No change in bowel frequency, consistency, or stool size was recorded, but the oligofructose-fed volunteers reported a better sense of well-being during the holiday period ($p < 0.02$). Even though not statistically significant, these data are encouraging and should stimulate more research in that area either by using different inulin-type fructans (especially oligofructose-enriched inulin Synergy 1) or by using a synbiotic product (e.g., Synergy 1 and bifidobacteria and/or lactobacilli).

12.4.2.2 Effects of Inulin-Type Fructans on the Risk of Irritable Bowel Diseases (IBD)

It has been hypothesized that pathogenesis and pathophysiology of IBD (Crohn's disease and colitis) involve imbalance of the commensal intestinal microflora leading to a lack of tolerance and an unrestrained activation of the intestinal immune system. In support of that hypothesis, it has been observed that these diseases:

1. Tend to occur in the most distal part of the intestine where the most dense population of bacteria is located[196,197]
2. Are accompanied by elevated levels of antibodies against some species of bacteria present in the intestinal microflora[198]
3. Can be ameliorated, symptomatically, by antibiotic treatment or fecal stream diversion[199,200]

Moreover, using different models of experimental colitis, the role of overgrowth of some bacterial genera (e.g., bacteroides and clostridium) that are commensal in the normal resident microflora has been demonstrated for the development of chronic intestinal inflammation that characterizes IBD. These models are:

1. Chemically induced chronic colitis in rats treated with the hapten trinitrobenzene sulfonic acid (TNBS) or by dextran sulphate that resemble human ulcerative colitis
2. A transgenic model, i.e., the HLA-B27 rats that spontaneously develop chronic intestinal colitis when raised under specific pathogen-free conditions but not when maintained under germ-free conditions[201]

3. Two knockout models, i.e., the IL-2 or IL-10 gene-deficient mice in which the development of intestinal inflammation is dependent on the presence of the colonic microflora[202,203]
4. A hamster model of *C. difficile* colitis in which specific germ-free animals (female Syrian hamsters) are treated with a broad spectrum antibiotic followed 6 d later by a challenge with *C. difficile* that overgrow in the large bowel[204]

Using these models, it has also been demonstrated that if, indeed, some bacterial genera are implicated in the induction of intestinal inflammation, others are considered beneficial. This is especially true for the lactic acid bacteria used as probiotics (see Section 12.3.2.3) including bifidobacterium and lactobacillus. It is thus not surprising that investigators have looked at the effect of inulin-type fructans, known to selectively stimulate the growth and the activity of these two genera in the colonic microflora, on the development and symptomatology of IDB in these experimental models:

1. In antibiotic-compromised mice (C57BL/6NHsd) fed a low-fiber diet, oligofructose (30 g/l in drinking water) has been shown to positively modulate the large intestinal inflammatory response caused by *Clostridium difficile* infection by improving a series of immune parameters essentially in the cecum where the majority of *C. difficile* induced lesions are located. Indeed, and as compared to the mice treated with the antibiotic alone, oligofructose prevented the raise in the number of dendritic and gd T cells as well as in the concentration of prostaglandin E_2, while increasing the number of macrophages in the cecum. At the same time it increased the concentrations of total anaerobes and prevented the raise in fecal toxin A excretion.[71]
2. In the hamster model of *C. difficile* colitis, the most significant effect of oligofructose (30 g/l in drinking water or ±2.5 g/kg bw) was an increase in median survival time.[204]

However, the experimental models of *C. difficile* colitis are not considered very relevant to demonstrate antiinflammatory effect, and the interpretation of the partial down regulation of inflammatory parameters is difficult. Still, the two studies suggest an effect of oligofructose in the prevention of *C. difficile* colitis (F. Guarner, personal communication).

3. In the rat model of distal colitis induced by oral administration of dextran sulphate (MW 40,000, 50 g/l in drinking water for 5 d), inulin (400 mg/d equivalent to ±1.75 g/kg) given orally (1% in drinking water) or by gavage, but not by enema, produced an antiinflammatory effect as evidenced by the reduction of both inflammatory activity and morphological damage of the mucosa. Indeed, oral inulin prevented colonic inflammation as shown by lower scores of mucosal damage, reduced the total area of mucosa showing any degree of crypt lesion, confined lesions to the distal colon, and reduced tissue myeloperoxidase activity, as well as the release of inflammatory

mediators (prostaglandin E_2, thromboxane B_2, and leukotriene B_4) in the colonic lumen. By treating rats with daily enemas of fecal water, it was shown that fecal water obtained from inulin-fed rats induced lower lesion scores and tissue myeloperoxidase than fecal water obtained from control rats. Moreover, fecal water from inulin-fed rats had antiinflammatory activity. Inulin feeding also extended the normal area of acidic environment, with pH values <7 shifting towards the left colon down to the rectum, acidic pH being associated with attenuated scores of mucosal damage. In rats treated with dextran sulphate + inulin vs. rats treated with dextran sulphate alone, the mucosal lesion scores were 10–30 vs. >30, 5–10 vs. 10–30, and <5 vs. 5–10 for the descending, transverse, and ascending colon, respectively, and in the first two segments of the colon, pH values were 6.48–7.13 vs. 7.35 and 6.32–6.66 vs. 6.48–6.9, respectively. These data suggest that oral inulin might have induced a shift from proteolytic to saccharolytic fermentation in the distal part of the colon possibly *via* an inhibition of growth of bacteroides and clostridium that are known to possess extracellular, proteolytic–putrefactive enzyme activities.[205]

4. In the rat model of TNBS-induced colitis, intragastric infusion (2 times daily for 14 d) of oligofructose (1 g/d equivalent to ±4 g/kg bw) attenuated the anorexia associated with the colitis, inhibited the loss of body weight induced by TNBS, reduced the gross score for inflammation, myeloperoxidase activity, and pH, while at the same time increasing lactate and butyrate concentrations, as well as counts of lactic acid bacteria in the cecum. The data of that study strongly suggest that the beneficial effects of oligofructose on side effects and on the intestinal damage associated with chemically induced colitis are mediated *via* its prebiotic effect leading to a stimulation of growth of lactic acid bacteria.[206]
5. These data have, recently, been confirmed in the transgenic rat model of colitis (HLA-B27 TG rats) by showing that the oral administration of the mixture of oligofructose and long-chain inulin (Synergy 1) (1.5 g/rat/d equivalent to 7.5 g/kg/bw/d) between week 7 (before colitis develops) and weeks 14–16 (when colitis has developed) of age significantly reduced gross as well as histology inflammation scores, and reduced IL-1 b but increased TGF-b production in the cecum (personal communication by L. Dieleman, University of Alberta, Division of Gastroenterology, Edmonton, Alberta, Canada).

The effects of inulin-type fructans on IBD in humans, have, so far, only been studied in patients with an ileal pouch–anal anastomosis but without signs of clinical pouchitis. In almost all those patients, mucosa of the ileal reservoir show signs of chronic inflammation that sometimes (10–50% of patients) leads to pouchitis that is classified as an IBD. As in the other IBDs, the inflammation of pouch mucosa is accompanied by a more acidic pH, a reduction in the number of lactic acid bacteria, an increase in the number of clostridia, and the occurrence of fungi in the effluent of the pouch. Results show that, compared with placebo, 3 weeks of supplementation with inulin (24 g/d) lowered pH, decreased numbers of *Bacteroides fragilis*, reduced

the concentration of secondary bile acids, and increased butyric acid concentration in the effluent of the pouch, all signs of a beneficial and preventive effect of inulin on the risk of inflammation. Endoscopic and histologic examinations indeed confirmed reduction of inflammation of the mucosa of the ileal reservoir (Table 12.6).[207]

All these data strongly support the hypothesis that inulin-type fructans have the capacity to reduce the risk of inflammation associated with IBD and that they act *via* their prebiotic effect to rebalance the intestinal microflora, thus preventing the overgrowth of commensal potentially pathogenic microorganisms such as *Bacteroides* or *Clostridium* that may induce inflammatory responses.

Even though the etiology and the physiopathology are different, it is the place to summarize here a doubleblind crossover trial of oligofructose (3 ¥ 2 g/d for 4 weeks) vs. sucrose (3 ¥ 1 g/d for 4 weeks) that was performed in patients suffering from irritable bowel syndrome (IBS). IBS is an extremely common gastrointestinal disorder (±15–20% of adult population of the Western world at some time in their

TABLE 12.6
Effects of Inulin-Type Fructans on Biomarkers and Symptoms of Irritable Bowel Diseases (IBDs) in Experimental Models

Models	Treatment	Effects	Refs
Mice, antibiotic-compromised + *C. difficile*	Oligofructose (3% w/vol. in water)	Modulation of large intestine inflammation; improved immune parameters (i.e., macrophages, dendritic cells, T cells, and PGE_2)	71
C. difficile colitis in hamsters	Oligofructose (3% w/vol. in water)	Increased media, survival time	205
Rat distal colitis (dextran sulphate p.o.)	Inulin (1% w/vol. in water)	Antiinflammatory effects (inflammatory activity; morphology of damage to mucosa; tissue myeloperoxidase; inflammatory mediators such as PGE_2, thromboxane B_2, and leukotriene B_4)	206
Rat colitis (TNBS)	Oligofructose (±4 g/kg bw)	Antiinflammatory effects (scores of inflammation, myeloperoxidase activity) Attenuation of anorexia Inhibition of body weight loss	207
Transgenic rats (HLA-B-27 TG)[1]	Synergy 1 (oligofructose -enriched inulin)	Antiinflammatory effect (scores of inflammation, decreased IL-1 b and TGF-b)	

[1] Personal communication, Dieleman, L.

lives). Symptoms include abdominal pain, distention, flatulence, and constipation or diarrhea plus some extracolonic symptoms such as headache, nausea, tiredness, fluid retention, and joint pain. The results of the trial show that oligofructose at a dose of 6 g/d had no beneficial effect on any of the parameters, i.e., fecal weight and pH, whole-gut transit time, and fasting breath hydrogen concentrations.[208]

12.4.2.3 Effects of Inulin-Type Fructans on Risk of Neonatal Necrotizing Enterocolitis

Despite significant advances in neonatal practice, neonatal necrotizing enterocolitis (NEC), a gastrointestinal disease in preterm infants characterized by abdominal distension, gastrointestinal bleeding, mucosal ulcerations, and intestinal pneumatosis remains a major cause of gastrointestinal emergency in neonatal intensive care units and the first cause of death in extremely premature infants. Its physiopathology remains unclear and several factors including immaturity of the intestine, enteral feeding, and bacterial colonization may be involved.[209] Thus, it has been hypothesized that "the intestinal injury in NEC may be result of synergy of the three risk factors, in which feeding results in colonization of the uniquely susceptible premature intestine with pathogenic bacteria, resulting in exaggerated inflammatory response."[210] In addition, and as none of the preventive treatments, i.e., antibiotic therapy, prophylactic aspects, and parenteral feeding, is considered fully satisfactory, it has been hypothesized that modulating the autochthonous microflora by probiotics and/or prebiotics could help in reducing the risk of that disease.[211]

