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Dyslipidemias

Pathophysiology, Evaluation and Management

Humana Press

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To

My wife, Sandeep, for her enduring encouragement, support and love; to my children, Ooshma and Aashima, for providing sparks in my life; to my parents, Anand Swaroop and Shyam Lata, for their nurturing; and to Scott Grundy, for guiding me through my research in lipids and lipoproteins.

Preface

For the last 20 years, there has been a growing recognition worldwide of the importance of managing dyslipidemia for the primary and secondary prevention of atherosclerotic vascular disease, especially coronary heart disease. This has been mainly due to the publication of the guidelines of National Cholesterol Education Program's Adult Treatment Panel and Pediatric Panel from the USA. These guidelines have stimulated generation of similar recommendations from all over the world, particularly Europe, Canada, Australia and Asia. Thus, it is important for the treating physicians and other providers to understand the pathophysiology, epidemiology, clinical evaluation and management of dyslipidemias. This book entitled, "Dyslipidemias: Pathophysiology, Evaluation and Management" has a clinical focus and is aimed at General Internists, Pediatricians, Cardiologists, Endocrinologists, Lipidologists and Geneticists.

A striking feature of this book is the fact that all the authors are at the forefronts of their disciplines, thereby ensuring inclusion of the latest scientific developments in their chapters. These authors have international reputation in their fields and represent global leadership. The authors were chosen by the Editor in view of their scientific contributions, reputation and most importantly not to have any direct conflicts of interests due to their employment in the pharmaceutical industry. A unique feature of this book is that all chapters have been peer-reviewed by an equally qualified group of experts and have undergone extensive revisions. This process has accomplished at least two goals: (a) improved the scientific quality of the chapters and (b) eliminated the bias of the authors, if any. Thus, I thank all the reviewers who provided constructive critiques but also appreciate the efforts of the authors in revising the chapters according to the comments of the peer reviewers. I hope that this book can provide practical guidance to the clinicians to provide the best care and new opportunities to the patients with dyslipidemias. The online version of the book provides useful links for those who seek an in-depth understanding of a particular topic.

This book could not have been edited without the dedicated administrative help of Erica Sawczuk. I also acknowledge the special contributions made by Michael Griffin at the Springer Science + Business Media.

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Lipoprotein Physiology

1

Daniel J. Rader and Sumeet A. Khetarpal

Introduction

Lipoproteins evolved due to the need to transport extracellular hydrophobic lipids within an aqueous environment. The two major lipids they transport are triglycerides (TGs) and cholesterol (both esterified and unesterified). Lipoproteins play an essential role in the absorption of dietary lipids, the transport of TGs from the liver to peripheral tissues, and the transport of cholesterol from peripheral tissues to the liver. Lipoproteins contain a core of hydrophobic lipids (TGs and cholesteryl esters, CEs) surrounded by hydrophilic lipids (phospholipids (PLs), unesterified cholesterol) and proteins that interact with body fluids. The plasma lipoproteins are divided into five major classes based on their relative density (Table 1.1): chylomicrons, very low density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). The proteins associated with lipoproteins are called apolipoproteins (Table 1.2). They serve a number of roles, including the assembly, structure, and function of lipoproteins, the activation of enzymes, and as ligands for cell surface receptors.

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Physiology and Metabolism of ApoB-Containing Lipoproteins

Lipoproteins containing apoB exist to transport hydrophobic lipids within the blood. A major role is the transport of energy in the form of TGs, and another key role is the transport of cholesterol largely in the form of CEs. The intestine produces chylomicrons containing apoB-48 and the liver produces VLDL-containing apoB-100. The role of intestinal chylomicrons is the postprandial transport of (exogenous) dietary fatty acids (within TGs) to tissues that use or store them, whereas a key role of hepatic VLDL is the fasting transport of (endogenous) fatty acids to tissues that use them. In each case, the by-product of lipolysis of the TGs is a remnant lipoprotein—chylomicron remnant or VLDL remnant (also known as IDL)—that contains residual TG as well as cholesterol and is removed from plasma by the liver. In the case of IDL, some of the particles are further converted to LDL before removal. These two related pathways are discussed in greater detail below.

First, however, is a discussion of the key structural protein in both chylomicrons and VLDL, namely apoB. ApoB is one of the largest proteins in the human genome and provides the unique structural and functional features of these lipoproteins. Importantly, there is one single molecule of apoB protein per lipoprotein particle. There is a single *APOB* gene that is expressed in both the enterocyte and the hepatocyte. However, whereas the hepatocyte synthesizes a full-length apoB known as apoB-100 (for 100 %), the

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Table 1.1 Lipoprotein classes

Lipoprotein	Density (g/mL)	Size (nm)	Apolipoproteins
Chylomicrons	< 0.930	75–1200	B-48, C-II, A-V
Chylomicron remnants	0.930–1.006	30–80	B-48, E
VLDL	0.930–1.006	30–80	B-100, C-II, C-III, A-V
IDL	1.006–1.019	25–35	B-100, E
LDL	1.019–1.063	18–25	B-100, C-III
HDL	1.063–1.210	5–12	A-I, A-II

HDL high-density lipoprotein, *VLDL* very low density lipoprotein, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein

Table 1.2 Apolipoproteins with known functions

Apolipoprotein	Primary source	Lipoprotein association	Function
ApoA-I	Intestine, liver	HDL, chylomicrons	Structural protein for HDL
			Activates LCAT
ApoA-II	Liver	HDL, chylomicrons	Structural protein for HDL
ApoA-V	Liver	VLDL, chylomicrons	Promotes LPL-mediated triglyceride lipolysis
ApoB-48	Intestine	Chylomicrons	Structural protein for chylomicrons
ApoB-100	Liver	VLDL, IDL, LDL	Structural protein for VLDL, LDL, IDL
			Ligand for binding to LDL receptor
ApoC-II	Liver	Chylomicrons, VLDL, HDL	Cofactor for LPL
ApoC-III	Liver	Chylomicrons, VLDL, HDL	Inhibits lipoprotein binding to receptors
ApoE	Liver	Chylomicron remnants, IDL, HDL	Ligand for binding to LDL receptor

HDL high-density lipoprotein, *LCAT* lecithin-cholesterol acyltransferase, *VLDL* very low density lipoprotein, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein, *LPL* lipoprotein lipase

enterocyte synthesizes a protein just less than half the size called apoB-48 (for 48% the size of apoB-100). The molecular basis of this differential apoB production is a result of messenger RNA (mRNA) editing. The apobec-1 editing complex is expressed in the enterocyte but not in the hepatocyte. It "edits" a specific cytidine to a uracil in the apoB mRNA in the intestine, creating a nonsense "stop" codon that results in cessation of protein translation. Thus, the shorter apoB-48 in synthesized. In the hepatocyte, apobec-1 is not expressed and thus no editing occurs and the full-length apoB-100 is synthesized. The reason for this difference is not clear, but apoB-48 assumes a structure that allows the much larger chylomicron to form. One key difference is that the LDL receptor (LDLR)-binding region in apoB is in the carboxyterminus and thus present only in apoB-100 and not in apoB-48. Thus, chylomicron remnants are dependent on other pathways and mechanisms for uptake by the liver (see below).

The Intestine Mediates Absorption of Dietary Fat and Cholesterol and Synthesis of Chylomicrons During the Fed State

The intestinal enterocyte is a key player in apoB-containing lipoprotein metabolism through its synthesis and secretion of chylomicrons (Fig. 1.1; [1]). The physiology of the processing of dietary lipids by lipases within the intestinal lumen is beyond the scope of this chapter. Lumenal fatty acids, particularly longer chain fatty acids, are absorbed by the enterocytes in the small intestine. Furthermore, luminal cholesterol, derived either from the diet or from the bile, is absorbed by enterocytes via the transport protein NPC1L1 [2]. Enterocytes esterify fatty acids to TGs through the action of several enzymes. One critical, rate-limiting enzyme in the synthesis of TGs is diacylglycerol acyltransferase 1 (DGAT1), which catalyzes the final addition of a fatty acid to diacylglycerol

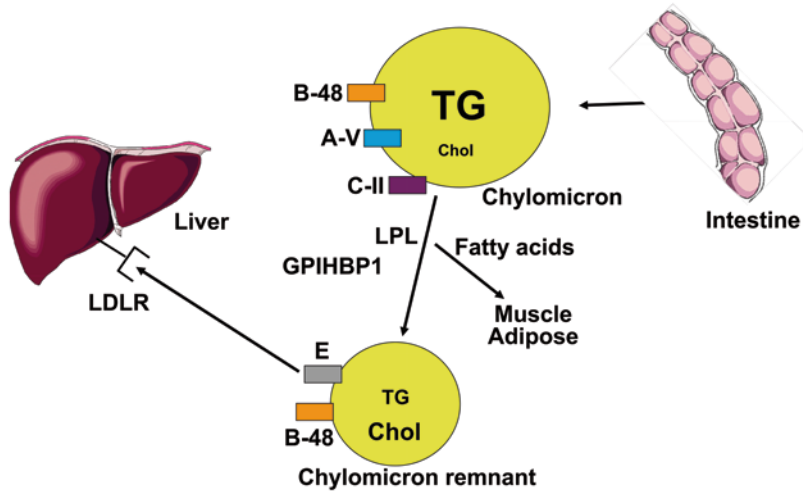

Fig. 1.1 Chylomicron metabolism. *A-V* apolipoprotein A-V, *B-48* apolipoprotein B-48, *C-II* apolipoprotein C-II, *Chol* cholesterol, *E* apolipoprotein E, *LDLR* LDL receptor, *LPL* lipoprotein lipase; *TG* triglyceride, *GPIHBP1* glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1

to form TGs [3]. Absorbed cholesterol is partly esterified in the enterocyte to CEs largely by the enzyme acylcholesterol acyltransferase 1 (ACAT1) [4]. TGs, and to a lesser extent CEs, are loaded on the nascent apoB-48 in the endoplasmic reticulum (ER) by the microsomal TG transfer protein (MTP) complex [5] and additional TGs are subsequently added to the core of the particle, ultimately forming mature chylomicron particles. The protein secretion-associated, Ras-related GTPase 1B (SAR1B), part of the coat protein *complex* II (COPII), is required for the transport of chylomicrons from the ER to the Golgi [6]. Studies of human Mendelian disorders have led to critical insights into the roles of specific proteins in chylomicron assembly and secretion [7]. For example, mutations that impair the synthesis of apoB-48 or abolish the activity of MTP, DGAT1, or SAR1B all result in reduced or eliminated assembly and secretion of chylomicrons, with attendant fat malabsorption and reduced postprandial plasma TG levels. Dietary fat intake is a key driver of chylomicron production. It is interesting that chylomicron secretion has been shown to be sensitive to insulin, with increased production of chylomicrons in insulin-resistant states [8].

Chylomicrons are secreted into the intestinal lymph and delivered via the thoracic duct directly to the systemic circulation, thus critically bypassing the liver. This process allows the direct transport of dietary fatty acids to peripheral tissues, where they are hydrolyzed and release their fatty acids. The lipolysis of chylomicrons has been extensively studied. The key enzyme in this process is lipoprotein lipase (LPL), which is expressed at high levels in tissues that utilize fatty acids for energy (skeletal and cardiac muscles) or that store fatty acids in cytoplasmic lipid droplets (adipose). LPL is synthesized by parenchymal cells (myocytes and adipocytes) and then transported to the luminal surface of the capillary endothelium. There it is anchored to the glycosylphosphatidylinositol-anchored protein (GPIHBP1) [9].

Chylomicrons enter the capillary and bind to LPL, bringing with them apolipoprotein C-II (apoC-II), which is a required cofactor for LPL. Activation of LPL results in hydrolysis of TGs in the chylomicron core, releasing free fatty acids, which are taken up by adjacent myocytes or adipocytes. Some of the released free fatty acids bind albumin in the plasma and are transported to other tissues, including the liver. The importance of LPL and apoC-II in chylomicron hydrolysis in humans is established by the condition familial chylomicronemia syndrome (FCS), in which

loss-of-function mutations in both alleles of either the *LPL* or *APOC2* gene result in massive hyperchylomicronemia due to failure of chylomicron hydrolysis [10]. Rare humans with loss-of-function mutations in *GPIHBP1* have also been reported to have hyperchylomicronemia. Conversely, a well-studied gain-of-function mutation in *LPL* gene 447X allele is associated with increased LPL activity, reduced levels of plasma TG, and reduced risk of coronary heart disease.

The expression and activity of LPL are highly regulated at the transcriptional and posttranscriptional levels [11]. The transcription of LPL is regulated by a number of nutritional, hormonal, and inflammatory factors. In addition, LPL is post-translationally regulated by proteases that cleave and inactivate the enzyme. Both angiopoeitin-like 3 and 4 (ANGPTL3 and 4) have been reported to target LPL for inactivation [11]. Indeed, loss-of-function mutations in either of these genes are associated with reduced plasma TG levels (likely due to increased LPL activity). Furthermore, apolipoproteins other than apoC-II on the chylomicron influence LPL activity. Apolipoprotein A-V (apoA-V) promotes LPL activity through mechanisms that are not fully understood [12]. Loss-of-function mutations in the *APOA5* gene are proven to be associated with increased plasma TG levels. Conversely, apolipoprotein C-III (apoC-III) inhibits LPL activity, and loss-of-function mutations in the *APOC3* gene are associated with decreased plasma TG levels and reduced risk of coronary heart disease [13].

The hydrolysis of chylomicron TGs results in progressive shrinking of the hydrophobic core of the particle and shedding of PLs and exchangeable apolipoproteins on the particle surface to other lipoproteins including HDL. Eventually, particles known as chylomicron remnants are created. Chylomicron remnants are atherogenic and when their catabolism is impaired, risk of cardiovascular disease is increased [14]. Importantly, chylomicron remnants still contain their core molecule of apoB-48, and have also acquired an apolipoprotein known as apolipoprotein E (apoE). As noted above, apoB-48 lacks the sequence in apoB-100 that binds to the LDLR and thus apoB-48 itself does not bind to the LDLR to mediate clearance. Instead, the apoE on chylomicron remnants binds to the LDLR and other receptors such as low-density lipoprotein receptor-related protein 1 (LRP1) and syndecan-4 in the liver and mediates their rapid removal from the circulation [15]. Mutations in apoE, such as the common apoE2 isoform, that impair binding to the LDLR can result in reduced clearance of chylomicron remnants and a distinctive phenotype of remnant accumulation in blood known as familial dysbetalipoproteinemia, or type III hyperlipoproteinemia. Once taken up by the liver, the residual TG and CE in chylomicron remnants are targeted to the lysosome for degradation by the lysosomal acid lipase (LAL), generating fatty acids and unesterified cholesterol [16]. As discussed below, these lipids are handled by the liver.

The intestinal chylomicron pathway is designed to efficiently mediate the absorption of dietary fat and its transport to skeletal and cardiac muscle for energy utilization and to adipose for storage. The average individual can ingest up to about 100 g of fat and yet demonstrate only modest elevations in postprandial TG levels, indicating the very high capacity of the system to handle a large dietary fat load. In individuals with normal LPL activity, chylomicrons are only seen in postprandial blood and are not present in the blood after a standard 12-h fast. The presence of chylomicrons in the blood after such a fast invariably indicates a defect in chylomicron metabolism, usually a primary or secondary defect in LPL activity.

The Liver Uses Adipose-Derived and De Novo Synthesized Fatty Acids for the Synthesis of VLDL

The hepatocyte is the other key cellular player in apoB-containing lipoprotein metabolism through its synthesis and secretion of VLDL (Fig. 1.2; [17]). VLDL is synthesized by the liver in both the fasting and the fed states. During the fasting state, dietary fat is not available and chylomicrons are not being actively synthesized. Another source of fatty acids is needed for tissues like cardiac and skeletal muscles that make abundant use

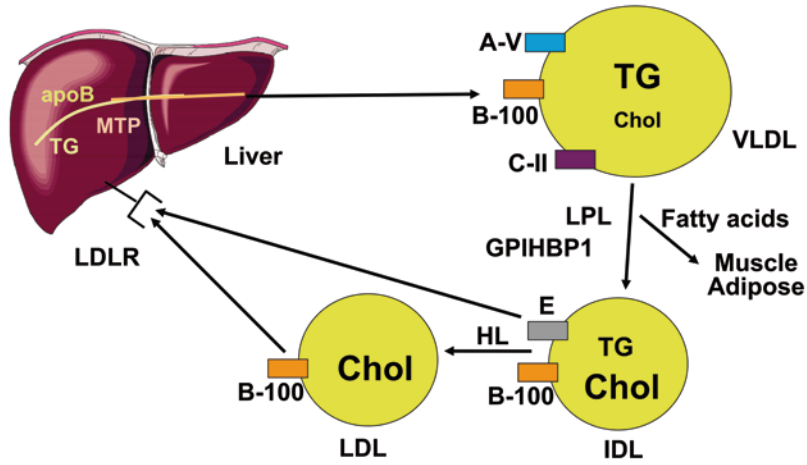

Fig. 1.2 Very low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL metabolism. *apoB* apolipoprotein B, *B-100* apolipoprotein B-100, *HL* hepatic lipase, *MTP* microsomal triglyceride (TG) transfer protein. *LDL* low-density lipoprotein, *A-V* apolipoprotein A-V, *C-II* apolipoprotein C-II, *Chol* cholesterol, *E* apolipoprotein E, *LDLR* LDL receptor, *LPL* lipoprotein lipase; *GPIHBP1* glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1

of fatty acids for energy. The primary source of fatty acids for VLDL synthesis by the liver during fasting is the adipocyte. A detailed discussion of the regulation of adipocyte TG hydrolysis is beyond the scope of this chapter [18]. Briefly, during fasting, insulin levels fall and pro-lipolytic pathways are activated that lead to the hydrolysis of adipose TG and release of fatty acids. These fatty acids are transported to the liver by albumin and other proteins, where they are taken up by hepatocytes. (Note that adipose-derived fatty acids can also be directly taken up and utilized by cardiac and skeletal muscle). VLDLs are also synthesized by the liver during the fed state. In particular, excess dietary carbohydrate is converted to fatty acids in the liver through de novo lipogenesis [19].

Fatty acids in the hepatocyte, whether derived from adipose or de novo lipogenesis, can be esterified to TGs by a series of enzymatic steps. In the hepatocyte, a critical enzyme in this process is diacylglycerol acyltransferase 2 (DGAT2), which catalyzes the final addition of a fatty acid to diacylglycerol to form TGs [3]. In addition, synthesis of CE appears to be important for VLDL assembly and secretion and the enzyme acylcholesterol acyltransferase 2 (ACAT2) is a critical enzyme in this process [4]. TGs and CEs are loaded on the nascent apoB-100 protein by the MTP complex. Additional TGs and CEs are subsequently added to the core of the particle, ultimately forming mature VLDL particles. The key roles of apoB-100 and MTP in VLDL assembly and secretion are demonstrated by the existence of inherited conditions in which mutations in these genes cause reduced or absent VLDL secretion and very low levels of TGs and LDL cholesterol [20]. As noted above, dietary carbohydrate intake can be an important determinant of hepatic TG synthesis and VLDL production. Insulin is an important positive regulator of hepatic TG synthesis and VLDL production. The inherited condition familial combined hyperlipidemia (FCHL) is characterized by VLDL overproduction, although the molecular mechanisms remain poorly understood [21].

VLDL are secreted into the blood and transported to peripheral tissues, where they are hydrolyzed and release their fatty acids. The LPL-mediated hydrolysis of VLDL TG is very similar to the process described above for chylomicron TG. The particle generated after lipolysis of VLDL is known as a VLDL remnant or IDL, which also acquires apoE from HDL. In a similar fashion to chylomicron remnants, many IDLs

are removed from the circulation by the liver through binding of apoE to the LDLR and other receptors. However, in contrast to chylomicron remnants, only about half of IDLs are directly removed from the circulation. ApoB-100 containing particles that remain are further converted by hepatic lipase (HL), a relative of LPL, to LDL. During this process, most of the TG in the particle is hydrolyzed, and most of the exchangeable apolipoproteins are transferred to other lipoproteins. Thus, the LDL particle consists primarily of apoB-100 and CE. It is the major cholesterol-carrying lipoprotein in the blood and accounts for more than half of the plasma cholesterol in most individuals. LDL is largely a by-product of the metabolism of VLDL, and despite being rich in cholesterol it is rarely if ever required as a cholesterol source by peripheral tissues. It appears to have a physiological role in the delivery of vitamin E to the retina and central nervous system through pathways that are poorly defined. LDL cholesterol is associated with cardiovascular disease and has evolved to be a major target of therapeutic intervention for reduction of cardiovascular risk.