Because quails present some interesting similarities with premature infants in terms of gastrointestinal histology and physiology, an experimental model of NEC has been developed using gnotobiotic quails associated with faecal flora isolated from premature infants that were fed a lactose diet sterilized by gamma irradiation and containing lactose 6% (w/w) to mimic the proportion in human milk.[212] Evaluation of the disease was made using macroscopic observations and histological examinations. In such an experimental model, bifidobacteria was shown to decrease populations of clostridia, to reduce the concentrations of SCFAs, i.e., butyric acid and branched SCFAs of protein origin, and to totally inhibit the development of the cecal lesions.[213–215] Similarly, in the same model, oligofructose feeding (lactose–oligofructose diet 3%:3% w/w) increased the number of bifidobacteria and decreased *E. coli* and clostridia colonization, depending on the initial composition of the microflora.[216] Therefore, it has been suggested that oligofructose may participate in the health-promoting effect of bifidobacteria by exerting a beneficial effect on microflora balance of the premature infants. Because of the delayed colonization by bifidobacteria in premature neonates, the effect of oligofructose has also been investigated in the absence of bifidobacteria and using clostridia to trigger cecal injury in two specific cases. In the first study, with gnotobiotic quails kept in the sterile isolator, oligofructose was shown effective in decreasing the numbers of potentially harmful bacteria species, i.e., *C. perfringens* and *C. paraputrificum*, reducing the incidence of NEC-like lesions caused by a multimicrobial infection, but these effects varied according to clostridia species, implantation level, and bacterial association. Furthermore, oligofructose did not affect cecal pH or the concentration of SCFAs. However, in another

set of experiments and although quails were housed in an environment containing bifidobacteria, oligofructose was unable to promote intestinal postcolonization by exogenous bifidobacteria in quails initially deprived of this bacterial genus. Nevertheless, like in the previous experiments, the level of *C. perfringens* decreased (1.6 log lower).[217] Comparing the different studies in gnotobiotic quails, the protective effect of oligofructose alone appeared less effective than that of a probiotic (i.e., bifidobacteria) or that of a synbiotic (i.e., oligofructose + bifidobacteria) that leads to a complete disappearance of the cecal lesions due to a sharp decrease in the number or even a disappearance of clostridia accompanied with a disappearance of cecal butyrate. Oligofructose was unable to promote bifidobacteria colonization when the microflora was initially deprived of that species. Nevertheless, oligofructose can act as an antiinfective agent and was demonstrated to be able to decrease the occurrence or severity of the intestinal lesions, depending on the bacteria involved in the onset of the lesions. Recently, preliminary tests have been performed with oligosaccharides added to premature formulae in an attempt to mimic the high levels of such compounds in human milk, particularly in milk from mothers delivering premature infants. Such preliminary tests have demonstrated the bifidogenic effect of a mixture of inulin and galactooligosaccharides. Regarding neonates with NEC, a supplementation of the formulae with prebiotics could offer — even in the absence of bifidobacteria — a new approach to improve their health and permit, when bifidobacteria are present, maintenance of the beneficial flora for a long period. These positive results support the need for further clinical investigations on the role of oligofructose in bacteria colonization of preterm infants and prevention of NEC.[211]

12.4.2.4 Effects of Inulin-Type Fructans on Risk of Colon Cancer

Cancer is a generic term for malignant neoplasia, a large group of diseases arising in practically all tissues composed of potentially dividing cells. Initiation, promotion, progression, and metastasis are the key steps in cancer development and they are multifactorial. Especially, several factors of environmental and genetic origin have been identified that affect cancer incidence. The most important environmental contributors are believed to be the diet that may contribute to some 35% of all cancer deaths and lifestyle factors (smoking, reproductive behavior, and high alcohol consumption).[218,219] Overall, it has been hypothesized that changing either diet and/or lifestyle could reduce the risk of approximately 75–80% of all cancers.

Inulin-type fructans could be effective food ingredients to be included in this type of strategy, and a series of experiments have been performed both in rats and mice to test this hypothesis.[220] In a first series of experiments the end-points were the preneoplastic lesions or intermediate endpoints, i.e., the aberrant crypt foci (ACF), induced chemically in the rat colon, a model that has been widely applied to study chemoprevention of colon carcinogenesis.[221–223] ACF are histological abnormal thickening of the wall in the pericarp of the colon crypts that can be stained and counted in the colon mucosa. It is believed that a few of these preneoplastic lesions develop into malignant adenocarcinomas, but most of the ACF are eliminated by repair mechanisms and only a few of them (mainly those with high numbers of aberrant crypts per foci) develop into tumors and cancers. A few of these experiments have thus targeted not only ACF

but also long-term tumors and adenocarcinomas. These experiments have looked at the effects of inulin-type fructans on the incidence (number of animals with lesions), yield (number of lesions per animal or preferably per animal bearing the lesions), and multiplicity (number of aberrant crypts per foci) of ACF, as well as on the incidence and yield of long-term tumors and cancers. The colon carcinogen is either dimethylhydrazine (DMH) or its alkylating metabolite azoxymethane (AOM) that specifically targets the rat colon (especially its distal part), where they damage DNA and initiate carcinogenesis.[224] In such a protocol, rats receive subcutaneous injections (6 ¥ 20 mg/kg bw/week) or oral administration (2 ¥ 15 or 4 ¥ 20 mg/kg bw/week) of DMH or subcutaneous injections (2 ¥ 10, 2 ¥ 12.5, or 2 ¥ 15 mg/kg bw/week) of AOM. A large number of ACF are detected already after a few weeks, and malignant tumors appear after a period of 45 to 52 weeks.[225] An overview of the variations of this model that has been used to study the effect of inulin-type fructans on the incidence, yield and multiplicity of ACF, and on the incidence of long-term tumors and cancers are presented in Table 12.7. These variations include animal species (mouse or rat),

TABLE 12.7
Summary Description of the Models of Experimental Carcinogenesis Used to Study the Effects of Inulin-Type Fructans

Animals	Carcinogenic Treatment	Dietary Treatment	Experimental Design	Endpoints	Refs
Mice CF_1 Female n = 20	DMH[1] s.c. 6 ¥ 20 mg/kg/week	Synbiotic: Oligofructose 5% + bifidobacteria **I + P[2]**	Sacrifices at weeks 18, 28, and 38 after last dose of DMH	ACF[3] Adenocarcinomas Incidence[4] Yield[5]	231
Rats (Wistar male) n = 15–20	DMH p.o. 2 ¥ 15 mg/kg/week	Synbiotic: oligofructose 2% + bifidobacteria Oligofructose **P[2]**	Start 1 week after last dose of DMH Sacrifice + 24 d and up to + 5 weeks	ACF/cm[2] Foci/cm[2] Crypts/foci	227–230
Rats F344 male n = ?	AOM[6] s.c. 2 ¥ 15 mg/kg/week	Oligofructose Inulin HP[7] 10% w/w **I + P[2]**	Sacrifice at week after last dose of AOM	ACF Yield/colon Multiplicity	228
Rats male Sprague- Dawley n = 15	AOM s.c. 2 ¥ 12.5 mg/kg/week	Inulin HP 5% w/w Synbiotic: Inulin HP + bifidobacteria **P[2]**	Sacrifice after 12 weeks of dietary treatment	ACF Total yield Multiplicity	229
Rats F344 male n = 12	AOM s.c. 2 ¥ 15 mg/kg/week	Inulin 10% w/w **I + P[2]**	Sacrifice at week 9 after last dose of AOM	ACF Yield/colon Foci/cm[2] Multiplicity	168

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TABLE 12.7 (continued)
Summary Description of the Models of Experimental Carcinogenesis Used to Study the Effects of Inulin-Type Fructans

Animals	Carcinogenic Treatment	Dietary Treatment	Experimental Design	Endpoints	Refs
Rats Male Sprague-Dawley n = 8	AOM s.c. 2 ¥ 15 mg/kg/week	Inulin HP 5% w/w Synbiotic: Inulin HP + bifidobacteria **P**[2]	Sacrifices at week 4 after last dose of AOM	ACF Yield Multiplicity	169
Rats F344 male, 1 year old n = 12	AOM s.c. 2 ¥ 10 mg/kg/week	Inulin HP 2.5, 5, and 10% w/w **I + P**[2]	Sacrifice at week 11 after last dose of AOM	ACF Yield/colon and yield/distal colon Multiplicity	226
Rats F344 male n = 10	AOM s.c. 2 ¥ 10 mg/kg/week	Inulin HP 10% w/w **I, P**[2]**, I + P**[2]	Sacrifice at week 45 after last dose of AOM	Long-term tumors Incidence Yield/colon and yield/distal colon	233
Mice B6C3F1 n = 25	DMH s.c. 6 ¥ 20 mg/kg/week	Oligofructose 10% w/w Inulin HP 10% w/w	Sacrifice at week for after the last dose of DMH	ACF Yield/colon	231
Rats F344 male n = 20	DMH p.o. 4 ¥ 20 mg/kg/week	Oligofructose 5 and 15% w/w Inulin HP 5 and 15% w/w **pretreat — I + P**[2]	Sacrifice at weeks 5 and 10 after first dose of DMH	ACF Yield/colon Multiplicity	234
Rats F344 male n = 32–33	AOM s.c. 2 ¥ 15 mg/kg/week	Synergy 1 10% w/w Synbiotic: Synergy 1 + bifidobacteria **I + P**[2]	Sacrifice at week 31 after last dose of AOM	Adenomas and cancers Incidence yield	235

[1] DMH =dimethylhydrazine.
[2] I = dietary treatment during initiation, P = idem during promotion, and I + P = idem during initiation and promotion of carcinogenesis.
[3] ACF = aberrant crypt foci.
[4] Incidence = number of animals with tumors.
[5] Yield = number of tumors by animal with tumors.
[6] AOM = azoxymethane, the carcinogenic metabolite of DMH.
[7] Inulin HP is long-chain inulin (see Chapter 3, Section 3.3.3).

protocol of chemical carcinogenesis (DMH or AOM, s.c. or p.o. administration, and doses), dietary treatment (oligofructose or long-chain inulin, prebiotic or synbiotic, doses, period of treatment, i.e., initiation or **I**, promotion or **P**, or initiation + promotion or **I+P**), and endpoints (incidence, yield, and/or multiplicity of ACF incidence of long-term tumors and adenocarcinomas). All studies have used young growing animals, but in one study chemical carcinogenesis and inulin treatment were applied to a mature 1-year-old rat.[226]

The results of these experiments are summarized in Table 12.8. They show that:

1. Inulin-type fructans have the capacity to reduce the risk of ACF, long-term tumors and cancers in the colon
2. Among the inulin-type fructans, oligofructose is less active than long-chain inulin or the mixture of long-chain inulin and oligofructose known as oligofructose-enriched inulin Synergy 1
3. Mixing long-chain inulin and a probiotic (essentially bifidobacteria and/or lactobacilli) in a synbiotic product increases the cancer risk reduction activity

Indeed, oligofructose has shown a low efficacy (24%) in significantly reducing the yield of ACF in only one[227] out of a total of six experimental groups of rats[137,169,228–230] in which it occasionally did, however, reduce the incidence of pre-neoplastic lesions[137] or the number of ACF with 1, 2, or 3 crypts[168,227] When associated with a probiotic in a synbiotic preparation, oligofructose either showed efficacy[231] or no effect.[228,229]

In contrast, feeding DMH- or AOM-treated rats or mice a diet supplemented with long-chain inulin (inulin HP) significantly reduces the yield of ACF but also tumors and/or cancers in all experiments[137, 170,226,227,232–234] reported so far, except in one experimental group that was fed a low-fat diet.[232] Evaluating the results of all the experimental groups and all the observations in these experiments, and even though in one experiment a linear dose-effect (2.5, 5, and 10%) relationship was established,[226] all together the data support the hypothesis that 5, 10, and even 15% (w/w in diet) of inulin HP are similarly active and reduce the yield of ACF by approximately 40%.[137,169,226,227,232–234] In those experiments inulin HP also reduces the number of ACF with 1–3 crypts[169,227,232,234] as well as larger foci with 4–5 or more crypts, and it reduces (by as much as 72 or 79%) the size of the tumors,[226] thus causing a shift towards smaller foci that are considered to be less prone to become malignant. By comparing the effect of an inulin HP-supplemented diet given during the initiation (**I**), the promotion (**P**) or both the initiation and promotion (**I + P**) phases of carcinogenesis Verghese et al. have demonstrated that inulin HP is the most active when it is given during the promotion, compared to the initiation, phases of the carcinogenic process.[233] Moreover, the same group has reported that the efficacy of inulin HP in reducing the risk of colon cancer increases with the duration of the treatment. Indeed, when feeding rats the inulin HP-supplemented diet for up to 45 weeks after the carcinogenic treatment, the reduction in ACF/tumor yield is 70% as compared to 40% in the other experiments with 4–12 weeks feeding time. Finally, inulin HP is also active in reducing the yield of tumors if carcinogenesis is induced by injecting AOM to mature 1-year-old rat.[226]

TABLE 12.8
Summary Presentation of the Results of the Studies Performed to Test for the Effects of Inulin-type Fructans on Experimental Colon Carcinogenesis

Treatment	Aberrant Crypt FOCI (ACF)					
	Incidence	Yield (number)		Multiple AC/FOCI		Ref
		Total	Per colon	n/FOCI		
DMH	100%	week 18 = 14			7.2	232
		week 28 = 20			2.5	
		week 38 = 24			2.2	
DMH +synbiotic	100%	week 18 = **¥ 0.56**[1]			**¥ 0.36**	
(Oligofructose 5%		week 28 = **¥ 0.51**			NS	
Bifidobacteria)		week 38 = **¥ 0.56**			NS	
AOM			120	n = 2	44	228
				n = 3	28	
AOM + Oligofructose			**¥ 0.76**	n = 1-2	NS	
				n = 2	**¥ 0.71**	
				n = 3	**¥ 0.75**	
AOM + Inulin HP			**¥ 0.65**	n = 1-2	NS	
				n = 2	**¥ 0.55**	
				n = 3	**¥ 0.59**	
AOM		132		n = 1-3	91	170
				n > 3	37	
AOM + Inulin HP		**¥ 0.71**		n = 1-3	**¥ 0.6**	
				n > 3	NS	
AOM + Synbiotic		**¥ 0.26**		n = 1-3	**¥ 0.2**	
				n > 3	**¥ 0.4**	
AOM			120			169
AOM + Oligofruct			NS	n = 1	**¥ 0.7**	
				n = 2-3	NS	
				n > 3	NS	
AOM						233
Low-fat diet		133				
High fat diet		138		n = 1-3	118	
AOM + Inulin HP						
Low-fat diet		NS				
High fat diet		**¥ 0.52**		n = 1-3	**¥ 0.46**	

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Table 12.8 (continued)
Summary Presentation of the Results of the Studies Performed to Test for the Effects of Inulin-type Fructans on Experimental Colon Carcinogenesis

Treatment	Aberrant Crypt FOCI (ACF)					Ref
	Incidence	Yield (number)		Multiple AC/FOCI		
		Total	Per colon	n/FOCI		
			Distal colon:			227
AOM		162	118	n = 1-2	34	
				n > 2	129	
AOM + inulin HP						
2.5%		**¥ 0.75**	**¥ 0.77**	n = 1-2	**¥ 0.53**	
				n > 2	NS	
5%		**¥ 0.49**	**¥ 0.50**	n = 1-2	**¥ 0.44**	
				n > 2	**¥ 0.49**	
10%		**¥ 0.35**	**¥ 0.36**	n = 1-2	**¥ 0.50**	
				n > 2	**¥ 0.32**	
AOM	90%	4.25[2]				234
AOM + Inulin HP						
I	**¥ 0.81**	**¥ 0.73**				
P	**¥ 0.77**	**¥ 0.32**				
I + P	**¥ 0.56**	**¥ 0.28**				
	Mid colon:		Mid colon:			184
DMH	71%	8.2	2.7			
DMH + Oligofructose	**¥ 0.625**	NS	NS			
DMH + Inulin HP	**¥ 0.56**	**¥ 0.56**	**¥ 0.44**			
	Distal colon:		Distal colon:			
DMH	74%		4.7			
DMH + Oligofructose	**¥ 0.71**		**¥ 0.57**			
DMH + Inulin HP	**¥ 0.70**		**¥ 0.625**			

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Table 12.8 (continued)
Summary Presentation of the Results of the Studies Performed to Test for the Effects of Inulin-type Fructans on Experimental Colon Carcinogenesis

Treatment	Aberrant Crypt FOCI (ACF)					Ref
	Incidence	Yield (number)		Multiple AC/FOCI		
		Total	Per colon	n/FOCI		
Week 5						235
DMH			152	n = 1-3	149	
DMH + Oligofructose						
5%			NS		NS	
15%			NS		NS	
15% + pretreatment			**¥ 0.72**		**¥ 0.70**	
DMH + Inulin HP						
5%			**¥ 0.60**		**¥ 0.59**	
15%			**¥ 0.51**		**¥ 050**	
15% + pretreatment			**¥ 0.72**			
Week 10						
DMH			144		113	
DMH + Oligofructose						
5%			NS		NS	
15%			NS		NS	
15% + pretreatment			**¥ 0.82**		**¥ 0.64**	
DMH + Inulin HP						
5%			**¥ 0.81**		**¥ 0.76**	
15%			**¥ 0.59**		**¥ 0.58**	
15% + pretreatment			**¥ 0.70**		**¥ 0.64**	
	ACF					
AOM	63%		48.2			236
AOM + Synergy 1	**¥ 0.91**		**¥ 0.55**			
AOM + Synbiotic	**¥ 0.82**		**¥ 0.55**			
	CANCER					
AOM	43%		15.5			
AOM + Synergy 1	**¥ 0.40**		**¥ 0.47**			
AOM + Synbiotic	**¥ 0.16**		**¥ 0.16**			

[1] = The figures in bold indicate the fold decrease in the parameter

[2] = Yield as number of tumors per rat bearing tumor(s)

So far, only one experiment tested the hypothesis that, like inulin HP, the mixture of oligofructose + inulin HP known as oligofructose-enriched inulin Synergy 1 would reduce the incidence and/or, yield of ACF tumors and/or cancers in chemically induced rat colon carcinogenesis. Data demonstrate this is indeed the case because after 31 weeks of Synergy 1 feeding, the incidence of ACF and cancers is reduced by 9 and 60%, respectively, whereas the yield is reduced by 45 and 53%, respectively.[235] The most important site of the cancer inhibitory action of inulin HP and oligofructose-enriched inulin Synergy 1 is the distal part of the colon, where also most of the ACF, the tumors, and the cancers are known to develop.

When tested, the synbiotic combination of inulin HP with a probiotic (bifidobacteria and/or lactobacillus) is also active in reducing the incidence and the yield of pre- and neoplastic colonic lesions, the synbiotic being, in most cases, more active than the prebiotic but in all cases much more active than the probiotic alone.[169,232,235] In one experiment, the synbiotic preparation was particularly efficient in reducing the risk of colon cancer, incidence and yield of adenocarcinomas being reduced by 84%.[235]

In conclusion, inulin HP and oligofructose-enriched inulin Synergy 1 but not oligofructose have the capacity to suppress chemically induced colon carcinogenesis both in mice and rats, and such an effect is likely to be potentiated in synbiotic preparations with lactic acid bacteria. It has been hypothesized that inulin HP and oligofructose-enriched inulin Synergy 1 are more active than oligofructose because the long-chain molecules are likely to be more slowly fermented in the large bowel, thus prolonging their effects in the transverse and distal colon.[227,234] Indeed, the small oligomers of oligofructose, and especially the short-chain fructooligosaccharides, are rapidly and quantitatively fermented already in the proximal colon and thus never arrive in the distal colon.

Inulin HP and oligofructose-enriched inulin Synergy 1 act mostly during the promotion phase of the carcinogenic process, and their effect is on the incidence and the yield, as well on the multiplicity of ACF, tumors, and even cancers. Not only do they reduce the number and the size of lesions but also they reduce the risk of progression of these lesions towards malignancy. They thus classify as negative modulators of the carcinogenic process. Negative modulation of carcinogenesis has been defined as "any procedure or experimental condition that, while not itself a prerequisite for cancer, is able to shorten the latent period, decreases the incidence and/or yield of cancers, and/or modifies the histological and the invasive nature of cancers."[236] When introducing that concept, we proposed that negative modulation of carcinogenesis may result from changes in metabolic and/or proliferative homeostasis, within the organ in which a neoplastic development had otherwise been initiated, that would create conditions that hinder the appearance of cancer as evidenced by a reduction in incidence and/or yield as well as invasiveness. Data so far available for inulin HP and oligofructose-enriched inulin Synergy 1 are well in line with that concept. Moreover, the mechanisms proposed to explain these beneficial effects include changes in the composition and/or activity of colonic microflora (the prebiotic effect), and in the composition of the SCFAs pool and especially an increased relative proportion of butyrate as a result of their anaerobic fermentation. These effects fit well with the concept of changes in

metabolic and proliferative homeostasis in the large bowel and especially the colonic mucosa. In addition, inulin-type fructans strengthen and stimulate the function of gastrointestinal defense and, especially, the intestinal immunity, two effects that certainly improve resistance to cancer development. They have been shown to reduce proliferative activity in crypts and they down-regulate the expression of a series of enzymes possibly involved in colon carcinogenesis,[235] i.e., placental pi type of glutathione S-transferase (GST-P) that is induced by K-Ras mutation during colon carcinogenesis and is associated with poor prognosis in cancer patients; cyclooxygenase-2 (COX-2); and inducible nitric oxide synthase (iNOS), the up-regulated expression of which is associated to resistance to apoptosis, DNA damage, increased proliferation, and metastatic potential.[237,238]