A major factor regulating plasma levels of LDL-cholesterol (LDL-C) is the rate of clearance of LDL by the liver via the LDLR. Approximately 70% of circulating LDL is cleared by LDLR-mediated endocytosis in the liver. Thus, the regulation of the LDLR activity in the liver is a major determinant of plasma LDL-C concentrations. The LDLR is regulated in a number of ways. First, the cholesterol content in the liver regulates the transcription of the LDLR [22]. As cholesterol content falls, it is sensed by the insulin-induced gene protein-sterol regulatory element-binding protein (SREBP) cleavage-activating proteins (INSIG-SCAP) complex in the ER, which leads to increased cleavage of the membrane-associated SREBPs, generation of the transcriptionally active SREBPs, which move to the nucleus and promote transcription of the LDLR (and other genes involved in cholesterol synthesis and metabolism). Second, the LDLR protein is targeted for ubiquitination and lysosomal degradation by the E3 ubiquitin ligase-inducible degrader of the LDLR (IDOL), which is regulated by the cholesterol-sensing nuclear receptor liver X-activated receptor (LXR) [23]. Third, the LDLR protein is targeted by the secreted protein proprotein convertase subtilisin/kexin type 9 (PCSK9) for lysosomal degradation [24]. PCSK9 is itself a cholesterol-regulated protein, and is paradoxically upregulated by cholesterol-depleted conditions that activate the SREBP system, with the effect of reducing LDLR-mediated uptake of LDL. Mutations in IDOL and PCSK9 that reduce the function of the proteins are associated with increased LDLR protein and activity and reduced levels of LDL-C.

Physiology and Metabolism of HDL

HDLs are so-named because they are the most dense of the lipoprotein classes, with less lipid and relatively more protein than the apoB-containing lipoproteins. A major function of HDL is classically thought to be to accept excess cholesterol from peripheral tissues which cannot metabolize it and transport it back to the liver for biliary excretion, a process known as "reverse cholesterol transport (RCT)." [25] HDL also serves as a circulating reservoir for apolipoproteins that transfer onto apoB-containing lipoproteins and modulate their function as described above. It is also becoming increasingly evident that HDL has functions in innate immunity and carries proteins that have specific roles in host defense. For example, HDL carries two proteins, apoL1 and haptoglobin-binding protein, that work together to lyse a species of trypanosomes [26]. This aspect of HDL function, which is still being actively explored, will not be discussed further in this chapter.

The major HDL protein is apoA-I, which is present in the vast majority of HDL particles and accounts for approximately 70% of HDL protein. ApoA-I (and thus HDL) are synthesized and secreted by the intestine and liver. HDL particles contain from one to four molecules of apoA-I. The second most-abundant protein in HDL is apoA-II, which represents about 20% of HDL protein. Approximately, two thirds of HDL particles contain both apoA-I and apoA-II, whereas

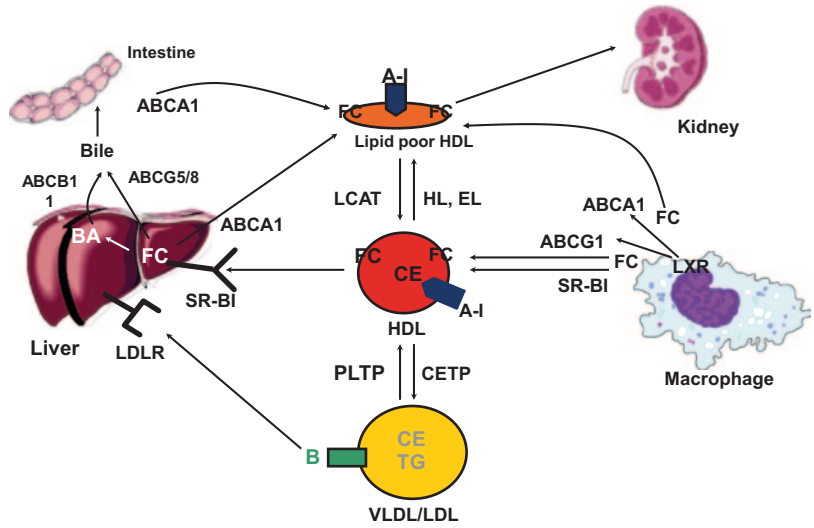

Fig. 1.3 HDL metabolism. *ABCA1* ATP-binding cassette transporter A1, *ABCB11* ATP-binding cassette transporter B11, *ABCG1* ATP-binding cassette transporter G1, *ABCG5/8* ATP-binding cassette transporter G5 and G8, *A-I* apolipoprotein A-I, *CE* cholesterol ester, *CETP* Cholesteryl ester transfer protein, *EL* endothelial lipase, *FC* free cholesterol, *LCAT* Lecithin-cholesterol acyltransferase, *PLTP* phospholipid transfer protein, *SR-BI* Scavenger receptor class B member 1, *ATP* adenosine triphosphate, *HDL* high-density lipoprotein, *LXR* liver X-activated receptor

one third contains only apoA-I without apoA-II. While these two types of particles differ in their metabolism and certain properties, the physiology of these particles remains uncertain. There are a large number of additional proteins found on specific HDL particles; indeed the study of the HDL proteome has provided important new insights into HDL structure and function [27].

HDL metabolism is complex (Fig. 1.3; [28]). It includes the biosynthesis of nascent HDL by the liver and intestine, the esterification of cholesterol to form the mature HDL, the remodeling of HDL by lipases and lipid transfer proteins, and the often separate catabolism of HDL cholesterol and HDL protein. In addition, the process of RCT has a number of molecular players acting in concert. The different steps in HDL metabolism and RCT are reviewed below.

HDL Biosynthesis and Production

HDL biosynthesis requires several steps to generate a mature HDL particle. The initial step in this complex process involves the synthesis of the major protein components of HDL, such as apoA-I and apoA-II, and their subsequent secretion. This phase is followed by the lipidation of these proteins with PL and cholesterol. The final stage of HDL maturation is the assembly of the mature HDL particle. ApoA-I is primarily synthesized in the liver (hepatocytes) and small intestine (enterocytes). The transcriptional regulation of apoA-I has been of substantial interest. Biochemical and genetic studies have demonstrated roles of peroxisome proliferator-activated receptor (PPAR)-α and liver receptor homolog-1 (LRH-1) in the transcription of apoA-I.

Once apoA-I is secreted it acquires lipids (PL and cholesterol) from the tissues of origin in order to assemble into nascent HDL particles. A critical mediator of lipid efflux from hepatocytes and enterocytes to apoA-I is the adenosine triphosphate (ATP)-binding cassette protein A1 (ABCA1), which is expressed at the basolateral surface of both cell types [29]. Patients with the rare genetic condition Tangier disease have mutations in ABCA1 and fail to effectively lipidate newly secreted apoA-I, leading to rapid catabolism. Consistent with the key roles of the liver

and intestine, HDL-C levels are reduced by about 80 % in mice that lack ABCA1 in the liver and by approximately 30 % in mice lacking ABCA1 in the intestine.

Although most of the initial lipidation of apoA-I via ABCA1 occurs in the liver and intestine, HDL derives much of its lipid mass from other sources, which includes other tissues and other lipoproteins. HDL also obtains lipids and apolipoproteins from TG-rich lipoproteins upon their lipolysis by LPL. Indeed, humans and mice with LPL deficiency have very low levels of HDL-C. Lipoprotein-derived PLs are transferred to HDL by the PL transfer protein (PLTP) [30]. Mice lacking PLTP have a significant reduction in HDL-C and apoA-I levels.

HDL Cholesterol Esterification by LCAT

The cholesterol that effluxes from cells is unesterified (or free) cholesterol. The esterification of cholesterol to form CE and the hydrophobic lipid core of HDL is a necessary step in the development of mature HDL (Fig. 1.1). An HDL-associated enzyme, lecithin-cholesterol acyltransferase (LCAT), catalyzes the transfer of a fatty acid from PL to free cholesterol, resulting in the formation of HDL-CE. Apo A-I activates LCAT, probably by organizing the lipid substrates for optimal presentation to LCAT [31]. CE is more hydrophobic than free cholesterol and moves to the core of the lipoprotein particle, allowing the formation of mature HDL. LCAT activity thus results in the conversion of nascent discoidal HDL particles to HDL_3, and then from smaller HDL_3 to larger HDL_2.

The activity of LCAT is essential for normal HDL metabolism. LCAT deficiency in humans results in substantially reduced HDL-C and apoA-I levels accompanied by rapid catabolism of apoA-I and apoA-II. It was originally hypothesized by Glomset that LCAT-mediated cholesterol esterification was important for RCT because it maintained a gradient of unesterified cholesterol from cell to HDL acceptors which helped to drive cholesterol efflux. Now that much cholesterol efflux is regulated by active transporters such as ABCA1 and ABCG1, the importance of LCAT-mediated cholesterol esterification for driving cholesterol efflux and RCT is less certain. Similarly, the relationship of LCAT activity to atherosclerosis is unresolved. While LCAT is clearly important for normal HDL metabolism, more studies are required to determine the effect of LCAT activity on RCT and atherosclerosis in humans.

HDL CE Transfer by Cholesterol Ester Transfer Protein

It is well established that HDL-CE can be transferred from HDL to apoB-containing lipoproteins by cholesterol ester transfer protein (CETP) in exchange for TG. The proof that CETP is important for human HDL metabolism came from the discovery of humans genetically deficient in CETP. These individuals, who have loss-of-function mutations in both alleles of the *CETP* gene, have extremely high levels of HDL-C and slower turnover of apoA-I. Confirmation that the CETP pathway is quantitatively important for hepatic uptake of HDL-CE in humans was obtained in a study in which injection of HDL labeled with a CE tracer showed that the labeled cholesterol that was excreted into bile was first largely transferred to apoB-containing lipoproteins [32]. The role of CETP in HDL catabolism and RCT is discussed in greater detail below.

HDL Remodeling by Lipases

Remodeling of HDL by lipases is a critical process that regulates HDL metabolism and apoA-I catabolism. HL, a cousin of LPL, hydrolyzes both TG and PL in HDL, and it has been shown that HL is most effective in hydrolyzing HDL lipids if the HDL is TG enriched, such as that generated by the action of CETP [33]. The hydrolysis of HDL lipids by HL leads to the release of apoA-I from HDL and increased apoA-I catabolism. Insulin-resistant states are associated with increased HL activity accounting at least in part for the reduced HDL-C levels. Genetic deficiency in HL

in humans has been reported to result in modestly elevated HDL-C and larger HDL particles. The relationship of HL activity to atherosclerotic cardiovascular disease in humans remains unclear.

A lipase closely related to HL, called endothelial lipase (EL), has relatively more phospholipase activity than TG lipase activity and a greater preference for HDL over apoB-containing lipoproteins [34]. Gain of function of EL in mice reduces HDL-C levels and loss of function increases HDL-C levels. Individuals with high HDL-C are more likely to have loss-of-function *EL* gene mutations. One low-frequency variant in EL with reduced lipolytic activity was shown to be associated with increased HDL-C levels, but was not found to be associated with protection from coronary heart disease.

Catabolism of HDL

The catabolism of HDL cholesterol and apoA-I is largely dissociated. The liver is the major site of HDL-C uptake (Fig. 1.3), and this process is mediated primarily by the scavenger receptor class BI (SR-BI) [35]. SR-BI promotes selective uptake of HDL cholesterol, but not HDL protein. In mice, SR-BI is a critical regulator of HDL metabolism: deletion of the SR-BI gene in mice results in elevated plasma HDL-C levels due to slow hepatic HDL-C uptake; it also results in reduced flux of RCT and increased atherosclerosis.

The physiologic importance of the hepatic SR-BI pathway for uptake of HDL-C remains to be definitively established in humans. Common variants at the *SCARB1* locus (gene encoding SR-BI) are significantly associated with plasma HDL-C levels, suggesting that SR-BI is relevant to HDL metabolism in humans. Furthermore, heterozygotes for a missense variant in SR-BI were reported to have modestly elevated HDL-C levels, but no evidence of increased risk of atherosclerotic cardiovascular disease. In humans, the CETP pathway is an alternative pathway for transport of HDL-derived cholesterol to the liver. Because CETP-deficient subjects have markedly elevated HDL-C levels, it suggests that SR-BI is not substantially upregulated to compensate for the lack of transfer of CE out of HDL in the absence of CETP activity.

ApoA-I is catabolized largely independently of HDL cholesterol. Kinetic studies in humans have shown that the turnover rate of apoA-I is an important determinant of plasma apoA-I and HDL-C concentrations. Using trapped ligands, studies in animals established that a substantial portion of apoA-I is catabolized by the kidneys, and the rest is catabolized by the liver. Lipid-free or lipid-poor apoA-I can be filtered at the glomerulus due to its small size. Following filtration, it is catabolized by proximal renal tubular epithelial cells through binding to cubilin, an extracellular receptor localized to the apical surface. Cubilin interacts with its coreceptor megalin, a member of the LDLR gene family, to mediate the uptake and degradation of apoA-I [36].

The rate-limiting step in the catabolism of apoA-I in the kidney is at the level of glomerular filtration. Thus, the degree of apoA-I lipidation plays a key role in determining the rate of apoA-I catabolism in the kidney. ApoA-I lipidation is determined by both lipid acquisition and maturation, as well as by remodeling of the mature HDL particle. There are several examples from human pathophysiology of impaired lipid acquisition by apoA-I leading to increased apoA-I catabolism. In Tangier disease, the failure of the liver and the intestine to lipidate newly synthesized apoA-I via the ABCA1 pathway results in a poorly lipidated apoA-I that is rapidly catabolized primarily by the kidneys. In LCAT deficiency, the lack of LCAT-mediated cholesterol esterification also results in accelerated apoA-I catabolism. Excess remodeling by lipases such as HL and EL or transfer proteins such as CETP can also promote shedding of apoA-I and faster renal degradation.

The liver is responsible for substantial degradation of apoA-I, but the mechanisms underlying hepatic uptake and degradation of HDL apolipoproteins are poorly understood. A portion of HDL particles contains apoE, and apoE-rich HDL is a ligand for LDLR and other apoE receptors, thus this pathway may contribute to the uptake of some HDL by the liver. HDL depleted of apoE may still be taken up and degraded by hepatocytes, however, and other mechanisms must

also exist. Substantial effort has been invested in identifying other hepatic HDL-binding proteins, but none have yet been proven to be bona fide HDL receptors in vivo in humans.

Cholesterol Efflux and RCT

Glomset introduced the concept of "RCT" in 1968 to describe the process by which extrahepatic cholesterol is returned to the liver for excretion in the bile and feces. Most cells acquire cholesterol through uptake of lipoproteins and de novo synthesis and yet, with the exception of steroidogenic tissues that convert cholesterol to steroid hormones, are unable to catabolize it. Macrophages take up large amounts of cholesterol from their environment and have evolved pathways to efflux cholesterol to HDL-based acceptors. Macrophages from ABCA1 knockout mice have substantially reduced cholesterol efflux to lipid-poor apoA-I as an acceptor. Macrophage ABCA1 plays an important quantitative role in macrophage cholesterol efflux and RCT in vivo.

In contrast to ABCA1, ABCG1 promotes macrophage efflux to mature HDL particles. Macrophage ABCG1 plays an important role in macrophage cholesterol efflux and RCT in vivo and is additive to ABCA1. Combined deletion of ABCA1 and G1 in macrophages leads to accelerated atherosclerosis and excess proliferation of hematopoietic stem cells [37].

ABCA1 and ABCG1 are upregulated by the nuclear receptors LXR α and β that are activated by oxysterols generated through intracellular enzymatic action on cholesterol. LXR agonists upregulate macrophage ABCA1 and ABCG1, promote RCT, and are antiatherogenic in mice. ABCA1 and ABCG1 mRNAs are targeted for degradation by miR-33, a microRNA that is embedded within the SREBP2 gene [38]. In vivo administration of antagomirs of miR-33, an miRNA that targets ABCA1 and ABCG1 mRNAs for degradation raises HDL cholesterol levels in mice and nonhuman primates, increases macrophage ABCA1 and ABCG1, promotes RCT, and is antiatherogenic in mice. These findings clearly indicate that interventions that increase macrophage cholesterol efflux and RCT are antiatherogenic. LXR agonism and miR-33 antagonism are targets for therapeutic development as a strategy to promote macrophage cholesterol efflux and reduce atherosclerosis.

In the classic model of RCT, HDL-C is transported to and taken up by cells in the liver by selective uptake by SR-BI. HDL-C can also be transferred to apoB-containing lipoproteins within the plasma compartment by CETP, with subsequent uptake of the apoB-containing lipoproteins by the LDLR and other hepatic receptors.

After delivery to the liver, there are several mechanisms for excretion of sterols into bile. ABCG5 and ABCG8 are half transporters which act together as heterodimers at the apical membranes of hepatocytes to promote transport of cholesterol (and other sterols such as plant sterols) into bile. Genetic deficiency of ABCG5 or ABCG8 causes sitosterolemia characterized by decreased biliary sterol excretion and increased tissue and plasma levels of cholesterol and plant sterols. ABCB11 (also known as the bile salt export pump, BSEP) transports bile acids from the hepatocyte apical membrane into the bile. In humans, hepatocytes express NPC1L1 on their apical surface where it can promote the reuptake of biliary cholesterol by the hepatocyte.

It has been suggested that some HDL-derived cholesterol may be transported directly from the plasma to the intestinal enterocyte, thus bypassing the hepatobiliary route, though the specific molecular pathways of this "trans-intestinal cholesterol excretion" pathway have not yet been elucidated.

Enterocytes express ABCG5 and ABCG8 on their apical surface which permits them to efficiently export cellular cholesterol into the intestinal lumen. As in other tissues, these transporters in the intestine are under the regulation of LXRs, which have the effect of promoting intestinal excretion of cholesterol and plant sterols. Intestinal-specific LXR agonism promotes RCT, reduces plasma cholesterol, and inhibits atherosclerosis.

There have been numerous attempts to measure integrated RCT in animal models, but experimental approaches that assess RCT from the entire periphery may not be sufficiently sensitive to

show specific effects of genetic or pharmacologic manipulations on macrophage RCT. A method for tracing cholesterol efflux and RCT specifically from macrophage to feces has been widely used and results generally support the concept that the rate of "macrophage RCT" is a better predictor of atherosclerosis than the steady-state measure of HDL-C [39]. Development of new techniques for evaluation of RCT in humans is essential for the assessment of new therapies aimed at enhancing RCT. However, a measure of ex vivo HDL cholesterol efflux capacity was a better predictor of coronary artery disease (CAD) than HDL-C levels [40]. Given recent challenges to the "HDL cholesterol hypothesis" that raising plasma HDL cholesterol will reduce CV risk, it may be worth considering a shift to the "HDL flux hypothesis" [41].

Other Properties of HDL

It should be noted that HDL has been described to have a variety of functions and properties based on in vitro studies and in some cases in vivo validation [42]. While a detailed description of these properties is beyond the scope of this chapter, they include antioxidant effects, anti-inflammatory effects on endothelial cells and macrophages, stimulation of endothelial nitric oxide production, inhibition of endothelial apoptosis, and antithrombotic effects. In addition, proteomic studies have demonstrated a remarkable number of proteins associated with HDL particles, including many proteins involved in innate immunity, inflammation, and other pathways. The scope of functions of HDL remains to be fully defined, and the relationship of these other functions to atherosclerosis remains poorly understood.