The effect of inulin-type fructans on the risk of intestinal cancer has also been tested in the C57BL/6J Min^+ mice that are heterozygous for a nonsense mutation of the APC gene that is likely to play a role in the early stages of colon carcinogenesis.[239] In Min^+ mice, intestinal tumors develop spontaneously but they are almost exclusively (more than 95%) located in the small intestine being extremely rare in the large bowel.[241] In that model, oligofructose (5.8% w/w in diet starting at day 42 of age), had no effect on the incidence (100%) nor on the total yield (per mouse with tumor) of tumors nor on the incidence and yield of tumors in the small intestine, but it significantly reduced the incidence and the yield of total (60% vs. 100% and 1 vs. 2) and small (diameter <1 mm) (20% vs. 80% and 1 vs. 1.4) but not large (diameter >1 mm) tumors in the colon.[161] Similarly, oligofructose did not affect the total incidence and yield of tumors nor the incidence and yield in the small intestine or the large bowel, but it significantly reduced the overall volume, as well as the volume of the tumors in the small (especially in the ileum) but not in the large intestine. Moreover, when mice were fed a Western-type diet rich in fats that significantly increased tumor yield in the ileum and the large bowel as well as the tumor volume (mm^3 per mouse) in the colon, oligofructose feeding prevented the increase in tumor yield only in the ileum and the increase in tumor volume both in the small and the large intestine, reducing these parameters to values that, in the small intestine, were lower than in mice fed the standard diet (Lipkin, personal communication). In a similar experimental model, i.e., C57BL/6J Min^+ mice fed a high-fat/low-fiber diet, standard inulin (i.e., an inulin with an average DP of 10 as compared to 20 for the long-chain inulin) fed at two doses levels (2.5 and 10% w/w in diet) from week 6 till week 17 of age did not significantly modify tumor incidence, size, or yield in the small intestine and in the large bowel.[241,242] A serious problem with these last experiments is that the reference diet is fiber-free and contains dextrose (a carbohydrate that is completely absorbed in the upper part of the small intestine) as the sole source of carbohydrate, as well an amount of fat that is unusually high for a rodent. This means that the gastrointestinal (and probably also the systemic) physiology of these mice is severely compromised, especially due to the high glycemic/high fat diet but also, and most importantly, to the lack of substrates for colonic microflora fermentation. The authors have not investigated the physiology nor do they know what consequences the introduction of an easily fermentable substrate such as inulin might have in these mice. For example, it has been reported that the protective effect of probiotics against cancer development is seen when the

diet contained at least 30% of a good carbohydrate substrate (i.e., nondigestible) for colonic fermentation.[243,244] Moreover, a diet containing high sucrose concentration (up to 40%) as readily digestible carbohydrate source has been shown to induce mutations in rat colon [245] and to increase the number of ACF in rats treated with chemical carcinogens.[246–249] Moreover, high intake of simple sugars affects colonic functions.[250] For all these reasons and besides the data concerning the effects of inulin on various markers of cell proliferation and cell differentiation, the speculations made by the authors of these last Min mice experiments about inulin being a cancer promoter appear irrelevant to human situation. As already discussed above (see Section 12.4.1.2), feeding mice a human-type diet does not make the rodent a human and certainly not if that severely unbalanced diet is likely to create physiopathological conditions that preclude any relevant conclusion. When used to help formulate hypotheses relevant to humans (the only reason for doing experimental research in functional food science), experimental animals must be maintained in or as close as possible to their normal physiological conditions.

12.5 INULIN-TYPE FRUCTANS AND SYSTEMIC DEFENSE FUNCTIONS

Besides their effect (both direct and indirect) on the risk of diseases related to dysfunction of the gastrointestinal defense, inulin-type fructans have also been shown to possibly contribute to reducing the risk of diseases related to dysfunction of systemic defense. Essentially, this has been shown using experimental models of:

1. Systemic infection
2. Chemically induced mammary carcinogenesis
3. Growth of implanted tumor
4. Metastasis
5. Cancer therapy

12.5.1 Effect of Inulin-Type Fructans on Risk of Systemic Infection

In a recent study, Buddington et al. have demonstrated a strong protection against a systemic (intraperitoneal) challenge by *Listeria monocytogenes* (1–5 ¥ 10^6 cfu/mouse) and *Salmonella typhimurium* (10^3 cfu/mouse) in mice (B6C3F1, n = 25) prefed with either oligofructose or inulin HP (10% w/w in diet) for 6 weeks.[137] Indeed, 2 weeks after the challenge, survival in the *L. monocytogenes* and *S. typhimurium* infected mice were 72% and 16%; 88% and 24%; 100% and 40% in the groups receiving the standard, the oligofructose (NS and +16%) or the inulin (+24% and +28%) diet, respectively. The protective effects of inulin and to a lesser extent oligofructose was correlated with a higher density of lactic acid bacteria in the intestine, an increased phagocytic activity of unactivated peritoneal macrophages, a heightened sensitivity of lymphocytes, and enhanced natural killer cell activity of splenocytes.

12.5.2 Effect of Inulin-Type Fructans on Risk of Chemically Induced Mammary Carcinogenesis

Only one preliminary experiment has been reported on the effect of oligofructose on chemically induced mammary carcinogenesis in rats. In that experiment, young (45 days of age) female Sprague-Dawley rats (n = 8) were injected s.c. with 50 mg/kg bw. of *N*-methylnitrosourea. One week later they were fed a standard diet either without or with oligofructose (15% w/w).[251] From week 4 after the injection of the carcinogen until week 26, number and position of mammary tumors were manually assessed and their volume was evaluated weekly by measuring three perpendicular dimensions. At week 27 rats were sacrificed and a detailed autopsy was performed with tumor counting, measuring, and histological examination. All along the observation period (week 4 to week 26) data show that the incidence of tumors as expressed by the number of rats bearing tumors was always lower in the oligofructose-treated than in the control rats (e.g., 25% vs. 50% and 62.5% vs. 90% at weeks 12 and 24, respectively). Similarly, the total yield of tumors (number of tumors per group) in the oligofructose fed group was lower than in the control group (e.g., 2 vs. 7 and 9 vs. 16 at weeks 12 and 24, respectively). As revealed by histological examination, all mammary tumors were adenocarcinomas and, in the rats fed the control but not the oligofructose diet, two renal fibrosarcomas and two metastases (one in the lung, one in lymphatic node) were detected. Oligofructose feeding had, however, no effect on mean mammary tumor volume. These data are consistent with the hypothesis that oligofructose has the capacity to negatively modulate the carcinogenic process by slowing down the kinetics of appearance of malignant tumors and by reducing the risk of metastasis. But these data need to be confirmed using a larger number of animals group and comparing oligofructose with long-chain inulin and/or oligofructose-enriched inulin Synergy 1.

12.5.3 Effect of Inulin-Type Fructans on Growth of Implanted Tumors

As indirect evidence for enhanced systemic defense functions, experimental studies have also been designed to test for the possible effect of dietary inulin and oligofructose on the growth of transplantable mouse tumors. Essentially three different tumor cell lines have been used, i.e., the mammary tumor cell line EMT6,[252] the transplantable liver tumor cell line TLT[253,254] and the B16F10 tumor cell line. In the first two protocols, the diets containing oligofructose or inulin (15% w/w) were introduced 7 d before an intramuscular transplantation of either EMT6 tumor cells into balb/e female mice or TLT tumor cells into young male NMRI mice. Two perpendicular tumor dimensions were measured weekly and the increase in the mean tumor surface was calculated for each time period for the different groups of control and treated animals. These experiments demonstrated that the *in vivo* the growth of both tumor cell lines was significantly (ANOVA $p < 0.01$) and similarly inhibited by both oligofructose and inulin that had basically the same effect (see Figure 12.3).[251,255]

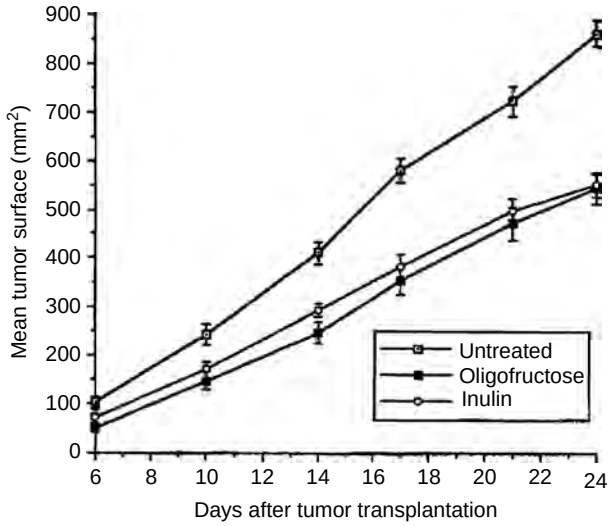

FIGURE 12.3 Effect ($p < .001$) of inulin-type fructans (15% w/w in diet) on the growth of a solid tumor after the intramuscular injection of TLT tumor cells in mice. (Each curve is the mean of 3 different experiments with a total of 30–36 mice.)

These results were confirmed in another series of experiments in which two different forms of TLT tumors, i.e., a solid and ascitic form, were transplanted intramuscularly and intraperitoneally, respectively. The growth of the solid intramuscular tumors was evaluated by measuring tumor dimensions as a function of time, whereas, for the development of the ascitic form, mortality rate was recorded and used as an indirect assessment. To evaluate the effects of the treatments, tumor growth and survival were compared with those in the control groups of mice fed the standard diet. Data show that supplementing the diet with oligofructose or inulin, similarly and highly significantly (Logrank test $p < 0.001$), inhibits (by 50%) the growth of the intramuscularly implanted tumor and prolongs the survival of the intraperitoneally implanted mice.[256] In further experiments (unpublished, Taper personal communication) aimed at comparing the effect of different doses of inulin and oligofructose on the growth of intramuscularly implanted TLT tumor cells, it was shown that the minimum effective does was between 5 and 10% (w/w in diet) for both inulin-type fructans. In the same series of experiments, oligofructose-enriched inulin Synergy 1 was also shown to inhibit the growth of intramuscularly implanted TLT tumor cells. Moreover, the Synergy 1 mixture appeared to be more potent than inulin and oligofructose fed alone. Indeed, in mice fed the diet containing 2.5% and 5% Synergy 1, the volume of the tumor at the end of the experiment (day 28) was still 16% and 30% lower than in the control group, whereas, at these two low doses neither oligofructose nor inulin had any inhibitory effect. In contrast to these data demonstrating an inhibitory effect of inulin-type fructans on the growth of implanted tumors, Buddington et al. have reported no effect of oligofructose or inulin (10% w/w in diet) on the incidence and yield of lung tumors in B16F10-challenged B6C3FI mice.[137]

12.5.4 Effect of Inulin-Type Fructans on Metastasis

In the experiments of chemically induced mammary carcinogenesis reported above (see Section 12.5.2), metastasis occurred only in the group of rats fed the standard diet but not in the rats then fed oligofructose-containing diet. These data are, however, preliminary, involving only eight rats/group, and the incidence of metastasis is very low. To further investigate the potential of inulin-type fructans to reduce the risk of metastasis, an experimental model was developed that involved the intramuscular transplantation of viable TLT cells that were transplanted into young male C3H mice. Dietary treatment with oligofructose (15% w/w) or inulin (15% w/w) was started 4 weeks before tumor transplantation and was continued until the end of the experiment. Sacrifice of the animals was performed at the time when mortality in the control animals bearing tumor appeared, i.e., ±47 d after tumor transplantation. Each sacrificed animal was then submitted to a detailed general autopsy followed by a histological examination of the metastases bearing other organs and tissues. The metastases were identified and counted and their mean diameter was measured. In all mice examined, metastases were detected only in lungs and the results show a distinct inhibition of their development in the animals fed oligofructose and inulin. Indeed, the percentage of mice with lung metastases was 59 in the control group, and 36% in the groups fed inulin or oligofructose. The total number of lung metastases was 37 in the control group, 18 in the inulin, and 16 in the oligofructose fed mice. In the majority of the cases the yield was one lung metastasis per mouse bearing metastasis, but multiple and large metastases were observed only in the control group. There were no metastases in other organs and tissues. In conclusion, dietary oligofructose and inulin significantly ($p \leq 0.05$) inhibit the development of lung metastases of a malignant, intramuscularly transplanted mouse tumor.[257]

12.5.5 Inulin-Type Fructans and the Potentiation of Cancer Therapy

Cancer treatment is a complex process that involves inhibition of tumor growth, immune activity, selective cancer-cell killing, prevention of metastasis, and resistance of healthy cells and tissues. Since inulin-type fructans have the potential to inhibit growth and to prevent metastasis of transplantable tumors, it appeared interesting to investigate whether the same treatment could positively affect the efficacy of cancer chemotherapy. To test that hypothesis, NMRI mice bearing an ascitic form of the TLT tumor were fed a diet supplemented with oligofructose or inulin (15% w/w), starting 7 d before the intraperitoneal transplantation of TLT tumor cells and continuing until the end of experiments. A single and subtherapeutic dose of six different cytotoxic drugs, commonly utilized in human cancer treatment, was then injected intraperitoneally 48 h after tumor transplantation. The effect of the inulin-type fructans on the efficacy of therapy was evaluated by calculating the increase of life span (ILS).[258] In all the experiments, both oligofructose and inulin had no direct significant effect on survival, but they considerably amplified the effects of cancer chemotherapy when compared with the untreated control groups. Indeed in 7 out of a total of 12 experiments, the increase in the efficacy of cancer therapy was

synergistically increased, and the majority of the effects are very highly statistically significant (Table 12.9). Obviously, the intensity of this adjuvant therapeutic effect was somewhat different for the various drugs. There was not any distinct difference between the effects induced by oligofructose or inulin.

Besides chemotherapy, radiotherapy is the main, if not in some cases the only, treatment of human cancer if it cannot be surgically operated. Unfortunately, many tumors are radio-resistant and the available radio-sensitizers are often very toxic or simply cannot be applied, e.g., in multiple or metastatic tumors. As a follow up of the experiments showing a possible potentiation of cancer chemotherapy by inulin-type fructans, the hypothesis has been proposed that these food ingredients might also improve radiotherapy. To test that hypothesis, the same basic protocol was applied and when tumors reached a volume of approximately 1000 mm^3, they were locally irradiated with a single X-ray dose of 10 Gy. To assess the efficacy of the

TABLE 12.9
Adjuvant Effects of Inulin-Type Fructans on the Chemotherapeutic Activity of Various Cytotoxic Drugs in Ascitic TLT[1] Bearing NMRI Mice, as Evaluated by the Changes in ILS[2]

Chemotherapy	Dietary Treatment	ILS D (%)	Type of Effect	*p* Value
5-Fluorouracil	Oligofructose	+21.9	Synergistic	<0.001
(ILS = 18.75%)[3]		+15.7	Synergistic	<0.001
	Inulin	+12.5	Additive	<0.001
Doxorubicine	Oligofructose	+2.9	Additive	<0.01
(ILS = 14.7%)				
Vincritine	Oligofructose	+13.4	Additive	<0.001
(ILS = 33.3%)	Inulin	+10	Additive	<0.01
Cyclophosphamide	Oligofructose	+33	Synergistic	<0.01
(ILS = 11%)	Inulin	+36	Synergistic	<0.001
Methotrexate	Oligofructose	+27	Synergistic	<0.001
(ILS = 2%)	Inulin	+18	Synergistic	<0.01
Cytarabine	Oligofructose	+12.1	Additive	<0.01
(ILS = 3%)	Inulin	+24.2	Synergistic	<0.001

[1] TLT = transplantable liver tumor cell line.[254,255]

[2] NMRI mice bearing an ascitic form of the TLT tumor were fed a diet supplemented with oligofructose or inulin (15% w/w), starting 7 d before the intraperitoneal transplantation of TLT tumor cells and continuing until the end of experiments. A single and subtherapeutic dose of six different cytotoxic drugs, commonly utilized in human cancer treatment, was then injected intraperitoneally 48 h after tumor transplantation. The effect of the inulin-type fructans on the efficacy of therapy was evaluated by calculating the increase of life span (ILS).[259]

[3] Values in () are the ILS due to the chemotherapeutic treatment of mice fed the control diet that is not supplemented with inulin or oligofructose.

Source: Adapted from Van Loo, J., Jonkers, N., Evaluation in human volunteers of then potential anticarcinogenic activities of novel nutritional concepts: prebiotics, probiotics, and synbiotics (the SYNCAN project QLK1-1999-00346), *Nutr. Metab. Cardiovasc. Dis.*, 11 (suppl. 4), 87–93, 2001.

irradiation, tumor dimensions were measured twice weekly and the mean tumor volume was calculated in each experimental group. The results show that dietary pretreatment with oligofructose or inulin (15%) increased the therapeutic efficacy of cancer radiotherapy, and this effect appeared to be similar for oligofructose and inulin. The difference in tumor growth between the inulin-type fructans-treated and the control mice was highly statistically significant ($p < 0.0001$).

In both the chemotherapeutic and radio therapeutic experiments, the treatment with inulin or oligofructose did not produce any adverse functional or morphological effects during either the observation or at autopsy of the animals including the histological examination of the main organs.[259] Based on these results, it might be hypothesized that introduction of such an dietary adjuvant cancer chemo- and radiotherapy into human clinics could help improve the efficacy of cancer treatment without any additional risk or side effects for the patients.[257,260]

12.6 INULIN-TYPE FRUCTANS AND DEFENSE FUNCTIONS: OVERVIEW, DISCUSSION, AND PERSPECTIVES

As discussed in the introduction of this chapter, the defense functions of the body are multiple, involving different organs, different mechanisms, and targeting different potential aggressors. The body is thus well protected, and in a healthy individual these multiple defense functions should exert an efficient protection. However, genetic predispositions, aging, stress, as well as lack of sufficient physical activity and unbalanced diet are all factors that are likely to weaken these functions and consequently create conditions for increased sensitivity to external aggressions both chemical and biological. It is one of the main objectives of functional food science to identify food components that have the capacity to positively modulate defense functions so as to help individuals strengthen, restore, or rebalance these functions. This chapter has shown that inulin-type fructans are classified among the potential functional food ingredients capable of playing such roles. As reviewed in the previous chapters, inulin-type fructans beneficially affect a series of gastrointestinal functions by modulating both the structure and composition as well as miscellaneous activities of the mucosa and the microflora. They also affect intestinal epithelia by improving mucosal morphology, thickening, and improving the composition of the mucins. As a consequence, they improve colonization resistance and prevent bacterial translocation (at least when tested in a appropriate model), and finally they contribute to improving both chemical and enzymatic safeguard functions in the gastrointestinal tract.

Inulin-type fructans also beneficially affect the immune system, especially the intestinal immune functions, by targeting the GALT and especially the Peyer's patches, and consequently they have been shown to reduce the risk of diseases related to dysfunction of the gastrointestinal defense functions, an indirect but strong evidence for a beneficial effect.

In particular, data strongly support the hypothesis that inulin-type fructans have the capacity to reduce the risk of inflammation associated with IBD, and that they

act *via* their prebiotic effect to rebalance the intestinal microflora, thus preventing the overgrowth of commensal, potentially pathogenic, microorganisms such as bacteroides or clostridium that may induce inflammatory responses.

Regarding the risk of colon cancer, inulin HP and oligofructose-enriched inulin Synergy 1 but, probably not oligofructose, have the capacity to suppress chemically induced colon carcinogenesis both in mice and rats, and such an effect is likely to be potentiated in synbiotic preparations with lactic acid bacteria. It has been hypothesized that inulin HP and oligofructose-enriched inulin Synergy 1 are more active than oligofructose because the long-chain molecules are likely to be more slowly fermented in the large bowel, thus prolonging their effects in the transverse and distal colon.[227,234] Indeed, the small oligomers of oligofructose, especially the short-chain fructooligosaccharides, are rapidly and quantitatively fermented already in the proximal colon and thus never arrive in the distal colon. Inulin HP and oligofructose-enriched inulin Synergy 1 act mostly during the promotion phase of the carcinogenic process, and their effect is on the incidence and the yield, as well on the multiplicity of ACF tumors and even cancers. Not only do they reduce the number and the size of lesions, but they also reduce the risk of progression of these lesions towards malignancy. They are classified as negative modulators of the carcinogenic process. The mechanisms proposed to explain these beneficial effects include changes in the composition and/or activity of colonic microflora (the prebiotic effect), and in the composition of the SCFAs pool, and especially an increased relative proportion of butyrate as a result of their anaerobic fermentation. These effects fit well with the concept of changes in metabolic and proliferative homeostasis in the large bowel and especially the colonic mucosa. In addition, inulin-type fructans strengthen and stimulate gastrointestinal defense functions and especially the intestinal immunity, two effects that certainly improve resistance to cancer development. Besides their effect (both direct and indirect) on the risk of diseases related to dysfunction of the gastrointestinal defense functions, inulin-type fructans have also been shown to possibly contribute to reducing the risk of diseases related to dysfunction of the systemic defense functions. Essentially, this has been shown using experimental models of systemic infection, chemically induced mammary carcinogenesis, growth and metastasis of implanted tumor, and cancer therapy.

The data available today are almost exclusively experimental data, and there is an urgent need for generating human data. As already stressed many times in this book, the experimental data are essential to help design a hypothesis-based human nutrition intervention trial. Regarding inulin-type fructans and body's defenses, many such hypotheses are available that are already supported by sound experimental data. The problem with the defense functions is that we still miss, in many cases, relevant and validated direct biomarkers of these functions that furthermore do not require invasive methods of clinical investigations. Today, indirect assessment of the state of well-being and health is thus, probably, the method of choice.

An example of such an approach is the SYNCAN project in which inulin-type fructans and especially oligofructose-enriched inulin Synergy 1 has been selected to be tested in a multicenter human intervention nutrition trial that was funded by the European Union (EU project QLK1-1999-00346 DG XII Research, EU)). In that trial, cancer patients (Dukes B and C) who are at high risk for developing colon

cancer are given a synbiotic preparation composed of oligofructose-enriched inulin Synergy 1 (10 g/d) + *Lactobacillus rhamnosus* GG (10^{10} cfu/d) and *Bifidobacterium bifidum* Bb12 (10^{10} cfu/d). To test for the efficacy of the synbiotic in reducing the risk of colon cancer, a series of markers is monitored in feces, blood, and rectal biopsies including immunological (GALT and systemic), microbiological (fecal flora composition), mucosal (DNA damage and repair), and fecal (water cytotoxicity, genotoxicity, and enzyme activities) parameters. By the end of 2003, the human intervention study was completed and data are expected to be available during year 2004.[261]

Other examples are the double-masked, randomized controlled studies specifically designed to assess several clinical parameters related to common acute pediatric illnesses or the immunological response to measles vaccine in infants and toddlers, supplemented with oligofructose (see Table 12.10).[262–265] That study involved 123 healthy youngsters, between the ages of 4 and 24 months who were already consuming cereal prior to enrollment and were all attending daycare in a large metropolitan area. The children were randomized to receive a commercially available infant cereal alone or the same cereal supplemented with oligofructose at a concentration of 0.55 g per 15 g of dry cereal. Once enrolled, children remained in the study as long as they continued to consume cereal. Data were obtained through a

TABLE 12.10
Summary of Results of a Double-Masked, Randomized Controlled Study Designed[1] to Assess the Effect of Oligofructose on Several Clinical Parameters Related to Common Acute Pediatric Illnesses

Clinical Paramters	Control Group	Oligofructose-Fed Group	*p* Values
Diarrhea + fever[2]	21.4	8.25 (¥[3]0.39)	<0.001
Diarrhea + medical examination	24.3	16.1 (¥0.66)	<0.001
Vomiting	4.5	2.9 (¥0.64)	=0.02
Discomfort	6.7	4 (¥0.59)	<0.001
Regurgitation	4.8	2.1 (¥0.44)	<0.001
Fever + cold symptoms	17.2	11.4 (¥0.66)	<0.001
Antibiotic use	12.2	8.2 (¥0.67)	<0.001

[1] Two groups of children (4–24 months of age) receiving either a control cereal (n = 60) or a cereal supplemented with oligofructose (3.3% w/w) (n = 63) for 6 months. Average intake of oligofructose was 3.3 and 3.45 g/kg bw for control and oligofructose-fed children, respectively.