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Epidemiology of Blood Lipids and Lipoproteins

2

Peter W.F. Wilson

Introduction

Research has shown that total cholesterol levels in the blood are highly associated with greater coronary heart disease (CHD) risk in middle-aged American adults, especially in men. Triglyceride levels are also associated with greater risk for cardiovascular disease (CVD) events, but the results were less consistent [1]. Associations between total cholesterol levels and CVD risk, which includes stroke as an outcome, have been less convincing in older adults. This chapter focuses on findings related to observational studies that investigated cholesterol levels and vascular disease risk, the determinants of low-density (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels, associations with blood triglyceride levels, information related to apolipoproteins and lipoprotein (a), data related to lipid levels in the setting of the metabolic syndrome and obesity, trends in lipoprotein cholesterol levels for the USA and around the world, and current population strategies to screen children and adults at high risk for CVD or hypercholesterolemia.

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Blood Lipids and Cardiovascular Risk

Higher concentrations of blood cholesterol are associated with greater risk for CHD death. The largest project that addressed this issue included screenees from the Multiple Risk Factor Intervention Trial (MRFIT), and more than 350,000 middle-aged men aged 35–57 years at baseline who were followed for more than a decade, as shown in Fig. 2.1 [2, 3]. Higher cholesterol levels and greater risk for CHD death and risk were synergistically associated with cigarette smoking, blood pressure levels, and diabetes mellitus [4].

Initial assessments of lipid levels in cardiovascular population studies such as Framingham, Chicago, MRFIT screenees, and the Seven Countries Study focused on total cholesterol levels [5–7]. Complementary to the MRFIT findings, the Seven Countries investigators analyzed the role of serum cholesterol levels as predictors of CHD death around the world and Fig. 2.2 shows the results according to cholesterol quartiles for sites in Japan, Southern Europe, Serbia, USA, Southern Europe coastal region, and Northern Europe. The relation between cholesterol and risk of CHD death was relatively flat at low cholesterol levels. On the other hand, cholesterol levels were uniformly much higher in Northern Europe and the relation between cholesterol and CHD death was relatively steep in that region [8].

Research has generally concentrated on the associations of risk factors and the development of CVD events over 5–15 years of follow-up, but

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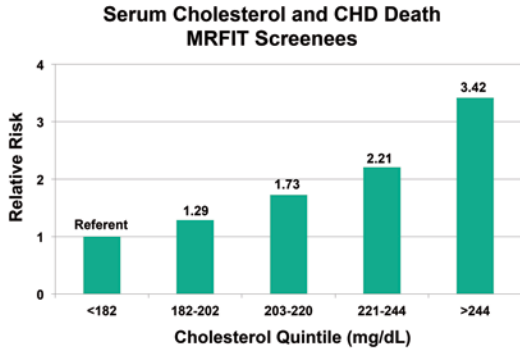

Fig. 2.1 Multiple Risk Factor Intervention Trial (MRFIT) screenees and relative risk for CHD death according to blood cholesterol in men aged 35–57 years at baseline [2, 3]

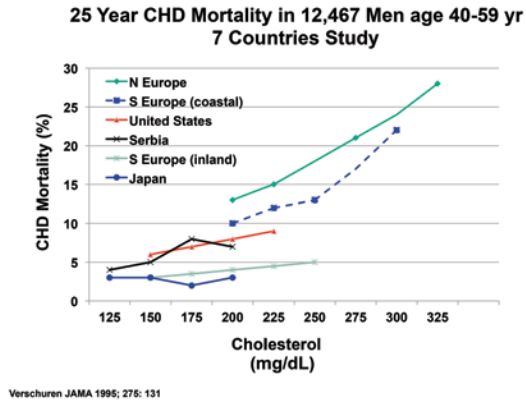

Fig. 2.2 CHD mortality over 25 years of follow-up in men aged 40–59 years at baseline in the Seven Countries Study [8]. *CHD* coronary heart disease

newer analytical methods have led to the development of estimates over a longer time frame and now it is possible to estimate risk for vascular disease over a person's lifetime. As shown in Fig. 2.3, both age and blood cholesterol levels are highly associated with a greater lifetime risk of CVD in both sexes for Framingham participants at all ages [9]. More recently, this approach has been widened to include data and estimates from a broad range of population groups [10].

Lipoprotein quantification was developed at the National Heart, Lung and Blood Institute (NHLBI) in the 1970s and the methods employed ultracentrifugation [11]. Subsequently, the Lipid Research Clinics (LRC) Program expanded the use of lipoprotein cholesterol quantification using ultracentrifugation and precipitation techniques that allowed estimation of LDL, HDL, and very-low-density lipoprotein (VLDL) cholesterol. The NHLBI subsequently sponsored a large LRC program that featured the use of these newer lipoprotein measurements. Quality control and standardization of the measurements were coordinated through the NHLBI and the Centers for Disease Control in several NHLBI observational studies and clinical trials that followed [12, 13].

The advent of lipoprotein cholesterol measurement led to epidemiologic analyses that considered the potential effects of the various particles on CVD risk. Reports from the late 1970s by Gordon, Miller, and other investigators using Framingham and other population data showed that both total cholesterol and HDL-C were highly associated with greater CVD risk, the effects were statistically independent, and the results persisted in multivariable risk formulations [14–17]. As an example of these findings, Figs. 2.4 and 2.5 show the risks for myocardial infarction in Framingham men and women over 12 years of follow-up after baseline measurement of lipids [18]. The heights of the vertical bars display the 12-year risk for myocardial infarction according to sex-specific quartiles of total cholesterol and HDL-C. Higher levels of total cholesterol were associated with greater risk of myocardial infarction and higher HDL-C appears to be cardioprotective in both sexes. Even in the lowest quartile of total cholesterol, the individuals with low HDL-C experienced greater risk for developing myocardial infarction.

The determinants of LDL-C are shown in Table 2.1. Dietary intake of fat is the most important determinant and research by Hegsted, Keys, and others showed that LDL-C levels vary according to the dietary composition [19]. Greater intake of dietary saturated fat and cholesterol increases blood cholesterol levels and greater intake of polyunsaturated fat decreases LDL-C [20]. Differences in blood cholesterol levels and vascular disease risk in populations around the world are believed to be greatly attributable to such dietary differences as shown in Verschuren's

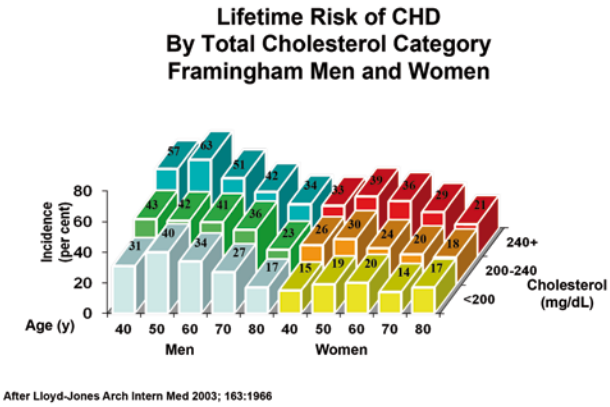

Fig. 2.3 Lifetime risk of coronary heart disease showed according to total cholesterol level groupings for men and women at various ages. (After Lloyd-Jones et al. [9])

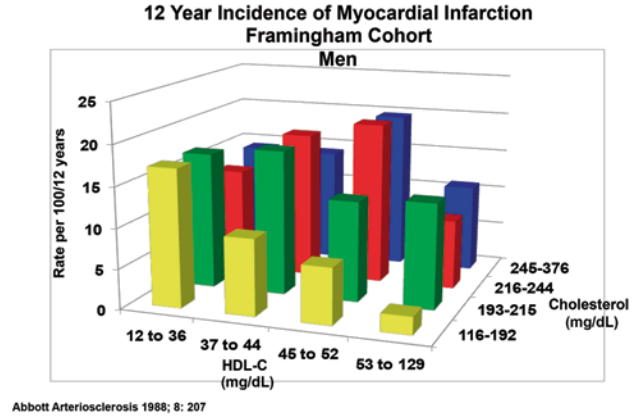

Fig. 2.4 Twelve-year risk of myocardial infarction shown for Framingham men according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol

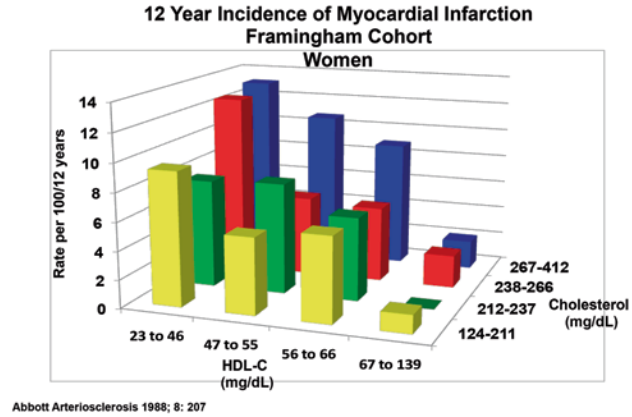

Fig. 2.5 Twelve-year risk of myocardial infarction shown for Framingham women according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol

Table 2.1 Determinants of LDL-cholesterol

Lower	Higher
Low dietary saturated fat	High dietary saturated fat
Low dietary cholesterol	High dietary cholesterol
High dietary polyunsaturated fat	Low dietary polyunsaturated fat
Estrogen	
Genetic	Genetic

LDL low-density lipoprotein

Table 2.2 Determinants of HDL-cholesterol

Lower	Higher
Male	Female
Androgens, progestins	Estrogen
Adiposity	Leanness
Cigarette use	No cigarettes
Low alcohol intake	High alcohol intake
Low dietary saturated fat	High dietary saturated fat
Genetic	Genetic

HDL high-density lipoprotein

Table 2.3 Prevalence* of dyslipidemia according to BMI levels in nonsmoker Framingham offspring study. (Adapted from Lamon-Fava et al. [69])

		Body Mass Index Level (kg/m^2)					
		<21	≥21 to <23	≥23 to <25	≥25 to <27.5	≥27.5 to <30	≥30.0
Men (n)		(27)	(72)	(188)	(347)	(253)	(240)
	Triglycerides (>200 mg/dL)	0	6.9	8.0	14.4	20.9	27.1
	Elevated LDL-C (>160 mg/dL)	7.4	11.1	18.6	24.5	26.9	25.0
	Low HDL-C (<35 mg/dL)	7.4	8.3	9.0	13.8	19.0	24.2
Women (n)		(163)	(264)	(207)	(194)	(119)	(194)
	Triglycerides (>200 mg/dL)	0.0	1.9%	3.9%	9.3	15.9	14.9
	Elevated LDL-C (>160 mg/dL)	8.6	15.2	15.5	28.4	28.6	28.9
	Low HDL-C (<35 mg/dL)	0.6	1.1	0.5	2.6	2.5	7.7

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol All trends across BMI level $P<0.001$
Entries in table are percents

report from the Seven Countries Study [8]. In addition to the dietary influences, there are important genetic determinants for high LDL-C that underlie familial hypercholesterolemia and for low LDL-C in association with hypobetalipoproteinemia [21]. The prevalence of heterozygous familial hypercholesterolemia is approximately 1 in 500 persons, but the condition is more common in South Africa, presumably because of a founder effect [22]. Finally, LDL-C levels are lower in adult women prior to menopause, lack of naturally occurring estrogen in post-menopausal women is associated with higher LDL-C, and exogenous products containing estrogens such as oral contraceptives and post-menopausal estrogens may reduce LDL-C [23, 24].

Table 2.2 summarizes the population-based determinants of HDL-C. The key lifestyle factors associated with higher HDL cholesterol levels are reduced adiposity, absence of cigarette smoking, greater exercise, and greater alcohol intake. For example, Garrison reported that relative weight was highly associated with HDL-C and there were weaker correlations between measures of obesity and VLDL-C or LDL-C [25]. There were very few lean individuals in some of the age groups, which prevented making firm conclusions concerning associations between lipoprotein cholesterol levels and adiposity in some men. Other associations between adiposity and lipoprotein cholesterol levels are shown in Table 2.3, as reported by Lamon-Fava. Greater body mass index was associated with hypertriglyceridemia, similar relationships tended to be observed for elevated LDL-C, and the opposite effect was observed for HDL-C

Table 2.4 Means for lipid levels according to self-reported weekly vigorous physical activity level Framingham offspring study. (Adapted from Dannenberg et al. [29])

Factor	Men		Women	
	<1 h	≥1 h	<1 h	≥1 h
HDL-C (mg/dL)	42.0	47.8*	53.5	61.1*
LDL-C (mg/dL)	133.5	135.0	126.3	131.6
VLDL-C (mg/dL)	29.3	20.5*	19.6	17.8

HDL-C high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *VLDL-C* very low-density lipoprotein cholesterol
*$P<0.001$

[26]. Longitudinal analyses were undertaken concerning weight change and lipid levels. Over an 8-year study interval in adults who were aged 25–34 years at baseline, their weight increased, HDL-C decreased, and both LDL-C and VLDL-C increased in both sexes [27].

Estrogen levels and treatments have been shown to have strong associations with HDL-C and LDL-C levels. As women go through menopause, their LDL-C levels typically increase, HDL-C declines or does not change, and LDL particles shift toward smaller sizes [24, 28]. Estrogen replacement therapy was associated with a shift toward higher HDL-C concentrations, lower LDL-C levels, and oral progestins tended to have unfavorable effects on the lipoprotein cholesterol levels [24].

Greater physical activity is highly associated with higher HDL-C levels. As shown in Table 2.4, among Framingham participants, an hour or more of vigorous physical activity was associated with HDL levels that were approximately 5.8 mg/dL greater in men and 7.7 mg/dL greater in women [29]. Research in runners and other competitive athletes has consistently shown much greater HDL-C levels in athletes and the differences are attributable to the training level, lack of adiposity, and lack of smoking in such individuals [30–32].

The determinants of triglyceride levels are shown in Table 2.5. For many of the factors, the associations are in the opposite direction from HDL-C. Obese type 2 diabetic patients who consume a diet that is high in saturated fat are especially prone to have elevated triglycerides. Greater alcohol intake and estrogen use have been associated with higher triglyceride levels, and

Table 2.5 Determinants of triglycerides

Lower	Higher
High intake of omega-3 fatty acids	Obesity
	Greater saturated fat intake
	Diabetes mellitus
	Greater alcohol intake
	Estrogens

persons with very high triglycerides are treated with diet and medications to lower triglyceride levels. Metabolic conditions such as chronic kidney disease, the nephrotic syndrome, pancreatitis, and diabetic ketoacidosis may all lead to higher concentrations of triglycerides in the blood [33]. Additionally, genetic variants associated with deficient or abnormal regulation of lipoprotein lipase are associated with increased concentration of triglycerides [34].

Greater prevalence of very atherogenic lipoprotein cholesterol levels was observed in Framingham offspring participants with diabetes mellitus, and these results are shown in Fig. 2.6 for men and women. The diabetic patients were much more likely than nondiabetic participants to have low HDL-C, elevated triglycerides, and combinations of lipid abnormalities. Interestingly, the diabetic patients did not tend to have elevated LDL-C levels [35].

On average, cigarette smoking has been associated with HDL-C levels that are approximately 4 mg/dL lower in men and 6 mg/dL lower in women compared to nonsmokers. On the other hand, greater alcohol consumption was highly associated with higher levels of HDL-C in the Framingham offspring studies [36, 37].

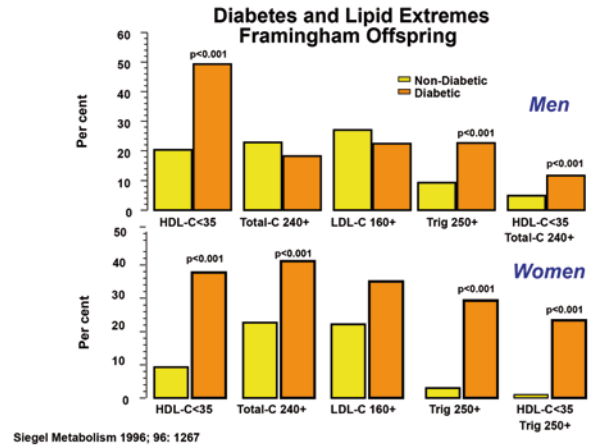

Fig. 2.6 Prevalence of lipid extremes in diabetic and nondiabetic participants is shown for Framingham offspring participant. (Adapted from Siegel et al. [35]). *HDL-C* High-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, Trig triglyceride

Table 2.6 Baseline lipoprotein risk factors and 14-year CVD incidence Framingham offspring study. (Adapted from Cromwell et al. [41])

Factor	Men			Women		
	No CVD	Yes CVD	*P* value	No CVD	Yes CVD	*P* value
HDL-C (mg/dL)	45	42	0.001	57	51	<0.0001
LDL-C (mg/dL)	134	138	0.09	126	143	<0.0001
Non-HDL-C (mg/dL)	158	168	0.0002	146	170	<0.0001
LDL Particle Number (nmol/L)	1509	1641	<0.0001	1344	1628	<0.0001

CVD cardiovascular disease, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Lipoprotein cholesterol, biomarker, and genetic investigations have increased greatly in the last two decades. Relevant to lipid research, these collaborations included measurement of insulin, apolipoproteins, lipoprotein particle number, and determination of gene variants such as apolipoprotein E that have been shown to be associated with lipid levels [38–40]. Lower HDL-C, higher LDL-C, higher non-HDL-C, and greater LDL particle number are all associated with greater risk of developing cardiovascular risk, as shown in Framingham analyses as well as others (Table 2.6) [41].

Metabolic Syndrome and Insulin Resistance

Since the late 1990s, it has been recognized that many individuals who develop CVD or diabetes mellitus tend to have greater adiposity, elevated triglycerides, low HDL cholesterol, elevated blood pressure, or impaired fasting glucose. Presence of three or more of these five traits was given the name metabolic syndrome, and it was felt that the syndrome was highly related to insulin resistance. As displayed in Fig. 2.7, principal components analysis showed that the metabolic syndrome traits clustered. The presence of three or more of the traits typically led to a doubling or tripling of risk for CVD, and more than a 20-fold greater risk for diabetes mellitus [42, 43]. A variety of other plasma biomarkers were subsequently used to study these phenomena, including laboratory biomarkers, traditional lipoprotein cholesterol levels, smaller LDL particles, and greater LDL particle number [28, 44–48].

Apolipoproteins

Lipoprotein particles include apolipoproteins, cholesterol, triglycerides, and phospholipid moieties. Protein assays became more prevalent

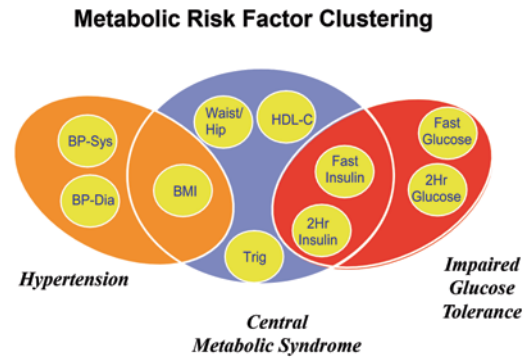

Fig. 2.7 Metabolic risk factor clustering is shown for domains related to hypertension, central metabolic syndrome, and impaired glucose tolerance. Models were developed from the Framingham offspring using principal components analysis. (Adapted from Meigs et al. [70]). *BMI* body mass index, *HDL-C* high-density lipoprotein cholesterol, Trig triglyceride

starting in the 1990s and associations with CVD were evaluated. For example, lipoprotein(a) originally tested using paper electrophoresis in Framingham was moderately associated with greater risk of heart disease and the effect was independent of LDL-C and HDL-C [49, 50].

Automated protein immunoassays were developed and apolipoprotein B was shown to be highly associated with LDL-C and greater CVD risk, especially in European studies [51, 52]. Concentrations of apolipoprotein A1 were highly associated with HDL-C and higher levels of each appeared to be cardioprotective. In analyses that compared prediction models with LDL-C and HDL-C versus models with apolipoprotein B and apolipoprotein A1, the overall ability to discriminate was similar. The results were interpreted as showing that measurement of apolipoproteins did not improve estimation beyond the traditional analytic approach with total cholesterol and HDL-C to estimate risk for initial CVD events [38].

Blood levels of an unusual lipid particle, lipoprotein (a), are very heritable and higher levels are very common in persons of African ancestry. An elevated level has been shown to be a risk factor for premature CVD in white populations and also in African American population groups [49, 53, 54].

Apolipoprotein E is of special interest because inherited deficiency is associated with increased atherosclerosis in animal models, and genetic variants have been associated with abnormal lipids, CVD, and dementia. Within the Framingham population cohorts, it was reported that higher concentrations of LDL-C were related to the presence and number of apolipoprotein E4 alleles present and lower levels of LDL-C were seen in persons with the E2 allele [40]. Results for triglycerides were slightly different, and both the E2 and E4 alleles were associated with higher triglyceride concentrations. The E4 allele was found to be present in approximately 24% of the Framingham participants and, on a population basis, it was estimated that approximately 10–15% of CVD could be attributed to the presence of the E4 allele. Separate analyses showed that the E4 allele was highly associated with greater risk for Alzheimer's disease and relative protection from dementia was found for persons with the E2 allele [55, 56].

Genetic research related to lipids led to a variety of collaborations with other laboratory scientists and other large population cohorts. Initially, these efforts included analyses with a limited number of genetic markers. Analyses were extended to include a large number of single-nucleotide polymorphisms and genome-wide association studies [57–60]. Genetic screening for gene variants associated with familial hypercholesterolemia has been used in tandem with screening blood cholesterol levels in families that include persons with very elevated cholesterol levels. Researchers in Europe have used these cased screening strategies to help identify persons with familial hypercholesterolemia at an early age in an effort to institute lipid lowering in the pediatric and young adult age groups [61].

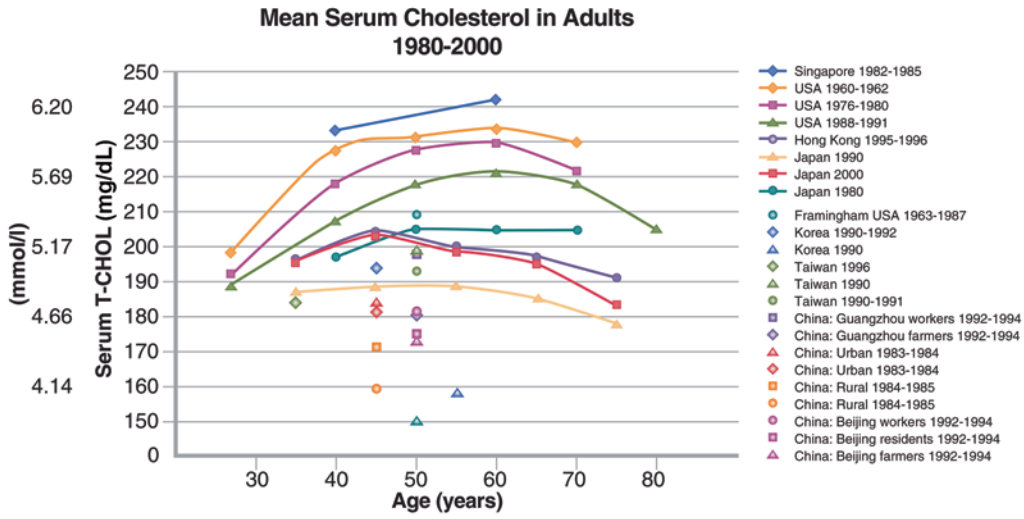

Fig. 2.8 Trends for mean serum cholesterol in adults from 1980 to 2000

Estimating Risk for CVD Outcomes

It was shown in the late 1980s that CVD risk could be predicted with reasonable accuracy using information obtained at the time of an outpatient clinical visit [62]. The variables used were age, sex, total cholesterol, HDL-C, systolic blood pressure, blood pressure treatment, diabetes mellitus, and cigarette smoking [63, 64]. A variety of lipid measures were assessed for potential use to estimate CHD and CVD risk. Concentrations of total cholesterol, HDL-C, LDL-C, non-HDL-C, and LDL particle number were shown to be highly associated with greater risk for CVD in the Framingham offspring [41]. Each of these measures has been used in modeling risk for initial CVD events, and specimens were most often obtained from healthy volunteers who were not taking lipid-lowering medications.

Debate has surrounded the utility of various lipoprotein cholesterol measurements and how they may be used in prediction equations. For example, the total/HDL-C ratio could be employed as a single lipid risk factor instead of using the total cholesterol and HDL-C as separate measures to estimate CVD risk. Alternatively, LDL-C and HDL-C could be used to estimate risk, but that approach did not appear to provide any advantage over simply using total cholesterol and HDL-C in the multivariable risk estimations [62]. As mentioned in the apolipoprotein section, use of the lipid measured apolipoprotein B and apolipoprotein A1 did not provide greater discrimination in estimation for risk of initial CVD events in comparisons with total cholesterol and HDL-C in multivariable models [38].

Mean Levels of Cholesterol Around the World

As seen in Fig. 2.8, cholesterol levels tend to rise in adulthood, peak between ages 50 and 60 years, and decline in older persons for a variety of population groups around the world. The review by Ueshima and coworkers shows that cholesterol levels that have historically been lower in Asia appear to be increasing in the past few decades [65]. Mean levels of total cholesterol in the control subjects from the INTERHEART participants who did not have a myocardial infarction are shown for men and women in Table 2.7 [66]. Among the male participants, the highest mean cholesterol levels (>200 mg/dL) were observed in Europeans and other Asians, intermediate levels (180–190 mg/dL)

Table 2.7 Means* and 95% confidence intervals for blood cholesterol levels INTERHEART controls. (Adapted from McQueen et al. [66])

Region	Men	Women
	Mean (95% confidence Interval)	Mean (95% confidence Interval)
European	203 (201–205)	216 (212–220)
Chinese	182 (181–184)	191 (188–194)
South Asian	184 (182–186)	193 (187–198)
Other Asian	208 (204–211)	222 (215–229)
Latin American	188 (188–190)	210 (205–215)
Arab/Persian	190 (187–192)	199 (194–205)
Black African	158 (153–164)	182 (175–190)
Colored African	190 (185–197)	211 (202–220)

*Means expressed in mg/dL units

were observed for most of the regions, and the lowest means (< 160 mg/dL) were seen in Black Africans. Similar patterns, with some notable differences, were observed for the female participants. Lower blood cholesterol in older persons partly explains why cholesterol levels in the elderly have not been highly associated with carotid artery disease or with stroke risk [67]. A Framingham analysis showed that cumulative exposures of cholesterol, blood pressure, and smoking were highly associated with greater carotid stenosis in person who underwent carotid ultrasound measurements at a mean age of 75 years [68].

Summary

This chapter has summarized many of the key findings related to lipid levels, risk factor levels, and vascular disease outcomes. At the outset of the study, the primary focus was simple measures such as total blood cholesterol and triglycerides and, over time, the scope expanded to include lipoprotein cholesterol quantification, apolipoproteins, genetics, lipid particles, and use of these measures in multivariable equations to estimate risk for the development of initial CVD outcomes. Research in lipids within populations continues to expand, and now we are beginning to trend over time effects of the treatments and the potential to assess CVD risk using on-treatment lipid measures in the future.

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Lipoprotein(a)

3

Byambaa Enkhmaa, Erdembileg Anuurad, Wei Zhang and Lars Berglund

Lipoprotein(a) Structure and Properties

Lipoprotein(a), Lp(a), was first described in 1963 by Berg [1]. After immunization of rabbits with human low-density lipoprotein (LDL) from different subjects, he noted an antigenic component present in the LDL fraction from some, but not all subjects, and termed this component p(a). Observations from early studies also demonstrated that the level of Lp(a) was determined genetically, different from other lipoproteins [2, 3]. Subsequent studies revealed that in contrast to LDL, Lp(a) had a limited species distribution and is present in humans and Old World nonhuman primates and likely evolved some 40 million years ago [3–5]. A variant of Lp(a) with a different molecular structure has also been described in the European hedgehog [6, 7].

Although Lp(a) shares properties with LDL, it has a number of features that set it distinct from other lipoproteins (Table 3.1a). The defining component of Lp(a) is its unique protein, apolipoprotein(a), apo(a), which has different properties from those of other apolipoproteins. Apo(a) is structurally heterogeneous and has many properties common with plasminogen as the apo(a) gene (*LPA*) evolved from the plasminogen gene during primate evolution (Table 3.1b) [8]. Both the *LPA* and plasminogen genes contain coding sequences for tri-loop structures stabilized by intrachain disulfide bonds, so-called kringle (K) domains. The plasminogen gene contains coding sequences for five different K domains (KI–KV), and two of these (KIV and KV) are present in the apo(a) gene (Fig. 3.1). In the apo(a) gene, the KIV motif has been expanded and diversified into 10 different types, referred to as KIV type 1 through 10. Of these, KIV types 1 and 3–10 are present as single copies, whereas the KIV type 2 motif is present as multiple copies, varying in number from 3 to more than 40 copies [9–13]. Reflecting the variable gene structure, there is a substantial size heterogeneity of the apo(a) protein ranging from overall 12 to more than 50 KIV repeats [13, 14]. As each KIV unit is coded by a 5.5-kb gene and represents a protein structure with a molecular weight of about 12 kDa, the result is a considerable size variability of the apo(a) protein between individuals. In addition, apo(a) contains one copy of the KV structure and also a variant of the carboxy-terminal protease domain, rendered inactive due to mutations compared to the original plasminogen template [3, 9, 10]. Resulting from the extensive size heterogeneity, most individuals have two apo(a) alleles of different size, and the degree of homozygosity is $<5\%$ [13, 15]. Thus, in the ma-

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Table 3.1 Properties and composition of (**a**) LDL and Lp(a) and (**b**) apoB, apo(a) and plasminogen

(a)

	LDL	Lp(a)
Buoyant density (g/mL)	1.04 (1.02–1.06)	1.07 (1.03–1.10)
Molecular mass (Da)	2.4×10^6	3.8×10^6
Molecular diameter (A)	210	250
Plasma half-life (days)	2.5–3.0	3.0–3.5
Fractional clearance rate (per day)	0.3–0.5	0.3
Lipid/protein mass	3.5	2.2

(b)

	ApoB-100	*Apo(a)*	*Plasminogen*
Molecular mass (kDa)	550	300–800	92
Chromosome	2	6	6
Plasma concentration (mg/dl)	50–200	0–200	100–200
Carbohydrate content (%)	9	28	2
Kringle structure	No	Yes	Yes

LDL low-density lipoprotein, *Lp(a)* lipoprotein(a)

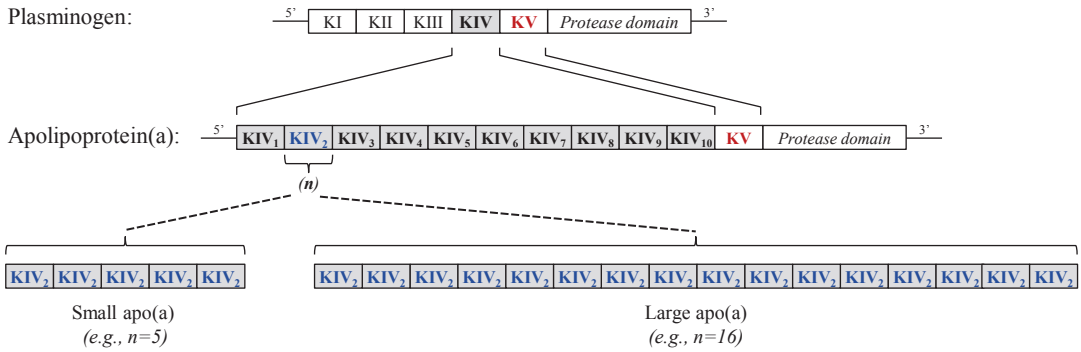


Fig. 3.1 Schematic models showing homology and differences between plasminogen and apolipoprotein(a) [apo(a)] genes. The plasminogen gene contains coding sequences for five different kringle (K) domains (KI–KV), and two of these (KIV and KV) are present in the apo(a) gene. In the apo(a) gene, the KIV motif has been expanded and diversified into ten different types, referred to as KIV_1 through KIV_{10}. Of these, KIV_1 and $KIV_{3–10}$ are present as single copies, whereas the KIV_2 motif is present as multiple copies, varying in number from 3 to more than 40 copies. Each KIV unit is coded by a 5.5-kb gene. The carboxy-terminal protease domain in the apo(a) gene is rendered inactive due to mutations compared to the original plasminogen template.

jority of all individuals, two different populations of Lp(a) particles carrying different-sized apo(a) contribute to the overall Lp(a) level [14–16]. As discussed below, there are some indications that the variability in particle distribution could impact on cardiovascular risk properties.

Lp(a) is primarily synthesized in the liver, but studies report an expression of apo(a) in human aorta and carotid arteries as well as in testes of cynomolgus monkeys [17]. The site of the formation of an Lp(a) particle, however, remains uncertain as studies using various cell systems [18–24] as well as in vivo kinetic studies in humans [25–28] have demonstrated intracellular, extracellular, and/or plasma membrane-associated assembly processes. A recent kinetic study using stable isotopes in humans found two different kinetic apoB pools within Lp(a) and LDL, supporting an intracellular Lp(a) assembly from apo(a) and newly synthesized LDL [29].

Apo(a) differs from other apolipoproteins also in its high carbohydrate content, about 30 % (Table 3.1b). The carbohydrate residues are mainly linked to the interkringle protein parts through

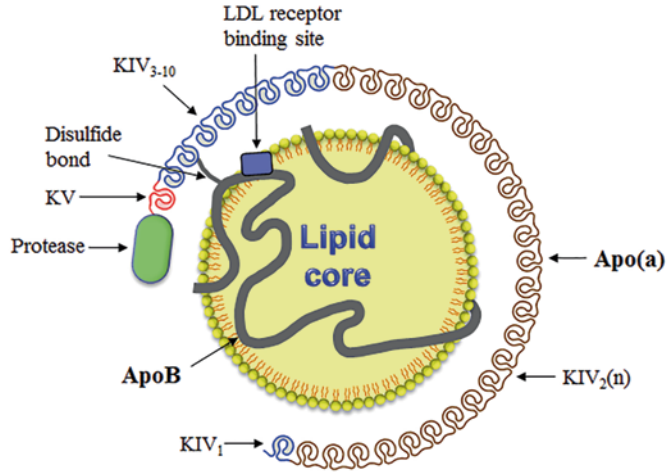


Fig. 3.2 Schematic model of Lp(a) particle. Lp(a) particle consists of an LDL-like part composed of cholesteryl-ester-rich lipid core and one molecule of apoB-100, and one molecule of a distinctive apolipoprotein—apo(a) evolved from plasminogen. Apo(a) in the liver binds to apoB-100 by a single disulfide bond. The unique structural and functional features of Lp(a) reside in apo(a), which has repeated tri-looped structures, i.e., kringles (K). Of the two kringles (KIV and *KV*) that are present in apo(a), KIV has 10 different types differing in amino acid sequence (KIV_1–KIV_{10}). KIV_2 represents copy-number variations that differ greatly interindividually, ranging from 3 to more than 40 copies. *LDL* low-density lipoprotein, Lp(a) lipoprotein(a), apo(a) apolipoprotein(a), apoB-100 apolipoprotein B-100

N- and O-linked glycosylations. The high carbohydrate content brings hydrophilic properties as well as a high degree of charges, likely to impact on Lp(a) physiological properties [30].

Physiological Properties

So far, no convincing physiological function has been ascribed to Lp(a). During formation of Lp(a), apo(a) is linked to an LDL-like particle through a single disulfide bond to apoB-100, and each Lp(a) particle contains one copy each of apo(a) and apoB-100 (Fig. 3.2). Cell-based experiments suggest that the assembly of Lp(a) occurs extracellularly but the exact mechanism remains to be elucidated [13, 18, 21, 31–36]. Apo B-100 and apo(a) are linked in their respective carboxy-terminal parts, and the binding site in apo(a) is located in KIV type 9, i.e., in a non-repeated portion of apo(a) [23, 37]. Beyond the covalent bond, noncovalent associations promote the association of apo(a) and apoB and contributes to bringing the putative unpaired cysteine residues in a position to form a covalent bond in the formation of Lp(a) [38–41]. The association of apo(a) occurs relatively close to the LDL receptor-binding site of apoB-100 [18, 35]. It seems likely that the attachment of a large, carbohydrate-rich, hydrophilic protein influences the binding of Lp(a) to the LDL receptor [15, 30], and this has been suggested to contribute to the apparent inefficiency of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors in modulating Lp(a) levels [42]. The lipid composition of Lp(a) reflects that of LDL and is dominated by cholesteryl esters, and the density distribution of the lipid moiety in Lp(a) mirrors that of LDL [43, 44].

Metabolic studies have demonstrated that Lp(a) levels are primarily impacted by the synthetic rate and that differences in the respective synthetic rates contribute to the interindividual variability in levels across apo(a) sizes [45, 46]. The mechanism of clearance of Lp(a) has not been resolved although a number of possibilities have been suggested [47–50]. Some studies have proposed a role of the kidneys as large apo(a) fragments have been found in the urine [51]. The narrow species distribution of Lp(a) limited to humans and nonhuman

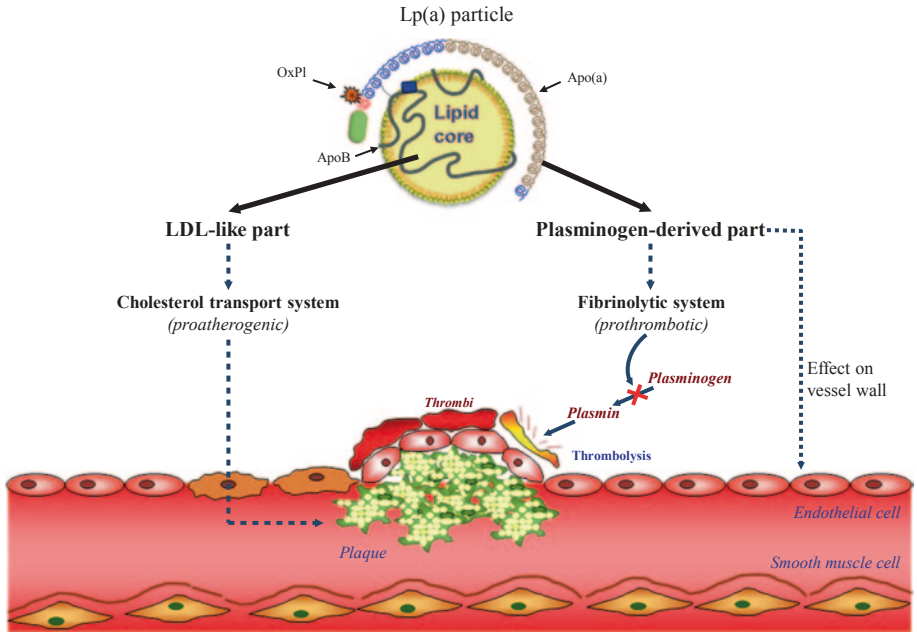


Fig. 3.3 Potential Lp(a) pathogenic mechanisms. Lp(a) can potentially promote the development of atherosclerotic cardiovascular disease by two key mechanisms. First, through its LDL-like lipoprotein component, Lp(a) can impact on cholesterol transport system exerting a proatherogenic effect. Second, through its unique plasminogen-derived protein structure—apo(a)—Lp(a) can interfere with fibrinolytic system exerting a prothrombotic effect. Lp(a) particles, in particular, those with small apo(a) sizes possess high potency to suppress fibrinolysis through tissue factor pathway inhibition with a simultaneous enhancement of coagulation. For more potential pathogenic mechanisms associated with a specific K unit of apo(a), see Fig. 3.4. Lp(a) lipoprotein(a), K kringles, LDL low-density lipoprotein, apo(a) apolipoprotein(a)

primates raises difficulties, as the interpretation of results from experiments where Lp(a) or apo(a) is introduced into animals, where it physiologically is not found, is challenging.