[2] Frequency of occurrence over study period.

[3] ¥ = times.

Source: Adapted from Tschernia, A., Moore, N., Abi-Hanna, A., Yolken, R. H., Coletta, F., Emenheiser, C., Effects of long term consumption of a weaning food supplemented with oligofructose, a prebiotic, *J. Paedtr. Gastroenterol. Nutr.*, 29, A58, 1999; Firmansyah, A., Pramita, G., Carrie Fassler, A., Haschke, F., Link-Amster, H., Improved humoral immune response to measles vaccine in infants receiving infant cereal with fructooligosaccharides, *J. Paedtr. Gastroenterol Nutr.*, 31, A521, 2001.

weekly daycare visit and a weekly phone call with a parent or guardian, using a standardized questionnaire.

Both groups showed similar cereal consumption that corresponded to a daily average intake of 1.1 g oligofructose; all subjects exhibited normal growth during the study, and the cereal was well tolerated in both groups.

No significant differences were observed when comparing the occurrence of perceived flatulence, and stool frequency and consistency but a significantly lower frequency of reported emesis, regurgitation (spitting up), and perceived discomfort with bowel movements was noted in the supplemented group.

However, highly significant reductions were noted in the oligofructose-supplemented group when comparing the groups for:

- Occurrence of concurrent fever as an indicator of severity during episodes of diarrhea
- Medical attention-seeking during such an event
- Daycare absenteeism rate during reported diarrhea
- Occurrence of fever with cold symptoms
- Use of antibiotics during respiratory illness

This first study on the clinical benefits of oligofructose supplementation demonstrates an interesting impact on the occurrence of febrile illness, either associated with diarrhea or upper respiratory illness; both are usually of viral etiology in the pediatric population. The question of potential immunomodulatory activity through this novel nutritional prebiotic strategy is raised.

The study by Firmansyah et al. adds further weight to this observation.[265] The authors studied the effect of a cereal-weaning food, supplemented with oligofructose on the immunological response to measles vaccine. In this double-masked, placebo-controlled trial, 50 healthy infants aged 7 to 9 months of age were randomized to receive a standard cereal or one supplemented with a mixture of oligofructose and inulin, added at a concentration of 1 g per 25 g of dry weight cereal for 10 weeks (mean intake of cereal was 5 g/kg bw/d and mean intake of inulin-type fructans was 0.2 g/kg bw/d). After 4 weeks of participation, the children were immunized using a standard live attenuated measles vaccination. Statistical analysis did not reveal any differences between the groups at baseline, and both cereals were well tolerated. Importantly, there was no difference on growth parameters between groups during the entire study. However, a significant difference was noted when comparing anti-measles IgG; initial levels were low and similar between groups at baseline, but a significant difference postimmunization was clearly detected above baseline (supplemented group: 6.6-fold increase vs. control: 4.2-fold increase, $p < 0.03$). Anti-measles Ig M levels showed no inter- or intragroup significant differences throughout the study. The positivity rates for children with adequate IgG antibody response were 96% and 88% in the supplemented and control group, respectively ($p < 0.01$). Mild reactions in the period post vaccination were observed more frequently in the supplemented group. Although this study does not provide any further insight into the mechanism of the observed clinical benefit, a targeted nutritional strategy using

prebiotics during childhood immunization certainly deserves further attention and investigation.

In terms of perspectives, the future of research is thus clearly in designing human nutrition intervention trials.

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13 General Discussion, Perspectives, and Conclusions

13.1 INTRODUCTION

Over the last two decades, inulin-type fructans have become a topic of much interest for both the food industry and for researchers. A series of reviews and book chapters have already been published, and four specific international congresses plus a series of symposia have been organized to discuss the chemistry, technological properties, physiological effects, and nutritional benefits of these natural ingredients individually or as part of the prebiotic concept.[1–16] At the end of this book in which all data available (until early 2004) on the nutritional properties of inulin-type fructans have been reviewed and evaluated, the general discussion will concentrate on three aspects:

1. Inulin-type fructans and the functional food concept
2. Inulin-type fructans: health and well-being
3. Inulin-type fructans and specific food applications

13.2 GENERAL DISCUSSION

Inulin-type fructans are now well characterized mixtures of linear oligo- and polymers composed primarily of fructose with b-(1➞2) osidic linkages. Inulin-type fructans are available as (see Chapter 3, Section 3.3):

- Oligofructose, a mixture of oligomers (DP 2–7; DP_{av} 4)
- Inulin as such or native (standard) inulin as it is extracted from chicory root (DP 2–65; DP_{av} 12)
- Inulin HP or long chain, high performance inulin (DP 10–65; DP_{av} 25)
- Oligofructose-enriched inulin Synergy 1 that combines oligofructose and inulin HP

A validated methodology exists to analyze and quantify inulin-type fructans in food products, and it is possible to qualitatively analyze the composition of the different mixtures by using specific chromatographic methods (see Chapter 3, Section 3.3.5).

Inulin-type fructans are found naturally in a wide variety of edible plants, mainly in grains, fruits, and roots (see Chapter 3, Section 3.2.3). Because of their technological and nutritional attributes, they are added to miscellaneous manufactured food products (see Chapter 3, Section 3.3.4) or offered to consumers as food supplements.

13.2.1 Inulin-Type Fructans and the Functional Food Concept

As discussed in Chapter 1, what makes a food "functional" is the scientific demonstration that it beneficially affects functions in the body beyond what could be expected from basic nutrition. Thus, the questions to be discussed here concern the beneficial effects of inulin-type fructans. By reference to the strategy for functional food development (see Figure 1.4), the discussion will concentrate on:

- Basic scientific knowledge and experimental data to identify potential functional effects as well as to formulate hypotheses to be tested in human nutrition intervention studies
- Results of human nutrition intervention trials to substantiate claims (mainly type A but also a few type B)

As extensively discussed in different chapters (see Chapter 4 to Chapter 7 and Chapter 9 to Chapter 12), the effects of inulin-type fructans have been investigated in different domains of interest, using a wide variety of experimental models and human trials. These domains are classified in one of the following categories:

Category 1: Experimental results have been evaluated and used to justify human intervention studies; confirmatory data of these trials are available to substantiate claims. The domains included in this category are dietary fiber and bowel functions, intestinal microflora, gastrointestinal absorption of minerals, and lipid (triglycerides) metabolism (Table 13.1).

Category 2: Data from different experimental models are convincing, and sound hypotheses exist, but more human nutrition trials are necessary to substantiate claims. The domains included in this category are cholesterol metabolism, intestine associated immune functions, inflammatory bowel diseases, and colon cancer (Table 13.2).

Category 3: Recent experimental investigations have generated promising results that justify more extensive studies including, in some cases, preliminary tests in human volunteers. The domains that are included in this category are bone health, gastrointestinal endocrinology, cancer therapy, and behavior and cognitive performance etc. (Table 13.3).

The claims that are or might become scientifically substantiated are presented in Table 13.4 and include:

1. Inulin-type fructans are fermentable dietary fiber and help improve gut functions especially by improving regularity, increasing stool frequency, and fecal bulking.
2. Inulin-type fructans are bifidogenic and prebiotic.
3. Inulin-type fructans increase calcium absorption.
4. Inulin reduces triglyceridemia in hyperlipidemic individuals.

TABLE 13.1
Experimental and Human Data Which Substantiate Claims on Inulin-Type Fructans: Summary Presentation

Property or Target Functions	Supportive Evidence	Section in Book	Claims for Inulin-Type Fructans
Dietary fiber	Oligo- and polysaccharide: Resistance to digestion Quantitative fermentation Validated analytical method	3.2.2 4.4 and 6.2.4.1 5.4.1 and 6.2.4.3 6.2.2	They are dietary fibers
Calorie value	Nondigestible oligo- and polysaccharides; colonic food	7.3 and 7.4	They are low calorie carbohydrates
Bowel functions Stool production	Bulking effect Regularization of stool production Improved stool consistency	6.2.4.5 6.2.4.5 6.2.4.5	They regularize bowel functions
Colonic microflora	Substrates for anaerobic saccharolytic fermentation Selective stimulation of growth of health-promoting bacteria (e.g., bifidobacteria) Prebiotic	5.4.1.1 9.4.1 and 9.4.2 9.5	They are prebiotic
Gastrointestinal absorption of Ca and Mg	Increased absorption of Ca Animal data Human data: adolescents, adult, postmenopausal women Increased absorption of Mg (animal data, human data) in adult, postmenopausal women	 10.5.1.2 10.5.1.3 10.5.2.1 10.5.2.2	They increase Ca absorption They increase Mg absorption
Lipid homeostasis	Reduction of triglyceridemia Animal data Human data (slightly hypertriglyceridemic) Mechanistic data (reduced hepatic lipogenesis; reduced gene expression)	 11.3.1 11.3.2 11.3.3	They reduce triglyceridemia in slightly hypertriglyceridemic persons

Inulin-type fructans thus classify as functional food ingredients that target not only gastrointestinal functions but also (most likely via their effects on the gut and the intestinal microflora) systemic functions that are known to be closely related to health and well-being.

TABLE 13.2
Data on Inulin-Type Fructans That Support Hypotheses to Be Tested in Human Nutrition and Clinical Intervention Studies: Summary Presentation

Property or Target Functions	Supportive Evidence	Section in Book
Bone health	Improved bone	10.5.3.1
	Bone improved in:	
	mineral content	10.5.3.2
	mineral density	10.5.3.3
	structure	10.5.3.1
Lipid homeostasis	Reduced cholesterolemia	11.3.1 and 11.3.2
Immunostimulation	Improved resistance to common infections in children	12.6 12.6
	Improved response to vaccination	
Colon cancer	Animal data in different experimental models	12.4.2.4
	+ SYNCAN	12.6
Irritable bowel diseases (IBDs)	Attenuation of inflammatory responses	12.4.2.2
	Improved defenses	

TABLE 13.3
Data on Inulin-Type Fructans that Require More Experimental Research to Support Hypotheses to Be Tested in Human Nutrition and Clinical Intervention Studies: Summary Presentation

Property or Target Functions	Supportive Evidence	Section in Book
Gastrointestinal absorption of minerals	Increased absorption of Fe, Cu, and Zn	10.6.1 10.6.2
Lipid homeostasis	Reduction of lipid pool in obese rats	11.3.1.2 11.3.1.3
Defense mechanisms	Improved barrier functions	12.4.1.1
	Improved resistance to intestinal infections	12.4.1.2
Cancer development	Slowing down of tumor growth	12.5.3
	Reduction of risk of metastasis	12.5.4
	Improved efficacy of cancer therapies	12.5.5
Inflammatory bowel diseases	Reduction of risk and improved management of necrotizing enterocolitis in preterm infants	12.4.2.3
Gastrointestinal endocrinology	Stimulation of production of intestinal hormonal peptides (GIP, GLP-1, PYY, and Ghrelin, etc.)	11.3.3

TABLE 13.4
Claims on Inulin-Type Fructans

1. Inulin-type fructans are fermentable dietary fiber and help improve gut functions especially by improving regularity, increasing stool frequency, and fecal bulking
2. Inulin-type fructans are bifidogenic and prebiotic
3. Inulin-type fructans increase calcium and magnesium absorption
4. Inulin reduces triglyceridemia in hyperlipidemic individuals

13.2.2 Inulin-Type Fructans: Health and Well-Being

It is not surprising that these ingredients are attracting interest as potential "feel-good" factors, as shown by the title of the last research conference, "Inulin and Oligofructose as Feel-Good Factors for Health and Well-being" (Paris, France, Cité de Sciences, February 12–13, 2004).