Overall, the presence of an LDL-like lipoprotein component together with a plasminogen-derived protein structure in Lp(a) suggests the possibility of an impact on both lipid transport and fibrinolytic systems (Fig. 3.3) [8, 52]. Indeed, in vitro, Lp(a) inhibits thrombolysis [53], and the apo(a) component inhibits a key positive feedback step of the plasmin-mediated Glu-plasminogen to Lys-plasminogen conversion [54]. In addition, small apo(a) isoforms have been reported to possess high potency to inhibit fibrinolysis and influence the ability of Lp(a) to interfere with fibrinolysis and promote thrombosis [55]. These findings suggest that Lp(a) or apo(a) might express prothrombotic, antifibrinolytic actions via inhibition of fibrinolysis with enhancement of clot stabilization and through enhanced coagulation by inhibition of tissue factor pathway inhibitor. However, while experimental studies provide data supportive of a prothrombotic role of Lp(a), these findings remain to be confirmed at the clinical level. Another mechanism suggested for Lp(a) is delivery of cholesterol to sites of injury and a role in wound healing [56].

Supporting a proatherogenic role, Lp(a) has been detected in the vessel wall, where it appears to be retained more avidly than LDL [57, 58]. The presence of highly charged hydrophilic carbohydrate structures in apo(a) might offer opportunities for interaction with vessel wall elements [30]. Several additional potential mechanisms have emerged [35, 59], including stimulation of endothelial cell permeability [60], induction of monocyte chemoattractant activity [61, 62], reduced tissue factor pathway inhibitor activity [63], increased endothelial plasminogen activator inhibitor-1 expression [35, 63], smooth muscle cell migration and proliferation [64, 65], interaction with

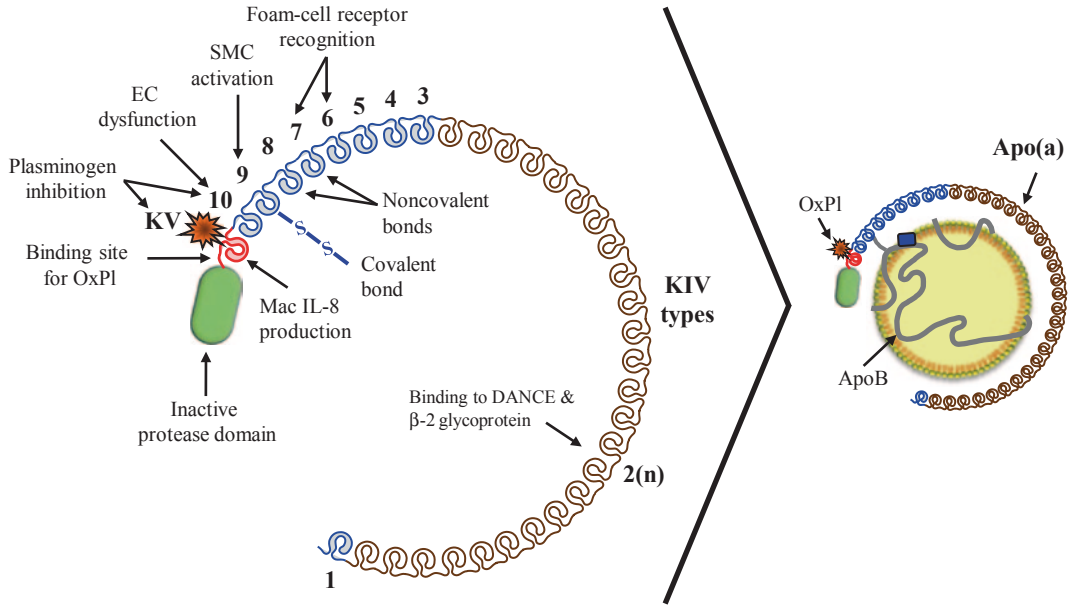


Fig. 3.4 Functional units in apo(a) that are potentially involved in the development of cardiovascular disease. Many of apo(a) domains have been identified as potential functional units that may play important roles in promoting atherosclerotic cardiovascular disease. Oxidized phospholipids (OxPl) with strong pro-inflammatory potentials are preferentially bound to Lp(a) particles via the KV unit of apo(a) [71, 73]. Apo(a) through its KV domain-stimulated interleukin (IL)-8 productions in human THP-1 macrophages derived from a human acute monocytic leukemia cell line [61]. Also, together with KIV_{10}, KV domain plays a role in the inhibition of the plasminogen activation [63]. Moreover, the strong lysine-binding sites in KIV_{10} mediate effects of apo(a) on human umbilical vein endothelial cell (EC) barrier dysfunction [60]. KIV_9 is found to be involved in smooth muscle cell (SMC) migration and proliferation [64], and through its unpaired cysteine (the only one in the molecule) mediates disulfide bond formation between apo(a) and apoB in the LDL-like part of an Lp(a) particle [23]. KIV_8 and KIV_7 domains have been shown to play critical roles in Lp(a) assembly through their effects on the formation of non-covalent bonds between apo(a) and apoB [38–41]. KIV_7 and KIV_6 domains mediate binding of Lp(a) to a receptor expressed on macrophage to promote foam cell formation [48]. Furthermore, KIV_2 is responsible for apo(a) protein heterogeneity, a major determinant of plasma Lp(a) levels, and mediates interactions with extracellular matrix protein—developmental arteries and neural crest epidermal growth factor-like (DANCE) [66] and β-2 glycoprotein [67]. The protease domain of apo(a) is considered to have lost its activity due to mutations. LDL low-density lipoprotein, Lp(a) lipoprotein(a), K kringles, apoB-100 apolipoprotein B-100

extracellular matrix proteins [66], and β-2 glycoprotein (Fig. 3.4) [67]. Lp(a) also contains lipoprotein-associated phospholipase A2 (Lp-PLA_2) that recently has emerged as a potential cardiovascular risk factor [68]. While these findings lend support for an atherogenic role of Lp(a), further studies are needed to establish specific mechanisms.

Although any physiological role of Lp(a) remains unclear, a potential role has emerged from the studies of Tsimikas et al. [69, 70], demonstrating that Lp(a) may have a unique physiological role to bind and transport pro-inflammatory, oxidized phospholipids (OxPl). Notably, Lp(a) is the preferential carrier of OxPl/apoB in human plasma [71, 72], and the OxPls are preferentially bound to the nonrepeated KV structure [73]. These results suggest that Lp(a) may act as a primary acceptor involved in the transport of OxPls from tissues or other lipoproteins [69, 70]. Thus, when present at low levels, Lp(a) would have an anti-inflammatory role participating in the transfer and degradation of OxPls. In contrast, the presence of OxPls in Lp(a) at higher levels may be proatherogenic, with an increased vessel wall uptake [74–76]. On the other hand, the existence of apo(a) does not necessarily imply a useful function as there is no evidence of evolutionary pressure to favor the development of

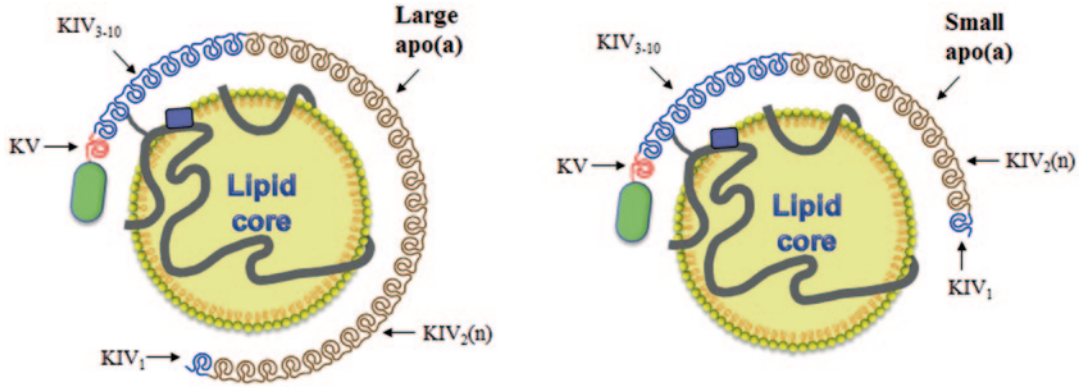


Fig. 3.5 Lp(a) particles with large versus small apo(a). Mirroring the variable gene structure (see Fig. 3.1), there is a substantial apo(a) protein size heterogeneity. Each KIV unit in apo(a) represents a protein structure with a molecular weight of about 12 kDa, and apo(a) size can differ considerably between individuals depending on the number of KIV_2 repeats. An example of a subject with a large apo(a) containing 25 KIV_2 (i.e., a total of 34 K) repeats and a small apo(a) containing 11 KIV_2 (i.e., a total of 20 K) repeats are shown. As the degree of apo(a) homozygosity is <5 %, the majority of individuals have two different populations of Lp(a) particles carrying different-sized apo(a). Lp(a) particles with smaller apo(a) isoforms are considered to be more atherogenic than those with larger apo(a) isoforms. Lp(a) lipoprotein(a), apo(a) apolipoprotein(a), K kringles

Lp(a). Thus, having undetectable or very low Lp(a) levels does not seem to bring any apparent disadvantages. Further, the differences between Africans and non-Africans regarding Lp(a) levels and apo(a) size allele variation could result from the distribution of the apo(a) allele in the subset of the population who left Africa and subsequently gave rise to other population groups [77]. While a positive function may have been important for survival during early stages of primate evolution, the importance of such a function may have decreased over time. In a state of neutral evolution, it could be possible for a variety of apo(a) sizes to evolve with no evolutionary disadvantage [15].

Lp(a) and Cardiovascular Disease

In view of its structure and similarity to LDL, much interest has focused on elucidating a cardiovascular risk role of Lp(a). A number of case–control studies, studying patients with established coronary artery disease (CAD), such as survivors of myocardial infarction, patients with symptoms of angina, and patients with angiographically diagnosed coronary disease, have shown a significant association between an elevated concentration of Lp(a) and CAD [78–83]. However, results from prospective studies have yielded conflicting results, ranging from a strong positive association between Lp(a) and CHD, to no association at all [84]. In a meta-analysis based on 27 prospective studies, Danesh et al. [85], demonstrated that subjects with an Lp(a) concentration in the top third were at 70 % increased risk of CAD compared with those of in the bottom third. Recently, Bennet et al. [86], reporting on data from 31 prospective studies, involving 9870 coronary heart disease (CHD) cases showed a significant association between Lp(a) and CHD after adjustment for established risk factors. Another recent meta-analysis of 36 prospective studies, involving more than 126,000 participants, provided additional support for the notion of Lp(a) as a cardiovascular risk factor and demonstrated a continuous association of Lp(a) levels with risk of CHD and stroke independent of traditional risk factors [87]. Furthermore, results from a study involving 58,000 participants reported that subjects with smaller apo(a) isoforms had a twofold higher risk of CHD or ischemic stroke than those who express larger isoforms [88]. Taken together, these studies underscore that elevated Lp(a) levels robustly and independently are associated with increased cardiovascular risk. Further, this association was continuous and without threshold and

Table 3.2 Hypothetical cardiovascular risk conferred by apo(a) isoform dominance pattern: Examples of two heterozygotes for apo(a) gene with the same apo(a) sizes and Lp(a) levels (60 mg/dl), but different apo(a) size dominant pattern

	Apo(a) allele size (KIV repeats)		Apo(a) isoform dominance		Lp(a) relative distribution		Allele-specific apo(a) (mg/dl)		Relative risk
	Larger	Smaller	Larger	Smaller	Larger	Smaller	Larger	Smaller	
Person #1	30	12	Yes		70%	30%	42	18	Lower
Person #2	30	12		Yes	30%	70%	18	42	Higher

Lp(a) lipoprotein(a), *apo(a)* apolipoprotein(a), *K* kringles

independent of high levels of LDL cholesterol or the presence of other cardiovascular risk factors.

In defining cardiovascular risk associated with Lp(a), the issue arises whether the extensive apo(a) size variation modulates risk factor properties (Fig. 3.5). Many studies have reported that Lp(a) levels in subjects who carry at least one small apo(a) isoform are associated with cardiovascular disease (CVD) or preclinical vascular changes [89–92]. In patients with end-stage renal disease (ESRD), Kronenberg et al. demonstrated that apo(a) phenotypes of low molecular weight were better predictors for the prevalence and the degree of carotid atherosclerosis than was the plasma Lp(a) concentration [93]. In prospective results from Bruneck's study, the same investigators have reported that apo(a) phenotypes of low molecular weight were independent predictors of advanced stenotic carotid atherosclerosis [90, 94]. Similar results have been reported from other studies, in Caucasians as well as African Americans [95, 96]. This has stimulated interest in assessing the amount of circulating Lp(a) associated with small apo(a) sizes, as these results support the view that Lp(a) particles carrying small apo(a) sizes might convey cardiovascular risk (Table 3.2). The approach to assess allele-specific apo(a) levels based on the combination of genotypic and immunoblotting data have been shown to be informative in this regard (Fig. 3.6) [97, 98].

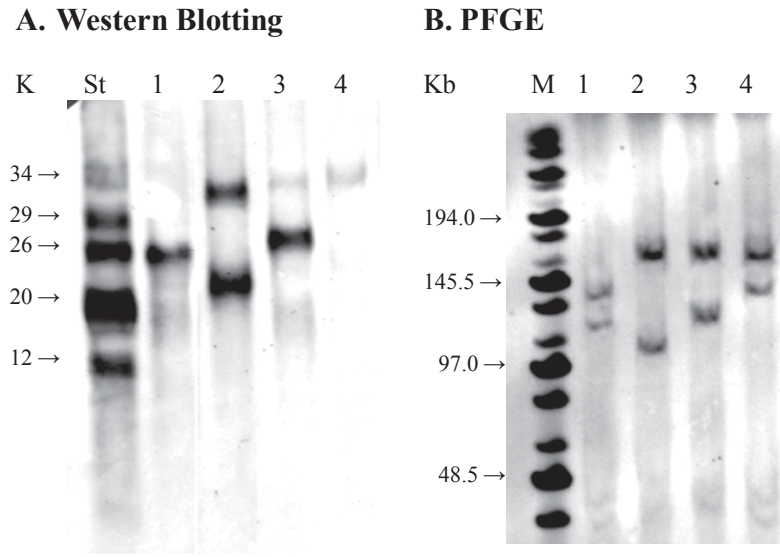
While an association with small isoform-specific Lp(a) levels and CVD has been found in men, the results are less convincing in women [95, 99, 100]. Furthermore, although Lp(a) levels have been reported to be increased in women with myocardial infarction [83], a prospective study on cerebrovascular disease demonstrated an association between Lp(a) and stroke in men but not in women [101]. This does not necessarily conflict with an association between plasma Lp(a) levels and CVD among women, although it could suggest that any risk carried by specific apo(a) sizes may be subject to modulation by gender-specific factors.

Based on a growing body of evidence, an European Atherosclerosis Society (EAS) Consensus Panel has recommended screening for elevated Lp(a) in patients at moderate-to-high risk of atherosclerotic CVD (Table 3.3) [102]. In addition to these patients, statin-treated patients with recurrent heart disease were also identified as targeted for screening of Lp(a) levels.

Regulation of Lp(a) Levels: Role of *LPA* Size Variability

As mentioned above, Lp(a) levels are largely genetically determined through the *LPA* gene [35, 59, 103]. Several transcription factors, including hepatocyte nuclear factor 1 alpha (HNF1A), sex hormones, and mediators of an acute phase response have been identified as potential regulators of Lp(a), and, in recent studies, bile acids acting through the farnesoid-X receptor have been demonstrated to have an Lp(a)-lowering effect [104–109]. While these studies contribute to increase our understanding of the regulation of Lp(a) levels, future studies are needed to translate these findings into any potential therapeutic effect.

Lp(a) levels are to a large extent influenced by apo(a) properties, most profoundly by the size polymorphism of apo(a), i.e., the number of KIV units. As described earlier, the plasma Lp(a) level in each individual represents the sum of Lp(a) carried by two apo(a) isoforms, coded by two apo(a) size alleles [14, 16]. In some cases, one or both of



PHENOTYPE Kringles (L/S)	-/ 26	33/ 23	33/ 26	33/ -
Lp(a) (mg/dl)	54	60	40	5
ASL, L (mg/dl)	-	24	4	5
ASL, S (mg/dl)	54	36	36	-

GENOTYPE Kringles (L/S)	29/ 26	33/ 23	33/ 26	33/ 29

Fig. 3.6 Determination of allele-specific apo(a) levels by phenotyping (Western blot, a) and genotyping (PFGE, pulsed field gel electrophoresis, b). The most common tool in assessing allele-specific apo(a) levels has been to estimate protein isoforms by sodium dodecyl sulfate (SDS)-agarose gel electrophoresis followed by immunoblotting (*panel a*). Apo(a) allele sizes are determined by PFGE using intact leukocytes embedded in agarose plugs (*panel b*)—providing the number of KIV repeats for each individual allele. The illustration here shows an example of different dominant expression patterns of apo(a) isoforms in plasma (**a**) and identification of null alleles that are not detected on the protein level by apo(a) genotyping (**b**) in four different individuals. The apo(a) isoform standard in Western blot contains five different apo(a) isoforms (12, 20, 26, 29, and 34 K repeats), whereas the mid-range PFG Marker I for genotyping contains 18 fragments ranging in size between 15 and 300 kb. For individuals #1 and #4, the genotype results show two different apo(a) alleles with respective sizes of 29/26 and 33/29 KIV repeats (b), of which only one is expressed in the Western blot (a). For individual #1, only the smaller apo(a) size allele (26 KIV) is expressed in plasma, whereas only the larger apo(a) size allele (33 KIV) is expressed in individual #4. For individuals #2 and #3, both apo(a) alleles are expressed in plasma, although the relative expression levels of the smaller versus larger apo(a) size alleles are different. In individual #2, the relative expressions of the larger versus smaller apo(a) isoforms are 40 versus 60 %, respectively. In contrast, in individual #3, the expression levels of the larger versus smaller apo(a) isoforms are 10 versus 90 %, respectively, resulting in different allele-specific apo(a) levels. K kringles, Kb kilobase, St standard, M marker, ASL allele-specific apo(a) level, L larger allele, S smaller allele. PFGE pulsed-field gel electrophoresis, Lp(a) lipoprotein(a), apo(a) apolipoprotein(a)

the apo(a) alleles do not give rise to any detectable apo(a) protein. In the latter case, Lp(a) levels are nondetected and, in the former case, the phenotypic pattern is due to the presence of apo(a) protein representing a single apo(a) allele [3, 97].

In general, there is an inverse relation between apo(a) size and Lp(a) levels, i.e., the larger the apo(a) size, the lower the Lp(a) levels. In a series of studies in baboon hepatocytes, White et al. showed that larger apo(a) sizes were more likely to be degraded intrahepatically compared to smaller apo(a) sizes, giving rise to the hypothesis that the lower Lp(a) levels seen for larger apo(a) sizes were due to a decreased production rate, most likely due to intracellular degradation [34]. However, there are exceptions to this rule as apo(a) levels are highly variable for a given apo(a) size between individuals [15, 97, 110].