Recently, the World Health Organization (WHO) has defined health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." Thus, to study the impact of nutrition on health cannot be done without due consideration of the concept of well-being. In the occidental-industrialized world the life expectancy is now over 72 years and it is expected that within the next 20 years, more than 50% of the population will be over the age of 65. As such, the well-being aspect of human health is getting more and more relevance. But the aging population is by no means the only target group. Often facing stress as well as increasingly demanding working conditions, the younger generations are also concerned about health and well-being, especially in a stressful society where there is strong economic competition. Moreover, even in infants, nutrition is a key element of well-being through harmonization of physiological developments to guarantee improved quality of life, e.g., the key role of early life nutrition in the balanced development of the immune system (TH_1/TH_2 ratio).

In this context, gastrointestinal functions, especially colonic functions (e.g., control of the colonic environment, regulation of hormone-dependent metabolic processes, modulation of the brain–gut axis, systemic impact of gut fermentation products, and activities of the immune system) deserve special attention. Disturbances of the colon's functions may lead to dysfunction, not only in the gut but also in the whole body. The classical view that the human colon is an organ (or a tube) that absorbs salt and water and provides a mechanism for the orderly disposal of waste products of digestion is no longer appropriate. Obviously, the colon has a major role in digestion (as achieved by the microbial fermentation) through the salvage of energy; it also contributes to absorption of nutrients like minerals and vitamins; it plays a key role in protecting the body against translocation of bacteria; and lastly, it is active as an endocrine (via the gastrointestinal peptides) as well as an immune organ.[17] It is also involved in miscellaneous diseases from acute infections and diarrhea or constipation to chronic diseases like inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), or cancer.[17]

The reason the colon plays such an important physiological role originates in its unique composition that associates pluricellular eukaryotic epithelial tissue and unicellular (mostly prokaryotic) microorganisms, which collaborate in maintaining health. The microflora that symbiotically colonizes the large bowel is a key player in maintaining the colon (and thus the whole body) healthy. But this population of unicellular microorganisms is complex and highly diverse. To better understand the microflora and its symbiosis with the intestine, we hypothesize that, "stimulated" by the complexity of its host's pluricellular tissue, the intestinal microflora has itself developed during the evolution, and continues to develop during the individual's life, as a population of myriads of unicellular microorganisms belonging to hundreds of genera, species, and strains that not only live close together but actively collaborate to reach a (sometimes precarious) balanced activity, which becomes essential not only for health and well-being but simply for life. It can even further be speculated that it is, in fact, two "pluricellular worlds" (one eukaryotic and one mostly prokaryotic) that live together and cooperate in the large bowel. Such a hypothesis would suggest that, like the different eukaryotic cells of a tissue (especially the immune system that is composed of mostly isolated specialized cells that interact and cooperate to neutralize and eliminate antigens), the various genera, species, and strains of microorganisms that colonize the digestive tract are specialized cells that form a complex tissue-like structure in which the different types of "specialized" (but we still have to identify most of these specializations!) cells interact to perform a series of physiological functions. Intestinal health and well-being would then result from interactions within and between these two pluricellular worlds, the interaction between the two worlds being referred to as "crosstalk."[18] The multicellular prokaryotic tissue-like entity would benefit from and, at the same time, provide benefits to the intestinal mucosa (i.e., the whole and complex pluricellular tissue) and vice versa. A major determinant of these interactions would be the composition of these two worlds, especially that of the prokaryotic population that establishes very early in life immediately after birth. It can also be modulated later in life by diet and may become more complex or more fragile as the body ages. Through modulation of the composition of the colonic microbiota, it is possible not only to influence large bowel functions but also to act indirectly on systemic functions and host health and well-being. Inversely, it cannot be denied that systemic dysfunction elsewhere in the body's organs influences the composition of the colonic flora and, as a consequence, the activities and the colonic functions.

By their specific effects, inulin-type fructans have the capacity to improve the composition, activity, and functionality of both the colonic microflora (see Chapter 9) and the intestinal mucosa (see Chapter 12, Section 12.4.1.1 and Section 12.4.1.2), and to optimize the interactions between these two pluricellular tissues and tissue-like structures thus creating the conditions for better intestinal health and well-being (Figure 13.1).

Inulin-type fructans beneficially affect three essential processes in the colon:

1. *Fueling* — because of resistance to digestion and no effect on digestion of nutrients (see Chapter 4, Section 4.3 and Section 4.4 and Chapter 6,

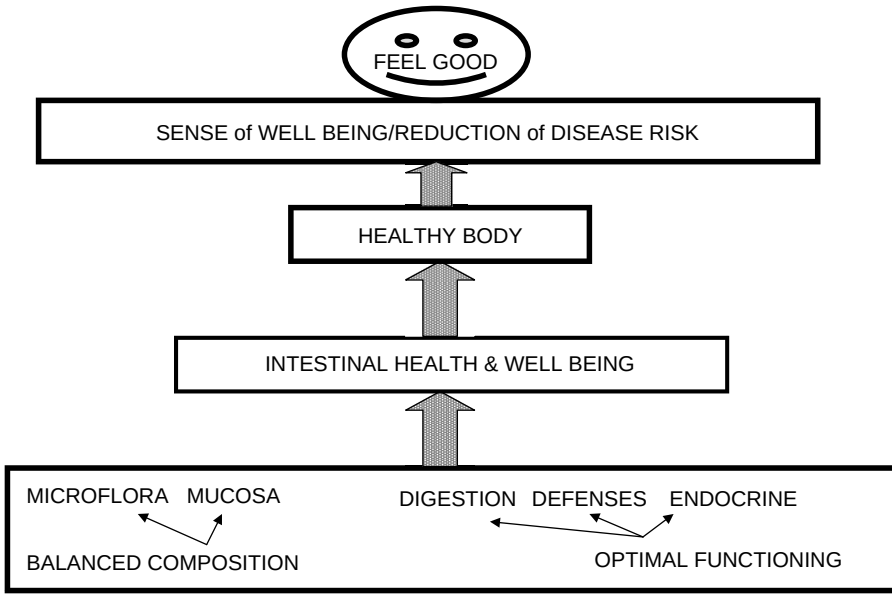

FIGURE 13.1 Intestinal health, a key to overall health.

Section 6.2.4.1 and 6.2.4.2), fermentation in the large bowel (see Chapter 5, Section 5.4 and Chapter 6, Section 6.2.4.3), and improved bowel functions (see Chapter 6, Section 6.2.4.4 and Section 6.2.4.5)

2. *Functioning* — due to activity of the microflora (i.e., more saccharolytic and less proteolytic fermentation or less putrefaction) producing a healthier pool of SCFAs (i.e., more butyrate) and a more acidic environment (see Chapter 5, Section 5.4 and Chapter 6, Section 6.2.4.3); composition and activity of the mucosa (i.e., in terms of cell proliferation, cell differentiation, mucin composition, and immune functions, see Chapter 12, Section 12.4.1); mineral absorption (see Chapter 10, Section 10.5 through Section 10.7); and endocrine activities (see Chapter 11, Section 11.4)
3. *Protecting* — by providing a barrier against pathogens, prevention of inflammation, and suppression of carcinogenesis (see Chapter 12, Section 12.4.2)

Inulin-type fructans are also likely to improve systemic health and well-being by their effects on lipid metabolism, in which they modulate the expression of genes of hepatic lipogenic enzymes (see Chapter 11, Section 11.3 and Section 11.4); on circulating levels of incretins and other gastrointestinal peptides (e.g., PYY, GLP-1, GLP-2, and Ghrelin; see Chapter 11, Section 11.4 and Delzenne's personal communication); systemic infections; systemic immunities; and tumor growth and tumor metastasis (see Chapter 12, Section 12.5).

13.2.3 Inulin-Type Fructans and Specific Food Applications

For all the reasons discussed previously and accumulating scientific evidence, supplementation of miscellaneous food products with inulin-type fructans is becoming a target for the food industry (see Chapter 3, Section 3.3.4 and Table 3.4) . Many different products (around 2500) are already sold worldwide, but still a minority uses claims or advocates nutritional benefits. Two of these applications, however, are almost exclusively developed with the objective to improve health and well-being and require additional comments because they have not been explicitly discussed in the main chapters — these are infant formulas, and feed for domestic animals and pets.

13.2.3.1 Inulin-Type Fructans and Infant Formulas

Oligosaccharides are the third most abundant solid constituent of human milk[19,20] in which these are believed to play two major roles, i.e., defense agents by acting as receptor analogues to inhibit the binding of enteropathogens to the host cell receptors and bifidogenic factors. At least 21 different kinds of these oligosaccharides have already been identified that are either linear or branched, composed of simple sugars like galactose, or sugar derivatives like uronic acids or uronic esters, some being acidic other being neutral.[21,22]

There is substantial evidence that the oligosaccharides secretion in mother's milk is a complex, variable, and dynamic process.[23] Not only does the amount of oligosaccharides in human milk change during lactation, but the composition of their mixture also varies among different samples, being influenced by many factors. The highest amount of oligosaccharides (i.e., 20/l) is reached on day four after birth,[21,22] but at days 30 and 120 of lactation, that content falls by 20 and 40% respectively, and is compensated by an increase in lactose content.[21,23]

Based on these considerations, and as cow's milk is very poor in oligosaccharides, it has been hypothesized that supplementing infant formulas with oligosaccharides could improve the nutritional value of formulas and help mimic some of the effects of mother's milk, especially the bifidogenic effect. Although breast-fed and formula-fed infants have similar gastrointestinal flora on day three or four after birth when it is dominated by bifidobacteria and lactobacilli, there is a substantial difference in colonic flora after several weeks of life in relation to feeding.[24] In order to improve formulas, either lactobacilli (probiotic) or oligosaccharides (synbiotic/prebiotic) were added with the aim of obtaining the same effect on gastrointestinal colonization as that of mother's milk.[25,26] The addition of lactobacilli to infant formula results in a gastrointestinal flora that is dominated by lactobacilli, comparable to the flora found in breast-fed infants, and the stools change accordingly.[27] However, this approach has been regarded as "nonphysiological" because lactobacilli are, of course, not present in human milk.[28] Moreover, the bacteria need to be administered in a viable form, necessitating specific guidelines of formula preparation. The addition of probiotics to infant formula has been shown to be associated with a decrease in the incidence and severity of episodes of infectious (rotavirus) diarrhea, in hospitalized children.[29]

By referencing the prebiotic concept, a mixture of long-chain inulin and galactooligosaccharides (10/90 w/w) has been added to an infant formula, and a series of studies show that like in breast-fed babies, the absolute number of bifidobacteria and lactobacilli or the proportion of bifidobacteria to the total number of anaerobic microorganisms increase in the prebiotic supplemented formula-fed infants (both preterm and term infants). Concomitantly, stool characteristics of the babies fed the supplemented formula were similar to those of the breast-fed babies.[30–32]

The growth and quality of growth of infants fed with the prebiotic formula containing b-palmitate and hydrolyzed proteins were similar to those seen in breast-fed infants and current infant formulas.[30,33] At first, the prebiotic approach seems more physiological, but the dynamic aspects of oligosaccharide content in mother's milk cannot be mimicked in an artificial feeding.