Table 3.3 Lp(a) and cardiovascular risk: Evidence supporting that elevated Lp(a) levels cause cardiovascular disease (CVD) and the European Atherosclerosis Society (EAS) recommendations [102]

Study findings	Elevated Lp(a)
Human epidemiology	Direct association in numerous studies
Human genetic studies	Direct association in numerous studies e.g. for kringle IV type 2 polymorphism
Mechanistic studies	Mechanism similar to that of LDL cholesterol and/or prothrombotic/anti-fibrinolytic effects
Animal models	Proatherogenic effect in numerous studies
Human intervention trials	Niacin lowers Lp(a) levels—CVD risk reduction remains to be demonstrated
Mendelian randomization	Probably causal
EAS recommended levels	*Desirable Lp(a) levels:*
	Patients with CVD and/or diabetes: <80th percentile (<50 mg/dl)
	Other patients and individuals: <80th percentile (<50 mg/dl)
Whom to screen	*All subjects at intermediate or high risk of CVD/CHD who present with:*
	Premature CVD
	Familial hypercholesterolemia
	A family history of premature CVD and/or elevated Lp(a)
	Recurrent CVD despite statin treatment
	≥3% 10-year risk of fatal CVD according to EAS guidelines [207]
	≥10% 10-year risk of fatal and/or nonfatal CHD according to US guidelines [143]

Lp(a) lipoprotein(a), *CHD* coronary heart disease, *LDL* low-density lipoprotein

Table 3.4 Lp(a) levels across different ethnic populations [114, 125, 208, 209]

	Median level (mg/dl)	IQR (mg/dl)
Hutterites[a]	2.9	3.6
Chinese	11	4-22
Non-Hispanic Caucasians	12	5–32
Japanese	13	5–26
Hispanics	19	8–43
South Asians	20	10–43
African Americans	39	19–69

[a] Expressed as mean and standard deviation. *IQR* interquartile range, *Lp(a)* lipoprotein(a)

Further, studies in humans have shown that the smaller apo(a) size in a given individual does not always represent the quantitatively dominating Lp(a) variant [97, 111]. In addition, numerous studies have demonstrated differences in Lp(a) levels across ethnic groups, where higher Lp(a) levels have been shown for individuals of African descent compared to Caucasians or Asians (Table 3.4) [95, 96, 112]. Notably, studies in South Asians have also shown elevated Lp(a) levels as compared to Caucasians [113, 114]. Given the relative constancy of Lp(a) levels over the lifespan in any given individual and the strong genetic impact on Lp(a) levels, the inter-ethnic difference in Lp(a) levels have attracted considerable interest. The difference between Africans and non-Africans have been found to reside primarily for medium-size apo(a) alleles (Fig. 3.7) [96, 97, 100]. In a large recent study, some genetic variants mapped to this area were found to contribute to the difference, although the association pattern between plasma Lp(a) levels and allele sizes is likely complex and only in part determined by apo(a) size [115].

Regulation of Lp(a) Levels: *LPA* Gene Nonsize Polymorphisms

While apo(a) size polymorphism is a major predictor of Lp(a) levels contributing between 30 and 70% of the variation in Lp(a) concentrations,

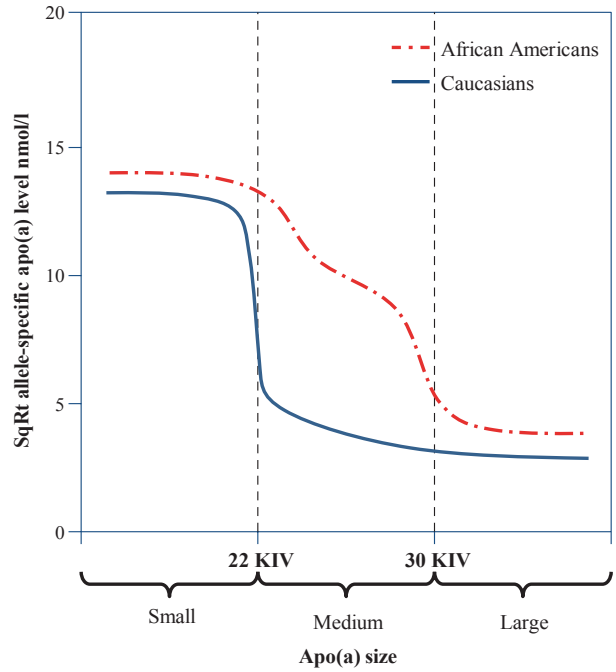


Fig. 3.7 Schematic graph demonstrating distribution of allele-specific apo(a) levels in Caucasians and African Americans across apo(a) size. Allele-specific apo(a) levels differ greatly across populations, being lower in populations of European descent (i.e., Caucasians) and higher in populations of African descent (i.e., African Americans). The major difference in Lp(a) levels between these two populations has been found to be due primarily to allele-specific apo(a) levels representing medium-sized apo(a) alleles. Lp(a) lipoprotein(a), apo(a) apolipoprotein (a) [97, 100]

other genetic variants at the *LPA* locus also contribute with varying effects across race/ethnicity (Table 3.5) [16, 116, 117]. In a biethnic study, variability at a pentanucleotide repeat (PNR; TTTTAn), locus, about 1 kb upstream of the *LPA* gene was found to influence allele-specific apo(a) levels in Caucasians, but not in African Americans, with a stepwise decrease with increasing PNR number >8 [118]. In a study of ESRD subjects, three single-nucleotide polymorphisms (SNPs) were reported to contribute to the interethnic African–Caucasian difference in Lp(a) levels [119]. One Lp(a)-increasing SNP (G-21A, reported to increase promoter activity) was more common in African Americans, whereas two Lp(a)-lowering SNPs (T3888P and G + 1/inKIV-8A, inhibiting Lp(a) assembly) were more common in Caucasians.

Several recent studies have addressed a role of a cluster of SNPs at the *LPA* locus in predicting Lp(a) levels. Two SNPs, rs3798220, located in the protease-like domain of apo(a) and rs10455872, which maps to intron 25, have repeatedly been associated with an increased Lp(a) level and a reduced copy number of KIV repeats. Thus, carriers of the I4399M, rs3798220 allele, had fivefold higher median Lp(a) level and a significantly smaller apo(a) isoform (17 KIV vs. 22 KIV) compared to noncarriers and a significantly higher risk for severe CAD [120]. A total of 19 *LPA* SNPs were tested in 7159 participants from three different subpopulations in the Third National Health and Examination Survey [121]. Fifteen out of 19 SNPs were associated with Lp(a) levels in at least one subpopulation, six in at least two subpopulations, but none in all three subpopulations. In non-Hispanic whites, three variants were associated with Lp(a) levels together explaining 7% of the variation in Lp(a) levels. In Mexican Americans, six SNPs were associated with Lp(a) levels and explained together 11% of Lp(a) variation. Non-Hispanic blacks had the greatest number of associations, i.e., with 12 SNPs, explaining 9% of variation in Lp(a) levels. However, although *LPA* genetic variants regardless of ethnic/race origin contribute to Lp(a) variation, lack of generalization of associations across subpopulations underscores a specific role of individual *LPA* variants as a contributor to the observed large interethnic and/or between-population variance in Lp(a) levels.

Table 3.5 Examples of studies that investigated the associations of the *LPA* and non-*LPA* gene polymorphisms with Lp(a) levels

Study	Year	Major polymorphisms	SNP/Chr/gene location/ function	Population	Association with Lp(a)
LPA gene					
Chretien et al. [119]	2006	G-21A	Increases promoter activity	CA and AA	All three SNPs contributed to higher Lp(a) levels in AA.
		T3888P G +1/in KIV-8A	Inhibits Lp(a) assembly		
Rubin et al. [118]	2006	C/T	Promoter region	CA and AA	A stepwise decrease in Lp(a) level with increasing PNR number >8 was observed in CA, but not in AA.
		Pentanucleotide repeat (TTTTAn)	1 kb upstream		
Luke et al. [120]	2007	I4399M/ rs3798220	Protease-like domain	CA	Carriers of the 4399M risk allele had five-fold higher median Lp(a) level and significantly smaller apo(a) isoform (17 KIV vs. 22 KIV) vs. noncarriers.
Clarke et al. [117]	2009	rs10455872	Maps to intron 25	CA	A total of 16 SNPs had significant effects on Lp(a) level. The two SNPs listed had the strongest associations with an elevated Lp(a) level and a reduced copy number of KIV repeat, and explained 36% of the variance in Lp(a) level.
		rs3798220	Protease-like domain		
Ober et al. [125]	2009	rs6919346	Maps to intron 37	CA and Hutterites	SNP rs6919346 was associated with Lp(a) level among CA. Both SNPs were significantly associated with an elevated Lp(a) level independent of the apo(a) size, and had a combined effect size of 4% on Lp(a) level among Hutterites.
		rs1853021 (+93C/T)	5′ untranslated region		
Lanktree et al. [114]	2010	rs10455872	Maps to intron 25	South Asians, Chinese and CA	Prevalent only in CA and associated with Lp(a) level and KIV repeat number.
		rs6415084	The same haplotype block as the KIV type 2 variation		Prevalent in all three ethnic groups and associated with Lp(a) level and KIV repeat number. Together with apo(a) size polymorphism, the SNPs explained 36% of variation in Lp(a) levels in CA, 27% in Chinese and 21% in South Asians.
Ronald et al. [122]	2011	rs3798220	Protease-like domain	CA	A total of nine SNPs were predictive of Lp(a) level and accounted for 30% of Lp(a) variance. The two SNPs listed associated with Lp(a) level adjusting for KIV repeat number, and explained 22% of Lp(a) variance. SNPs and apo(a) size polymorphism together explained 60% of Lp(a) variance.
		rs10455872	Maps to intron 25		

Table 3.5 (continued)

Study	Year	Major polymorphisms	SNP/Chr/gene location/ function	Population	Association with Lp(a)
Deo et al. [115]	2011	rs9457951	Intronic	AA	A number of SNPs were associated with Lp(a) level accounting for up to 7% of the variation, as well as >70% of the African-Caucasian interethnic difference in Lp(a) level. SNP rs9457951 expressed the strongest association and alone explained 5% of Lp(a) level variance.
		rs6930542	Intronic		
		rs10455872	Maps to intron 25		
		rs6922216	Intronic		
		rs1801693	Exonic, KIV type 9		
		T3888P G +1/inKIV-8A	Inhibits Lp(a) assembly		
Non-LPA gene					
Lopez et al. [128]	2008		Chr 2 *TFPI*	Spanish families	A new locus influencing Lp(a) levels has been identified, although any of selected 12 SNPs at the gene was not associated with Lp(a) levels.
Ober et al. [125]	2009		Chr 6	Hutterites	Significant effects of these eight genes on Lp(a) levels have been observed. Effects size of an individual SNP to total variance in Lp(a) levels varied between 0.1% (rs9364496) to 8.7% (rs9384296). Variations at least in six genes were significantly associated with Lp(a) levels independent of each other and of the apo(a) size polymorphism in this population.
		rs7745725	*SYNE1* intron 3		
		rs9384296	*TIAM2* intron 16		
		rs6917698	*ARID1B* intron 6		
		rs9364496	*SYTL3* intron 5		
		rs8191829	*IGF2R* intron 21		
		rs14224	*PLG* exon 7		
		rs4252125	*PLG* exon 11		
		rs2022991	*PARK2* intron 6		
		rs11966948	*PACRG* intron 5		
		rs14224	*PLG* exon 7	CA males	A replication study revealed that only a single SNP listed was associated with Lp(a) levels, and that the association was in linkage disequilibrium with KIV repeat numbers.
Berthold et al. [129]	2011	−174G/C	Chr 7 *IL-6* 5' flanking region	CA	The C-allele at the −174 locus was associated with elevated Lp(a) levels (>60 mg/dl).

Table 3.5 (continued)

Study	Year	Major polymorphisms	SNP/Chr/gene location/ function	Population	Association with Lp(a)
Zabaneh et al. [130]	2011	rs2296065	Chr 1 *GALNT2* intronic	CA	Variants in these four loci significantly associated with Lp(a) in WHII European cohort. A replication study in five other cohorts (EAS, NPHSII, PROCARDIS, SAPHIR and EPIC-Norfolk) failed to detect significant associations except for *TNFRSFF11A*.
		rs2919872	Chr 2 *FABP* 5′ upstream		
		rs2932971	Chr 4		
		rs2932976	*PPARGC1A* intronic		
		rs7231887	Chr 18		
		rs17069904	*TNFRSFF11A* intronic		

AA African Americans, *CA* Caucasians, *K* kringle, *SNP* single-nucleotide polymorphism, *Chr* Chromosome, *WHII* Whitehall II, *EAS* the Edinburgh Artery Study, *NPHSII* Northwick Park Heart Study II, *PROCARDIS* the Precocious Coronary Artery Disease Study, *SAPHIR* the Salzburg Atherosclerosis Prevention Program in subjects at High Individual Risk study, *EPIC-N* the European Prospective Investigation into Cancer and Nutrition study-Norfolk, *Lp(a)* lipoprotein(a)

Integrated Role of *LPA* Gene Size and Nonsize Polymorphisms

Given the strong genetic impact on Lp(a) levels, there is considerable interest in assessing the combined impact of different genetic variations affecting the *LPA* gene. In a study by Clarke et al., using a custom genotyping chip containing 48,742 SNPs in 2100 candidate genes tested in 6497 healthy subjects and patients with CAD, the genomic region 6q26-q27 spanning the *LPA* locus was significantly associated with presence of CAD [117]. The finding has been replicated in three independent cohorts, involving an additional 9440 subjects [117]. Further investigation revealed that of 16 SNPs at the *LPA* locus with significant effects on Lp(a) levels, two SNPs, rs10455872 and rs3798220, had the strongest associations with an increased Lp(a) level and a lower number of KIV repeats, explaining 36% of the overall variance in plasma Lp(a) levels and being associated with increased CAD risk. A total of seven SNPs including the former two SNPs, associated with Lp(a) levels in stepwise regression analysis, collectively explaining 40% of the variance in Lp(a) levels. In agreement with previous findings, these results demonstrated that the largest variability in Lp(a) levels was seen for smaller apo(a) sizes [96, 100]. Thus, variability in genetic loci mapped to small-size apo(a) can be expected to be a major predictor of Lp(a) levels. The results also confirmed the well-demonstrated previous association between small-size apo(a) and CVD.

Attesting to the importance of studying different population groups, a comprehensive analysis of genomic variation in the *LPA* locus conducted in a multiethnic population comprising of South Asians, Chinese, and Caucasians reported that the SNP rs10455872, reported by Clarke et al., was prevalent only among Caucasians [114]. In addition, SNP rs6415084 within the same haplotype block as the KIV type 2 variation, was significantly associated with both plasma Lp(a) level and KIV type 2 repeat number in all three ethnicities. Interestingly, SNPs and apo(a)-size polymorphism together explained a greater proportion of variation in Lp(a) level in Caucasians (36%) than in Chinese (27%) or South Asians (21%).

Furthermore, Ronald et al. [122], in a carotid artery disease cohort identified a set of nine SNPs that accounted for 30% of the variation in Lp(a) level, five of which overlapped with the set of seven SNPs described by Clarke et al. [117]. Six of these SNPs, of which four had previously been reported by others, were predictive of Lp(a) level conditional on the number of KIV repeats [114, 120]. After adjustment for KIV repeat number, SNPs rs3798220 and rs10455872 were strongly associated with Lp(a) levels, and together explained 22% of Lp(a) variance [114, 120]. It has been proposed that the nonsynonymous SNP rs3798220 may affect protein stability [120], whereas rs10455872 may be in linkage disequilibrium with regulatory variants [123].

There has been a considerable heterogeneity in estimating the portion of variance in Lp(a) level explained by SNPs alone or in conjunction with the copy-number KIV repeat. Thus, the two SNPs reported to explain 36% of variance in Lp(a) level in the study by Clarke et al., contributed 22% of the same variance in the study by Ronald et al. [117, 122]. Further, the combination of SNPs and KIV repeat polymorphism explained 36% of the variance in Lp(a) levels in a study by Lanktree et al. [114], whereas the corresponding contribution was considerably higher (above 60%) in the study by Ronald et al. [122]. An early study by Boerwinkle et al., indicated that 90% of the variance in Lp(a) level was attributable to variation at the *LPA* locus, of which the KIV repeat polymorphism accounted for 69% of the variance [124]. Among Hutterites (a founder population), two additional SNPs at the *LPA* locus (rs6919346 in intron 37 and rs183021 (+93C/T) in the 5′ untranslated region) have been significantly associated, although with a modest impact, with an elevated Lp(a) level independent of apo(a)-size polymorphism [125]. A replication study in unrelated Caucasian males overlapping with the study by Ronald et al. [122], confirmed a significant association between Lp(a) level and

SNP rs6919346, independent of the apo(a) gene size. The association between *LPA* +93C/T SNP and Lp(a) levels has been reported in African Americans [126], but in the opposite direction to that seen in Hutterites [127].

Recently, Deo et al., using a panel of ancestry information markers allowing an accurate estimation of the African ancestry proportion, investigated genetic variants in *LPA* that might contribute to the interethnic difference in Lp(a) levels in 4464 African Americans from the Jackson Heart Study [115]. A number of common SNPs, strongly associated with Lp(a) level, accounted for up to 7% of the variation in Lp(a) level, as well as >70% of the African–Caucasian interethnic difference in Lp(a) level. SNP rs9457951 expressed the strongest association and alone explained 5% of Lp(a) level variance. In contrast to previous findings in Caucasians [114, 117], no single common SNP has been found to explain a large portion of variation in Lp(a) levels in African Americans. These findings might reflect a possibility of limited linkage disequilibrium between the number of KIV repeats and common SNPs on the African ancestral background [114]. The variability in these reports illustrates the difficulty in accurately assessing the complex relationship between Lp(a), apo(a) gene size (copy number of KIV repeats), and other genetic variants at the *LPA* locus, and underscore the importance of identifying other common as well as rare genetic markers in the region.

Factors Beyond the *LPA* Gene Impacting on Lp(a) Levels

During recent years, the interest in an impact of genetic variability beyond the *LPA* locus in regulating Lp(a) levels has increased (Table 3.5). A genome-wide association study in a Hutterite population identified eight genes beyond the *LPA* gene on chromosome 6q26-q27 with significant effects on Lp(a) levels [125]. Variations in at least six of these genes were significantly associated with Lp(a) levels independent of each other and of the apo(a) size polymorphism. A replication study in Caucasian males reported an association of an SNP in the *PLG* gene with Lp(a) levels, where the association was in linkage disequilibrium with the number of KIV repeats [125]. Another genome-wide linkage study in Spanish families reported a locus influencing Lp(a) levels on chromosome 2 with several candidate genes, including *TFPI* gene [128]. Other studies have shown a positive association of the C-allele at the –174 locus of human *IL-6* gene with elevated Lp(a) levels (>60 mg/dl) [129].

Recently, a meta-analysis of candidate gene variants outside the *LPA* locus with plasma Lp(a) levels was undertaken in 14,500 participants representing six European cohorts [130]. In addition to the *LPA* locus, variants in other four loci were significantly associated with Lp(a) in one of these cohorts (Table 3.5). A further attempt to replicate these findings in the other five cohorts failed to detect significant associations except for one locus (*TNFRSFF11A*). At present, the use of specialized chips and variability across cohorts present limitations, further complicated by ethnic/race-specific differences in Lp(a) levels. Future studies employing a wider coverage of genetic variants (in- and outside of *LPA* locus) across different ethnic/race populations should bring more insights into the nature of Lp(a) heritability.