As it has been reported that differences in the gut microflora of neonates precede the development of atopy, suggesting a crucial role of the indigenous intestinal microflora for the maturation of human immunity,[34] it can also be hypothesized that prebiotics may help reducing the risk of allergy in infants by improving the composition of the intestinal microflora early in life.

Even though breast feeding must remain the gold-standard and the common recommendation, supplementing infant formulas with inulin-type fructans and other prebiotics is a promising approach that is likely to help in improving the intestinal health and well-being of babies who are not breast-fed at all, breast-fed only for a short period, or are mixed-fed. As compared to the probiotics, the prebiotics stimulate the growth of indigenous bacteria (especially indigenous bifidobacteria strains) and are thus expected to maintain and favor a more natural and physiological microflora, creating the conditions for an optimum crosstalk between the prokaryotic and the eukaryotic worlds that live in symbiosis in the intestine. An optimum crosstalk situation such as this is more and more recognized as a condition for a healthy development during early life which contributes to good health throughout life.

13.2.3.2 Inulin-Type Fructans and Feed for Domestic Animals and Pets

Based on what is known about the nutritional effects of inulin-type fructans, it is not surprising that these ingredients are increasingly applied in the feeds of domestic animals and pets. But there are many specific questions when including oligofructose and inulin in such feeds. First, it is essential to recognize the different forms of fructans because differences in chain lengths can elicit varying responses, especially in the intestine where fermentation of the longer chains is slower than that of the shortest oligomers, resulting in their arrival into the most distal parts of the intestine. This is particularly important for animal feed, because different types of animals (poultry, pigs, pets, and horses) have different intestinal tract morphologies and different types of bacterial populations. Furthermore, it is important to consider the type of domesticated animal or pet that will consume the supplemented feed. Different animals usually have very different levels of colonic bifidobacteria,[35] and an effect demonstrated in one species may not necessarily be seen in others or may require different doses. An example of great importance concerns the geriatric pet

population, which tends to have a less favorable intestinal microbial composition, is more prone to intestinal dysfunctions and has diminished digestive efficiency as compared to younger animals.[36–38] The nature of the diet is also of vital importance; the supplementation with prebiotic being more appropriate in high protein, meat-based diets that are, usually, associated with elevated production of putrefactive metabolites such as ammonia, amines, and phenols and indols;[39] this causes alkalinization of the colonic content[40] and may produce unpleasant fecal odors (especially in poultry, rabbits, pigs, and horses). Another pertinent consideration in the inclusion of inulin-type fructans in companion animal diets is determining the effective dose while minimizing potential negative side effects like flatulence and loose stools that may occur at very high levels (i.e., >20% of dry matter), or at more moderate levels (i.e., >10% of dry matter) in nonadapted animals. This is particularly important for pets that, usually, consume large, infrequent meals and thus could potentially be exposed to a rather large bolus of inulin-type fructans, with increasing risk of these adverse effects. Finally, oligofructose and inulin do not induce persistent effects but must be consumed on a regular basis in order to be effective;[41] there are practical considerations when designing and preparing supplemented food products; and the manufacturing processes should retain the structure and activity of inulin-type fructans.

Several studies have been conducted to investigate the effects of inulin-type fructans (mainly oligofructose) on companion animals, but the results have still to be considered as preliminary. In dogs, oligofructose reduces small intestinal bacterial overgrowth but has a mixed effect on colonic microflora; it enhances small intestinal absorptive capacity, improves the balance between epithelial cell proliferation and differentiation in the colon, tends to decrease fecal excretion of putrefactive compounds. In the same animal, both oligofructose and inulin have only a modest effect on fecal characteristics and nutrient digestibility as compared to traditional fiber sources. In cats, data are limited but oligofructose may improve colonic bacterial balance.

The nutritional benefits of supplementing livestock feed with oligofructose or inulin have also been investigated to some degree, and the collected data has recently been reviewed.[42] Data show that ileal digestibility of nutrients is improved in pigs, colonic concentrations of beneficial bacteria, including bifidobacteria, are increased in pigs and quails, cecal and colonic epithelial cell proliferation is stimulated in young pigs, fecal excretion of ammonia is reduced in pigs and rabbits, and contamination and colonization of poultry by pathogen is reduced. In swine, oligofructose has only a modest effect on fecal characteristics, it increases total digestibility of zinc but not of other minerals. Inulin and oligofructose have mixed effects on weight gain and feed efficiency in pigs and early-weaned calves, but oligofructose improves growth performance and meat production of broilers. Furthermore, data from poultry suggest that oligofructose may be as effective as antibiotics in controlling the proliferation of pathogens and enhancing growth performance.

The use of inulin and oligofructose in the diets of domesticated animals and pets is a relatively recent endeavor, and many issues still remain unresolved even though the available data show promises. Although preliminary data show, as expected, that inulin-type fructans can modify the intestinal microflora and their

fermentative activity, it remains to be established if these effects directly influence measurable characteristics of animal health. Furthermore, we need to understand the mechanisms of these influences. Once these underlying mechanisms are better understood, supplementation with inulin or oligofructose can be implemented in a more precise manner.

Other unanswered questions are if inulin and oligofructose can be used interchangeably, at which dose level for the different animal species, and at which phase of its life cycle or production stage? Geriatric as well as young animals have vastly different microbial populations and, therefore, may require different concentrations of inulin or oligofructose supplementation. It may also be beneficial to combine different oligosaccharides to simultaneously elicit complementary beneficial effects. Inulin-type fructans function to enhance host health by improving the balance of the colonic microflora, but other oligosaccharides act via different mechanisms,[43] e.g., mannan oligosaccharides stimulate the immune system because of their high degree of antigenicity that triggers the hepatic secretion of mannose-binding protein which binds the capsule of invading bacteria (e.g., *Escherichia coli*, salmonella, and clostridia) and stimulates the complement fixation system and phagocytosis.[44–46] Combining inulin-type fructans and probiotics in what has been called a *synbiotic* is another practical approach that needs further investigation to help in improving the health and well-being of domestic animals and pets. Essentially because of the problem of resistance, antibiotic usage in animal feed has been challenged and new regulations will soon strictly regulate it. Therefore, there is an urgent need to determine the effectiveness of inulin and oligofructose in reducing dependence on or replacing antibiotic usage in animal diets.

Another but still poorly understood application of inulin and oligofructose is in the reduction of fecal odor. The fermentation of carbohydrates, including oligosaccharides, reduces fecal ammonia, amine, and phenol concentrations,[40] all of which have been implicated as the major sources of odorous feces.[47–49] Because of increased human contact with animals and heightened awareness of environmental considerations, fecal odor is of concern to companion animal owners and livestock producers alike. In conclusion, some research exists concerning supplementation of inulin and oligofructose in the diets of companion animals and livestock. Studies to date indicate a positive effect of fructans on colonic microbial ecology, and host health and performance. However, much more research remains to be done to determine the appropriate role of these oligosaccharides in animal nutrition.

13.3 CONCLUSIONS AND PERSPECTIVES

As discussed in the first chapter, and as described in the "Scientific Concepts of Functional Foods in Europe: Consensus Document:"[50]

> The design and development of functional foods is a key issue, as well as a scientific challenge, which should rely on basic scientific knowledge relevant to target functions and their possible modulation by food components.

Emphasis is then put on the importance of the effects of food components on well-identified and well-characterized target functions in the body that are relevant to well-being and health issue, rather than, solely, on reduction of disease risk. In order to achieve such a development, it is necessary to identify potential functional foods or functional food components and, at least partly, to understand the mechanisms by which they modulate target functions.

At the end of this book it can be concluded that inulin-type fructans are functional food ingredients. Particularly over the last two decades, extensive scientific research has investigated their effects on target functions in the body, demonstrating benefits that are relevant to health and that justify human-intervention nutrition studies including clinical trials on reduction of disease risks.

But rather than being a single chemical entity, inulin-type fructans include different products (i.e., oligofructose, inulin, long-chain inulin, oligofructose-enriched inulin Synergy 1) with different physicochemical and technological attributes. Although the effect of most functional food components target only one or a limited number of functions (e.g., cholesterol lowering for phytostanol/phytosterol), the inulin derivatives target a range of different physiological functions from bowel functions and colonic microflora activities to mineral absorption, lipid homeostasis, and immunity, etc. In this respect, their effects compare to those of some strains of lactobacilli or bifidobacteria that classify as probiotics, but the diversity of products offers a much greater flexibility for food applications.

The reason for the multiplicity of functions as targets for inulin-type fructans could be due to their primary effect, i.e., the beneficial modification in the composition of the gut microflora, and their prebiotic attribute. As discussed earlier (see Section 13.2.2), important interactions (crosstalk) exist between the intestinal tissue and the intestinal microflora, interactions that play major roles in controlling and modulating not only intestinal but also systemic functions. By modifying (improving) the composition of the intestinal microflora, inulin-type fructans modify (improve) theses interactions (crosstalk) and consequently all the body functions that are more or less directly dependent on these interactions and their physiological consequences. In this context, it is also not surprising to see that, for a large part, the most recent research on gut microflora, including the development of the new molecular methodologies to study its composition, has started as a consequence or with the objective to meet requirements (mostly methodological) of research projects on inulin-type fructans. This research has also contributed to stimulate new developments in basic research on colonic physiology and especially on the crosstalk between the intestinal tissue and the pluricellular population of microorganisms (the pseudo-tissue) that symbiotically live there.

In term of perspectives, the future in research on inulin-type fructans as functional food ingredients relies in the progress in understanding these interactions prokaryotes–eukaryotes (crosstalk). Data will certainly continue to accumulate in the different areas covered by different chapters in this book with the aim to support more claims, and more data will be available that will allow better understanding of the mechanisms of action. Even though all inulin-type fructans have probably the same pattern of activities, quantitative differences in their efficacy in specific functions will be confirmed by the use of long-chain molecules, or the mixture of

oligofructose and long-chain molecules (oligofructose-enriched inulin Synergy 1). This is likely to prove most effective, in many cases, simply because of delayed fermentation, a necessary condition for changing the composition of the microflora all along the digestion process and especially in the most distal part of the large bowel. The effect of a synbiotic association between inulin-type fructans and probiotics, which was already suggested in the paper introducing the concept of prebiotics,[51] has up to now not been extensively investigated, and some studies already show promising results. Even if food applications of such a mixed product remain limited as compared to inulin-type fructans alone, it might have specific benefits that must be explored.

Similarly, the mixing of inulin-type fructans and other dietary fibers and nondigestible oligosaccharides is a domain that has not really been explored. Such an approach might show interesting applications, for example, in the control of lipid homeostasis and the risk of cardiovascular diseases or type II diabetes. But positive modulation of carcinogenesis and reduction of cancer risk is of such prime importance that it certainly justifies testing all possible approaches.

With the development of the new methodologies that allow an almost unlimited investigation of gene expression and protein synthesis, it is clear that new biomarkers of body functions will become available and will be validated. The uses of such biomarkers in well-designed hypothesis-driven human intervention studies in healthy volunteers (of different ages), in persons identified to be at risk of diseases, and in patients, especially those who are at risk of disease recurrence, is the gold standard and should be applied in future research programs on inulin-type fructans.

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