Lp(a) levels remain relatively unchanged over the life span and are unaffected by most clinical conditions. Kidney disease represents an exception as one of the few clinical conditions shown to impact on Lp(a) levels, and Lp(a) increases have been reported in patients with nephrotic syndrome as well as in patients with ESRD undergoing dialysis treatment (Table 3.6) [131–134]. In some studies in patients with chronic kidney disease, a difference in response across apo(a) sizes was noted, as the increase in Lp(a) levels was seen among carriers of larger but not smaller apo(a) sizes. Together with the previous observations on the association of smaller apo(a) sizes with CVD, these results underscore the value of assessing Lp(a) levels contributed by particles carrying specific apo(a) sizes, i.e., allele-specific apo(a) levels [97, 98]. The presence of inflammation has also been shown to affect Lp(a) and

Table 3.6 Conditions influencing Lp(a) levels beyond apo(a) size

Conditions	Comment
Kidney disease	Presence of chronic kidney disease and a decrease in glomerular filtration rate increase plasma Lp(a) levels. The increase in Lp(a) levels is reported to be isoform-specific, with an increase in large, but not small apo(a) isoforms. Patients with end-stage renal disease undergoing hemodialysis and patients with nephrotic syndrome have been shown to have elevated Lp(a) levels.
Diabetes mellitus	A presence of association between Lp(a) and diabetes mellitus (DM) has not been firmly established as findings from studies up to date are inconclusive. Results from WHS indicated that women with Lp(a) levels in higher quintiles [2–5] had a 20 % lower risk of incident DM compared with those in the lowest Lp(a) quintile.
Sex hormones	Estrogens and androgens have an Lp(a)-lowering effect. Differences in the Lp(a)-lowering effect has been observed between various hormonal therapies as well depending on dosage levels and route of administration.
Thyroid hormones	Patients with hypothyroidism generally have increased Lp(a) levels, while hyperthyroidism is associated with decreased Lp(a) levels. Treatment with thyroid hormone analogues may reduce Lp(a) level, but could result in thyroid hormone-related side effects.
Inflammation	Presence of inflammation affect Lp(a) and allele-specific apo(a) levels. Lp(a) and allele-specific apo(a) levels were increased in the presence of chronic, low-grade inflammation. During sepsis and burns, a pronounced reduction in plasma levels of Lp(a) was observed.

WHS Women's Health Study, Lp(a) lipoprotein(a)

allele-specific apo(a) levels, most profoundly expressed during sepsis conditions [98, 135].

Mendelian Randomization Studies and Lp(a)

Until recently, despite accumulating evidence from many large observational prospective epidemiological studies indicating associations between elevated plasma Lp(a) levels and increased risk for CVD, the causal nature of this association has been elusive. Associations between exposures and disease seen in observational studies are subject to confounding by other environmental and behavioral factors. Further, the lack of a specific Lp(a)-lowering therapy to date, as well as comparatively modest effect size have been major limitations in elucidating a cause and effect relationship. A Mendelian randomization approach, incorporating genetic information into traditional epidemiologic methods has become increasingly useful in obtaining evidence for the causal role of risk factors, including Lp(a) in the development of CVD [136, 137]. In contrast to most cohort studies, in which a single exposure–outcome association is ascertained, there are three separate associations in a Mendelian randomization study (Fig. 3.8). In the case of Lp(a), the three associations would be: (1) *LPA* genetic variants and plasma Lp(a) levels, (2) plasma Lp(a) levels and CVD, and (3) *LPA* genetic variants and CVD. An association of genotypes with risk of disease points to causality because population distributions of risk alleles are usually not confounded by behavioral and environmental factors, and because associations due to reverse casualty can be ruled out. Although several limitations exist in Mendelian randomization design, including insufficient statistical power, confounding due to linkage disequilibrium or population stratification, pleiotropy, and canalization, such an approach will likely be commonly used due to more readily available genetic data obtained through genome-wide association studies and advancements in gene sequencing. To some extent, previous studies where the presence of small apo(a) sizes have been associated with the presence of CAD can be seen as examples of such Mendelian randomization studies [95, 116, 136, 137].

Based on a Mendelian randomization approach, Kamstrup and colleagues, using data obtained from three large Danish cohorts based on the sum of KIV type 2 repeats from both alleles estimated with a quantitative polymerase chain reaction (PCR) assay, demonstrated that with

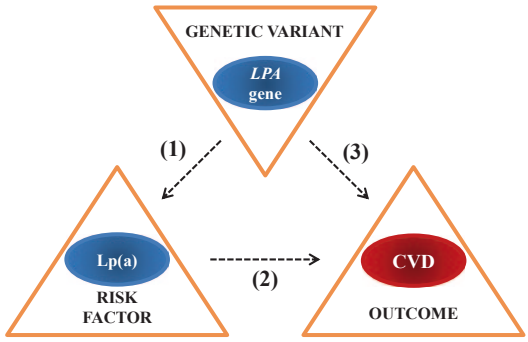


Fig. 3.8 Mendelian randomization—nature's randomized trial. A Mendelian randomization approach has become increasingly useful in obtaining evidence for a causal role of Lp(a) in the development of cardiovascular disease. There are three separate associations ascertained in Mendelian randomization studies focusing on Lp(a): (**1**) *LPA* gene polymorphisms (e.g., KIV repeat number) and plasma Lp(a) levels; (**2**) plasma Lp(a) levels and cardiovascular disease; and (**3**) *LPA* gene polymorphisms (e.g., KIV repeat number) and cardiovascular disease. CVD cardiovascular disease, Lp(a) lipoprotein(a), K kringles

increasing number of KIV type 2 repeats, plasma Lp(a) levels decreased as expected, and the KIV type 2 genotype explained about 25 % of the variation in plasma Lp(a) levels [136]. An increase in risk for myocardial infarction was observed with increasing levels of Lp(a), as with decreasing numbers of KIV type 2 repeats, consistent with findings in earlier studies [88]. However, contrasting results have also been published where Mendelian randomization showed no association between Lp(a) and early atherosclerosis among young Finns, demonstrating the need for a robust experience [138].

Employing the same Mendelian randomization approach, Kamstrup and colleagues recently attempted to answer the question about whether elevated Lp(a) levels primarily promote thrombosis or atherosclerotic stenosis [137]. Verifying their previous findings, Lp(a) levels and apo(a) KIV type 2 repeat tertiles were associated with risk of coronary, carotid, and femoral atherosclerotic stenosis, but not with the risk of venous thrombosis. These findings support the notion that Lp(a) promotes CVD through atherosclerotic stenosis rather than venous thrombosis.

Treatment and Intervention Options to Reduce High Lp(a)

Currently, there are no proven dietary or therapeutic interventions that effectively lower Lp(a) levels and at the same time reduce CHD outcomes, except apheresis procedures [139]. Many well-known lipid-lowering drugs, including statins, are ineffective in lowering Lp(a) levels. Among regimens, nicotinic acid [140], nateglinide [141], and estrogens for hormone replacement [142] can reduce Lp(a) levels, but by a relatively limited amount (Table 3.7) (10–30 %). In regard to guidelines and recommendations, the National Cholesterol Education Program Adult Treatment Panel III recommendations concluded that no clinical trial evidence supports a benefit from lowering Lp(a) levels with particular agents in the asymptomatic population [143]. More recently, the EAS Consensus Panel concluded, based on a robust and specific association between elevated Lp(a) levels and increased cardiovascular risk together with recent genetic findings, that elevated Lp(a), like elevated LDL cholesterol, is causally related to premature CVD [102]. The EAS Consensus Panel recommended a desirable Lp(a) level <50 mg/dl, as a secondary priority after LDL cholesterol reduction (Table 3.3). The arbitrary cutoff value of 50 mg/dl recommended by EAS is higher than the level of 30 mg/dl that for long time has been used to define the upper limit of the "normal" range. The most recent 2011 Guideline for Healthcare Professional from the American Heart Association recommended to treat other traditional cardiovascular risk factors in patients with high Lp(a) and to consider the administration of nicotinic acid up to 2 g/day, in conjunction with appropriate attention to blood pressure, LDL cholesterol, and glycemic control [144]. The lack of knowledge of Lp(a) metabolism, both regarding its formation and catabolism, raises considerable challenges in devising strategies to lower Lp(a) levels [13].

Nicotinic acid is the only approved major hypolipidemic agent that at present has proven efficacy in lowering Lp(a) levels [140]. The Lp(a)-lowering effect of niacin is graded and dose dependent, with a 25 % decrease in Lp(a)

Table 3.7 Treatment and intervention options to reduce plasma Lp(a) levels

Drugs	Regimen type	Cohort description	Sample size	Dosage	Duration	Δ Lp(a), %
Niaspan [151]	Extended-release niacin	Patients with established CVD, on simvastatin and ezetimibe	$n=1,718$	1500–2000 mg daily	3 years	−25%
Eprotirome [172]	Thyroid hormone analogue	Patients with hypercholesterolemia	$n=137$	25/50/100 μg daily	12 weeks	−27/−32/−43%
Aspirin [175]	Acetyl-salicylic acid	Patients with CAD or cerebral infarction	$n=70$	81 mg daily	6 months	−20%
Mipomersen [189]	Antisense inhibitor for apoB mRNA	Patients with homozygous familial hypercholesterolemia	$n=34$	200 mg/week s.c.	26 weeks	−25%
Anacetrapib [193]	CETP inhibitor	Patients with CHD or at high risk for CHD	$n=762$	100 mg daily	24 weeks	−36%
REGN727 [197]	PCSK9 inhibitor	Patients with heterozygous familial heper-cholesterolemia	$n=77$	150 mg every 2 week	12 week	−24%
Lipid apheresis [139]	LDL apheresis	CAD patients with Lp(a) >95th percentile	$n=120$	Apheresis every week, 2 week or 10 week	5 years	−73%

CETP cholesteryl ester transport protein, *CVD* cardiovascular disease, *CAD* coronary artery disease, *CHD* coronary heart disease, *PCSK9* proprotein convertase subtilisin/kexin type 9, *s.c.* subcutaneous, *Lp(a)* lipoprotein(a), *mRNA* messenger RNA

with 2 g/day and 38% decrease with 4 g/day [140]. However, use of niacin is commonly associated with broad range of side effects such as flushing, pruritus, and hyperuricemia, although extended-release niacin has been shown to have fewer dose-limiting adverse effects than regular or immediate-release niacin [145, 146]. Extended-release niacin monotherapy has been shown to decrease Lp(a) up to 40% from baseline, and a significant reduction in Lp(a) levels has been shown in diabetic patients with Lp(a) levels greater than 25 mg/dl [147–151]. A recent report from the Atherothrombosis Intervention in Metabolic Syndrome with Low High-Density Lipoprotein (HDL)/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial showed that extended-release niacin reduced Lp(a) levels by 21% but did not reduce CVD risk [152]. Of note, baseline median Lp(a) levels were modestly elevated, and further studies in subjects with high Lp(a) levels are warranted.

The efficacy of statins in reducing Lp(a) levels is not well established. While some studies have shown no effect of statins [153–157], or even an increase of Lp(a) concentrations during statin therapy [158–160], others have shown Lp(a)-lowering effect of statins [161–165]. A recent meta-analysis of randomized controlled trials suggests that atorvastatin may reduce Lp(a) levels [166].

Several types of sex hormones have been found to affect Lp(a) levels. Both androgens, such as danazol and tibolone, and estrogen treatment significantly reduce Lp(a) levels [167–170]. In a recent study, Danik et al. showed lower Lp(a) levels among women taking hormone replacement therapy and a higher hazard ratio for future CVD for the highest Lp(a) quintile compared to the lowest quintile in women not taking hormone treatment, while this was not seen for women taking hormone treatment [142].

Thyroid hormones play a critical role in differentiation, growth and metabolism, and have

substantial effects on lipid metabolism. Thyroid hormones increase expression of hepatic LDL receptors, decreasing apoB levels by a different mechanism than statins [171, 172]. In a recent study, eprotirome, a thyroid hormone analogue stimulating hepatic thyroid receptors, was found to decrease LDL cholesterol and Lp(a) to a similar extent when given on a statin background [172]. However, the development of eprotirome was recently stopped due to adverse effects seen in animal studies.

Aspirin has been widely used in patients with atherosclerotic diseases, and its efficacy in preventing CAD has been well established [173, 174]. Since Lp(a) has been suggested as an inflammatory marker with potential prothrombotic effects, it has been proposed that aspirin could decrease Lp(a) concentrations via its anti-inflammatory and antithrombotic potential. Low-dose (81 mg/day) aspirin treatment significantly lowered serum Lp(a) levels in patients with CAD or cerebral infarction, with a more pronounced effect in patients with higher baseline Lp(a) values [175]. In another study, serum Lp(a) decreased by 46% after 4 weeks of treatment with 150 mg aspirin. Kagawa et al. demonstrated that aspirin therapy may reduce apo(a) production from hepatocytes via suppression of apo(a) messenger RNA (mRNA) expression [176]. Findings from the Women's Health Study (WHS) demonstrated that benefits from aspirin therapy in a general primary setting may vary depending on the apo(a) genetic variation and similar results have been reported from the Atherosclerosis Risk in Communities (ARIC) study [177, 178].

Dietary approaches to decrease plasma Lp(a) levels have generally been disappointing and no influence of diet has been found in a number of studies [179–181]. Some notable exceptions include effects of *trans*-fatty acids and saturated fat. Mensink et al. reported an Lp(a)-increasing effect from diets rich in *trans*-monounsaturated fatty acids and a similar result was seen by Nestel et al., using a diet enriched in elaidic acid [182, 183]. In several studies, reduction of saturated fat has been associated with increased Lp(a) levels, and, in primates, addition of saturated fat resulted in a decrease in Lp(a) levels [184–187]. Several studies have demonstrated an increase in plasma Lp(a) level during low-fat, high-carbohydrate diet [187, 188]. Although the magnitude of change has been relatively modest, taken together the findings suggest a modification of Lp(a) levels by saturated and *trans*-fatty acids.

As conventional lipid-lowering agents, with the exception of nicotinic acid, has had limited impact of Lp(a) levels, much interest has been placed on development of novel agents. As Lp(a) is an apoB-containing lipoprotein, it would seem reasonable that approaches to inhibit apoB production would impact also on Lp(a) levels. Recent studies on mipomersen, an investigational antisense inhibitor of apoB synthesis in hypercholesterolemic subjects receiving statins and in patients with familial hypercholesterolemia (FH), support this concept as a reduction in Lp(a) levels of about 30% has been reported [189–192]. In addition, a recent study on anacetrapib, a cholesteryl ester transport protein (CETP) inhibitor, that raises HDL cholesterol and reduces LDL cholesterol, reported a 36% placebo-adjusted decrease in Lp(a) levels [193, 194]. A similar finding was reported in a recent small study among healthy subjects, where 150 mg/day anacetrapib treatment for 2 weeks resulted in a significant decrease in Lp(a) levels from baseline [195]. Recently, inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9) serine protease have been shown to reduce Lp(a) levels [196–199]. These findings are encouraging and also provide new avenues to increase our understanding about Lp(a) metabolic pathways.

Lipid apheresis is indicated to treat patients with extremely high cholesterol levels and has been successfully used in cases of severe FH [200]. As Lp(a) particles contain apoB, the apheresis approach to reduce apoB-containing lipoproteins has resulted in reduction of Lp(a) levels [201, 202]. In a recent longitudinal study in patients with CHD, regular lipid apheresis resulted in a significant reduction of Lp(a) levels and a decrease in the number of cardiovascular events [139].

Table 3.8 Methods used for quantification of Lp(a)

Methods	
Nonimmunologically based techniques	Lp(a) cholesterol assay
	Ultracentrifugation (sinking pre-β-lipoprotein)
	Lectin-affinity chromatography
	Electrophoretic separation followed by in situ enzymatic assay
Immunochemical techniques	Enzyme-linked immunosorbent assay (ELISA)
	Immunonephelometric assay (INA)
	Immunoturbidimetric assay (ITA)
	Fluorescent immunoassay (FIA)
	Electroimmunodiffusion (EID)
	Radioimmunoassay (RIA)
	Dissociation-enhanced ligand fluorescence immunoassay (DELFIA)

Lp(a) lipoprotein(a)

Measurement of Lp(a)

There are several issues related to measurement of Lp(a) levels and their use in guiding intervention strategies. One issue relates to the ability to accurately and precisely analyze circulating Lp(a) levels. The other issue relates to the interpretation of LDL cholesterol levels, as any cholesterol carried by Lp(a) would be interpreted as LDL cholesterol in clinical settings.

Regarding the first issue, as measurement of Lp(a) in plasma samples is commonly based on immunological methods, the apo(a) size heterogeneity provides a considerable challenge, and many of the commercially available Lp(a) assays are impacted by an isoform size-dependent bias (Table 3.8) [111, 203, 204]. Methodologies based on antibodies recognizing a repeated epitope will generally tend to underestimate Lp(a) levels in samples with smaller apo(a) sizes, while overestimating Lp(a) levels in samples with larger apo(a) isoforms. Other challenges include the association of apo(a) with apoB-100 and the high degree of similarity between apo(a) and plasminogen. At present, standardized and validated methodologies to measure Lp(a) that are insensitive to isoform size are not widely available. Although this lack of standardization of Lp(a) measurement have contributed to an uncertainty with regard to interpretation of results from clinical studies addressing the role of Lp(a) as a cardiovascular risk factor, the degree of uncertainty introduced is relatively modest in relation to the wide distribution of Lp(a) levels in the population. In view of this, the biological variability is unlikely to contribute to any misclassification of an individual's risk attributable to Lp(a) [59, 204].

Although high Lp(a) levels are found in a relatively limited number of subjects, this may lead to misinterpretation of LDL cholesterol levels. Any cholesterol carried in Lp(a) is currently interpreted as LDL cholesterol. In cases where patients are undergoing treatment aiming for an optimal LDL cholesterol (< 70 mg/dl), a considerable portion of this level could be due to Lp(a) and thus not likely to be affected by conventional lipid-lowering treatment, such as statins. Studies in patients with FH, where Lp(a) levels commonly are increased, have illustrated this phenomenon [205, 206]. Together, these issues illustrate some pitfalls regarding use and interpretation of Lp(a) levels and also the need to take Lp(a) into account when evaluating LDL cholesterol levels.

Conclusion

The putative role of Lp(a) as a cardiovascular risk factor has been subject to much debate over the years. Much progress has recently been made in understanding the genetic regula-

tion of Lp(a), and the role of genetic variability in assessing circulating Lp(a) levels and atherogenicity. A number of large studies, many using genetic data, have unequivocally shown a strong association between Lp(a) and CVD and some have indicated causality. Guidelines regarding screening and treatment of high Lp(a) levels have been published. Despite considerable progress, many questions regarding Lp(a), such as basic pathophysiology, metabolism, and function remain unresolved. Novel agents showing promise in modulating Lp(a) levels open opportunities for new advances and therapeutic possibilities.

Addendum

A recent pooled analysis of data from > 1,300 patients in four phase II trials with a 12-week intervention of a PCSK-9 inhibitor reported significant mean dose-related reductions in Lp(a) levels compared to controls [210]. The highest corresponding reduction (approximately 30 %) was observed with a regimen of 140 mg dosed every 2 weeks. Presently clinical guidelines do not specify if Lp(a) concentrations should be measured in the fasting or nonfasting state. In the Copenhagen General Population Study and the Copenhagen City Heart Study participants, Lp(a) concentrations were minimally affected in response to normal food intake (17 mg/dl at fasting vs. 19 mg/dl at 3–4 h since last meal) [211]. The LPA SNP rs10455872 was associated with aortic-valve calcification across multiple ethnic groups (European, African-American, and Hispanic-American cohorts) [212]. In two of these cohorts, where data on Lp(a) concentrations were available, rs10455872 was strongly associated with Lp(a) levels, and Lp(a) levels were associated with the presence of aortic-valve calcification. After adjustment for Lp(a) levels, the association between the rs10455872 SNP and aortic-valve calcification was attenuated in both cohorts. Further, a recent study suggested that the SNP rs10455872 influences mRNA levels of LPA (transcription or stability), while the SNP rs3798220 influences Lp(a) levels through effects on translation or protein stability [4]. In this study, Lp(a)-cholesterol level was significantly associated with a SNP near the APOA5–APOA4–APOC3–APOA1 gene cluster on chromosome 11q23 [213]. Finally, in our own study, a differential association of Lp(a) and allele-specific apo(a) levels with other apoB-containing atherogenic lipoproteins across African-American/Caucasian ethnicity, despite similar levels of these apoB-containing lipoproteins in the two groups, was seen [214]. After adjustment for the contribution of Lp(a)-cholesterol or Lp(a)-apoB, ApoB and apoB/apoA-1 remained consistently and positively associated with both Lp(a) and allele-specific apo(a) levels in African-Americans.

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Lipoproteins and Cardiovascular Disease Risk

4

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Introduction

Several lipoprotein subfractions are currently utilized in general practice to predict the future risk of cardiovascular disease (CVD). While some lipids such as serum triglycerides (TG) are generally measured in the fasting state, serum total cholesterol and other lipoproteins are less affected by a fasting versus a nonfasting state [1]. The risk stratification for CVD using only lipoproteins is complex because an individual's CVD risk (in addition to lipoprotein measurement) also is influenced by the concomitant presence or absence of other traditional CVD risk factors such as age, sex, diabetes mellitus (especially insulin resistance), high blood pressure, smoking, obesity, and prior CVD. Each of these risk factors influences lipoprotein levels through different pathophysiological mechanisms and therefore can influence an individual's overall global CVD risk. Additionally, several lipoprotein abnormalities also exist as part of various clinical syndromes such as polycystic ovary disease, familial combined hyperlipidemia, and type 2 diabetes mellitus. Lastly, several clinical characteristics such as central obesity, hypertriglyceridemia, hyperglycemia, hypertension, and low high-density lipoprotein (HDL) cholesterol coexist as part of the metabolic syndrome, and perhaps are important in the assessment of global CVD risk for an individual because of possible differences in treatment goals (compared to those without metabolic syndrome).

In this chapter, we focus on epidemiological data related to the common lipoproteins that are used to estimate an individual's risk for developing new CVD events, and then discuss some advantages and disadvantages of using different lipid components in select situations. We also include a brief discussion of how risk assessment tools utilize lipoproteins values in general assessment of global CVD risk, the concept of residual CVD risk, and the differences between various current dyslipidemia guidelines.

Total and LDL Cholesterol

Investigators from Framingham Heart Study, a large cohort study of Framingham, Massachusetts residents, without any history of coronary heart disease (CHD) first reported a positive association between total cholesterol and CHD risk [2]. Subsequently, the Multiple Risk Factor Intervention Trial (MRFIT) showed a J-shaped curvilinear relationship between serum total cholesterol and CVD mortality [3]. The Johns

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Hopkins Precursor Study, [4, 5] the Atherosclerosis Risk in Communities (ARIC) Study [6], Young Finns Study [7], and the Bogalusa Heart Study [8] subsequently confirmed that elevated serum cholesterol in young adulthood is related to the development of CHD later in life.

Studies across different populations reveal that those with higher serum cholesterol levels have a greater atherosclerotic burden and an elevated risk of CHD events compared to those with lower circulating cholesterol levels [9]. Based on all these observations and animal models of hypercholesterolemia, it was widely accepted that high serum cholesterol elevated CHD risk, and the majority of this effect was attributable to circulating levels of LDL-cholesterol (LDL-C). This hypothesis was further supported by observations from several research studies that used drug therapy to lower elevated serum cholesterol levels; most of these therapies that lowered serum LDL-C levels also lowered the risk of CHD in a continuous graded fashion [10, 11]. These observations then led to the concept of a causal relationship between LDL-C and CHD risk, and to the so-called cholesterol hypothesis. These data were complemented by other studies that were performed on "high-risk" patients (such as those with diabetes and prior CHD events) that also demonstrated a higher risk of subsequent CHD with higher levels of circulating LDL-C [12, 13]. Lastly, several genetic variants have been reported to influence blood LDL-C concentration [14]. Thirteen of these genetic loci that elevate circulating LDL-C levels were modeled jointly in the form of a risk score, and the score was associated with higher risk of myocardial infarction (Mendelian randomization design) [15]. Moreover, in a genome-wide association study of different genetic loci linked to coronary artery disease, ~20% of these loci were associated with higher LDL-C at a genome-wide significance level, [16] further strengthening the causal link between LDL-C and CVD. Therefore, it is widely accepted across the world and recommended that we can lower CHD risk by lowering blood LDL-C concentrations [17]. Of note, serum proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to LDL receptors and is involved in the degradation of LDL receptors. Interestingly, activating missense *PCSK9* mutations cause familial hypercholesterolemia. Null (loss-of-function) *PCSK9* mutations result in low LDL-C levels and protection from CHD [18]. Recent randomized trials using antibodies to *PCSK9* in injectable forms have shown successful results in decreasing LDL-C concentration [19, 20] although subsequent decrease in lowering CHD risk is yet to be seen.

The mean serum LDL-C level in the US population was 115 mg/dL in 2005–2006 in the National Health and Nutrition Examination Survey (NHANES) [21] compared with approximately 50 mg/dL in native hunter-gatherers, healthy human neonates, free-living primates, and other wild mammals. It is estimated that serum LDL cholesterol concentrations as low as 25–60 mg/dL are physiologically adequate for body functions [22]. Animal species that do not develop atherosclerosis generally have circulating LDL-cholesterol levels below 80 mg/dL. The circulating LDL-cholesterol concentration in the newborn infant is approximately 30 mg/dL or less, indicating that such low levels are safe [23]. Moreover, persons who have extremely low levels of LDL throughout life due to familial hypobetalipoproteinemia have documented longevity [18, 24].

LDL particle size also has been linked to atherosclerosis and indeed some investigators believe that the LDL particles concentration measured by nuclear magnetic resonance (NMR) predicts CVD better than LDL levels [25, 26]; however, the technique of measurement remains expensive and this lipid trait still needs confirmation in future studies.

HDL Cholesterol

In 1977, Framingham Heart Study investigators [27] and others [28] observed the protective role of serum cholesterol (HDL-C) concentrations in the context of CHD risk. Since then, multiple studies in different cohorts across the world have observed that lower the circulating HDL-C, the higher is the CHD risk. Moreover, studies from Framingham cohort have also

shown that higher HDL-C levels can potentially mitigate the higher risk posed by higher levels of other lipoproteins subfractions (such as LDL-C) [29]. Subsequent studies analyzing the risk of CHD among patients who are already taking therapy to reduce their LDL-C have also suggested that HDL-C continues to be inversely related to future risk of CVD even among those with lowest circulating levels of LDL-C [30]. However, more recent data from a larger sample of patients and using more potent statin therapy for primary prevention of CVD have suggested otherwise [31]. Nonetheless, strategies to increase circulating HDL concentrations with drug therapy have so far failed to show benefit in terms of lowering CVD risk. In particular, drug therapies with cholesteryl ester transfer protein (CETP) inhibitors [32–34] or niacin [35, 36] have not shown any significant advantage of lowering CVD risk by increasing HDL-C concentrations. In fact, the Heart Protection Study 2: Treatment of High Density Lipoprotein to Reduce Incidence of Vascular Events (HPS2-THRIVE) Trial, which was presented in March 2013, was stopped early [37] due to side effects from niacin and inability of study participants to tolerate the drug [36]. It is, therefore, unclear whether pharmacological ways of increasing HDL has any proven benefit in lowering CHD risk although nonpharmacological lifestyle measures such as increasing exercise, quitting smoking, and weight reduction still can raise circulating HDL-C concentrations and lower CVD risk. Additionally, genetic studies using Mendelian randomization have so far failed to show any link of increased cardiovascular risk from the genetic variants causing HDL-C deficiency, [38] or any protective effect from genetic variants which are associated with increased HDL-C [15]. In addition, it is noteworthy that CVD risk is not exaggerated in Tangier disease where apolipoprotein A-1 and HDL are absent [38].

Current estimates from NHANES data show a favorable trend of increasing circulating HDL-C concentrations in year 2007–2010 among US residents compared to corresponding values in the previous decade [39]. Specifically, the mean circulating values of HDL-C for men and women were 47.0 mg/dL and 57.6 mg/dL, respectively, in the latest US survey, being higher compared to corresponding values of 45.9 mg/dL (men) and 56.3 mg/dL (women) from 1999 to 2002 [39].

Non-HDL Cholesterol

In 1998, the use of non-HDL-C over LDL-C in CVD risk assessment was first proposed [40]. Thereafter, several studies have shown significant correlation between higher non-HDL-C and increased atherosclerosis, [41] CVD risk [42], and recurrent CVD in those with established disease [43]. National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines also recommend the use of non-HDL cholesterol as a secondary target after correcting LDL cholesterol levels in high-risk individuals. Data from long-term follow-up studies have also observed a strong association of circulating non-HDL-C concentrations and higher CVD risk [44]. The main advantage of measuring non-HDL-C is that it can be measured without the requirement for fasting, which can sometimes prove to be cost-effective and beneficial in clinical practice [45].

The distribution of circulating non-HDL-C concentration among adults varies by gender, with women generally having lower levels compared to men, and circulating levels continue to increase until age of 65 year (with a steeper increase in women) but decreases thereafter, based on the NHANES data [46]. Overall, the mean circulating levels of non-HDL-C have declined from 155 (95% CI, 153–157) mg/dL in 1988–1994 to 144 (95% CI, 143–145) mg/dL in 2007–2010 ($P < 0.001$ for linear trend) in serial national surveys in the USA [39].

Data are also available for circulating concentrations of non-HDL-C among children that clearly show a positive cross-sectional correlation with adverse risk factors such as BMI, waist circumference, and smoking, and a negative correlation with serum HDL concentrations [47].

Apolipoproteins

As has been discussed in previous chapters, all atherogenic particles such as very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), LDL, and lipoprotein (a) contain one molecule each of Apo B-100, and each chylomicron contains one molecule of Apo B-48. Therefore, theoretically it seems reasonable to consider that the measurement of circulating Apo B-100 concentrations will provide a definite measure of a person's atherogenic burden, or in some sense can also provide a definite measure of circulating non-HDL-C concentrations. Indeed, data from several population-based cohort studies have suggested that circulating Apo B-100 concentration is a better measure of CVD risk compared to LDL-C [48–51]. In addition, it is worth noting that the INTERHEART study, a study conducted in 52 countries, also reported that the Apo B/Apo A1 ratio was strongly associated with the risk of a future myocardial infarction [52]. Prior studies had also shown circulating Apo B levels to provide a direct measure of severity of coronary disease in patient undergoing cardiac catheterization [53].

However, data from ARIC [6] and Framingham Heart Study [54] did not show any added advantage of measuring Apo B-100: Apo A-1 ratio above and beyond traditional cholesterol measures (serum total cholesterol and HDL-C) in assessing CVD risk. Other investigators have reviewed the comparisons of the predictive utility of the circulating apolipoprotein measures and traditional serum cholesterol measures [55, 56]. Specifically, the Emerging Risk Factor Initiative that combined 68 study cohorts examined the risk associated with traditional blood cholesterol measures versus circulating apolipoproteins. The authors concluded that there is a limited advantage of using apolipoproteins in CVD risk assessment, with the only major benefit being that apolipoproteins can be measured in a nonfasting state [56]. Very recently, a publication from Emerging Risk Factor Collaboration investigators compared the predictive utility offered by Apo B-100 and Apo A-1 versus that provided by traditional lipid measures, and observed a very marginal increase in the prediction of CVD risk using the c-statistic as a metric [57]. To date, if the cost associated with obtaining additional newer lipid biomarker measures is considered, it may be hard to justify the measurement of circulating apolipoproteins for assessing CVD risk in the general population. Moreover, National Cholesterol Education Program ATP III guidelines had also favored the measurement of circulating non-HDL-C concentrations over assessing blood apolipoprotein levels for assessment of CVD risk [58]. Lastly, American College of Cardiology and American Heart Association's most recent guidelines conclude that currently, there is insufficient data in favor or against measurement of circulating apolipoprotein B concentration for assessment of CVD risk [59].

Triglycerides

Traditionally, the association between high TG and risk of CVD is not strong enough as assessed in older epidemiological studies [60]. There are several reasons that have been postulated to explain why the relations between TG and CVD risk may not be easy to demonstrate and may be challenging to interpret. Potential reasons include a greater intraindividual variability in circulating TG concentrations (sometimes up to >20%), a strong correlation of TG levels with other lipid parameters, and the complex relations between TG- and cholesterol ester-rich lipoproteins that may interact to increase risk of CVD. It is plausible that many of these factors together may influence the predictive utility of blood TG concentrations for CVD risk, especially when accounting for other lipid parameters.

Although many early cohort studies reported univariate associations of blood TG with CVD risk, these associations often became statistically nonsignificant upon adjustment for either total cholesterol or LDL-C [61]. Most of these earlier studies did not measure blood HDL-C concentrations, and some prospective studies have shown a stronger link between blood TG and CVD risk in people with lower circulating levels of HDL-C. Higher CVD risk associated with higher TG

levels is also observed among individuals with low serum LDL-C [61, 62] and in patients with diabetes mellitus [63]. Additionally, investigators from Women's Health Study have also shown that nonfasting TG levels are better predictors of CVD risk compared to the fasting TG levels [64].

The first meta-analyses of the TG–CVD relations, published in 1996, included 16 studies, (6 of these studies were from the USA). In that meta-analysis, the authors observed a 14% higher relative risk of CVD for men and 37% for women for each 1 mmol/L (88.5 mg/dL) increment in circulating TG concentrations, even after adjustment for HDL-C [65]. Subsequently, a second expanded meta-analysis evaluated 29 studies and reported a relative risk of 1.4 for the upper TG tertile compared with the lower tertile; this estimate improved to 1.72 with correction for "regression dilution bias" (accounting for intra-individual variation in TG levels) [66].

A more recent meta-analysis from the Emerging Risk Factors Collaboration evaluated 68 prospective studies and circulating TG showed a strong, stepwise association with both CVD and ischemic stroke; however, after adjustment for standard risk factors and for HDL-C and non-HDL-C, the associations for both CVD and stroke were no longer statistically significant [56]. The authors concluded that blood TG measurement provides no additional information above traditional risk factors including circulating concentrations of HDL-C and total cholesterol, although serum TG could be measured for other reasons such as prevention of acute pancreatitis [56]. However and in contrast, recent genetic studies have found a strong association of common variants with TG levels which are also linked to increased risk of coronary artery disease [67, 68]. One study showed that genotyped approximately 12,000 CHD patients found nonfasting remnant cholesterol which is rich in TG to be causally associated with higher odds of CHD, independent of reduced HDL-C [68], whereas investigators from another study revealed that strength of association between single nucleotide polymorphisms and TG levels correlated with the magnitude of its effect on CHD risk [67]. In fact more recently, investigators have successfully shown that loss-of-function mutations in *APOC3* are strongly associated with low serum levels of nonfasting TG and with a lower incidence of ischemic cardiovascular disease [69, 70].

Some investigators have related the change in circulating TG levels over time to the risk of CHD and have observed that among young men, a decrease in circulating TG levels after 5 years correlated with a parallel decrease in CHD risk, but the overall CHD risk in these people is still higher when compared to individuals who have persistently low blood TG concentrations [71].

Mean levels of serum TG after adjusting for age increased from 118 mg/dL in 1988–1994 to 123 mg/dL in 1999–2000 but then declined to 110 mg/dL in 2007–2010 [39]. These data were similar for men and women. NHANES data reveal that about 31% US adults have blood TG above the level of 150 mg/dL, the highest being among Mexican Americans (34.9%) followed by non-Hispanic whites (33%) and blacks (15.6%).

Controversy Between Guidelines for Risk Assessment

Several guideline recommendations have been made by different groups in Canada, Europe, and America and largely they seem to address dyslipidemia in similar way but have subtle differences in how to lower that risk. American Society guidelines from 2010 for cardiovascular risk assessment in asymptomatic individuals gave class III recommendation for measurement of circulating apolipoproteins or LDL particle size in addition to obtaining a standard fasting lipid profile [72], whereas the most recent guidelines from 2013 chose not to make any recommendation—for or against measurement of apolipoprotein B [59]. Interestingly, lipoprotein(a) has been reported to relate to increased vascular risk [73] by the Emerging Risk Factor Collaboration but the general consensus still remains against performing such additional testing in routine care because of the absence of clear thresholds for the initiation of treatment in those with higher circulating lipoprotein(a) concentrations, lack of therapeutic target levels, or lack of data regarding

efficacy of such treatments beyond those already recommended by lipid treatment guidelines directed by the standard lipid profile [74]. A consensus panel of lipid investigators also endorses the measurement of circulating lipoprotein-associated phospholipase A_2 concentrations in individuals with "intermediate-to-high" CVD risk in order to identify truly high-risk people in whom further aggressive risk reduction strategies to lower CVD risk can be justified [75].

Canadian national guidelines do differ in their recommendation regarding the use of circulating apolipoprotein B-100 as an alternative to LDL measurements and suggest that either of those two can be used as treatment targets [76]. These consensus guidelines from Canadian Cardiovascular Society also rated blood Apo B measurements as a class I recommendation (with level of evidence A) as an alternative to LDL cholesterol with treatment target as <0.80 g/L to lower CVD risk. In fact, a very recent publication from Women's Health Study also concluded that specifically in women, future risk of CHD may even be under- or overestimated when LDL-C alone is used compared to other measures such as apoliopoprotein B or non-HDL cholesterol [77]. Further, and in contrast, European guidelines do recognize the role of apolipoprotein B levels, and consider them equally informative as LDL-C; however, due to a paucity of available data on apolipoproteins as primary treatment targets, these guidelines give it a class IIa recommendation for measurement of apolipoproteins in assessment of CVD risk [78].

Concept of Residual Risk

In general, the term "residual risk" refers to individual's risk of developing a CVD event after the basic lipid profile measures (and other modifiable lifestyle risk factors) are reasonably well controlled with or without medical therapy. In this regard, first it is important to note that >90% of individuals with CHD have one or more elevated CVD risk factors [79]. In reality, the term of "residual risk" has been loosely defined by investigators and further treatment measures to reduce the residual risk have therefore been somewhat confusing. Largely, investigators believe that after appropriate level of therapy to reduce serum LDL cholesterol concentrations to target levels, the focus should be on raising blood HDL-C levels to reduce residual risk. This concept is further strengthened by studies which have examined the beneficial effect of HDL-C on endothelial function [80] and its anti-inflammatory effect [81] Additionally, investigators have observed reduction in level of atherosclerosis observed by using ultrasound of coronary arteries in patients with increase in blood HDL-C levels after treatment [82]. However, prior epidemiological evidence from studies supporting serum HDL-C as a predictor for subsequent CVD events after lowering circulating LDL-C to therapeutic goal levels [30] has been challenged [31]. Moreover, as noted above, to date the therapies that can raise blood HDL-C levels have not proven to be successful in reducing CVD risk [33, 83] and some also have resulted in increasing morbidity and mortality [84] It is conceivable that other biomarkers may be better predictors of residual risk, a premise that warrants additional research [85].

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Detection and Treatment of Children and Adolescents with Dyslipidemia

5

Peter O. Kwiterovich

Introduction

Dyslipidemia can present a particular challenge in infants, children, and adolescents due to their size, age, effects of sexual development, other medical conditions, use of medications, and demands of hygienic or pharmacologic treatment [1, 2]. In addition, there is controversy about screening for dyslipidemia in youth, and concerns about the long-term safety and efficacy of drug treatment starting in adolescence, and whether treatment starting early in life will decrease cardiovascular disease (CVD) events in adulthood [1, 2].

The clinical and scientific data supporting a strong link between pediatric dyslipidemia and other CVD risk factors and the early lesions of atherosclerosis in adolescence and young adulthood have expanded considerably over the past several decades [1, 2]. This chapter reviews these data, which provide a basis for updated recommendations and approaches to dyslipoproteinemia in youth [1, 2]. These data are conceptually divided into those related to youth with inherited disorders such as familial hypercholesterolemia (FH), and those from long-term observational studies of entire populations of children.

Disorders of Low-Density Lipoprotein Metabolism in Children and Adolescents Due to Altered LDL Receptor Activity

Familial Hypercholesterolemia

Heterozygous FH is the most common inherited disorder of lipoprotein metabolism with a prevalence of between 1/300 and 1/500. Due to founder effects, FH has a higher incidence in French-Canadians, Afrikaners, Christian Lebanese, and Finns. The University College London (UCL) low-density lipoprotein receptor (LDLR) variant database includes over 1288 different variants reported in FH patients, 79 % of whom are likely to be disease causing [7]. Examination of children aged 1–19 years, born to one parent with FH and a normal parent, showed that 45 % were affected with a mean low-density lipoprotein-cholesterol (LDL-C) of 230 mg/dL, compared to a mean LDL-C of 110 mg/dL in the unaffected children [8]. Cut points, which minimized misclassification, were 160 mg/dL for LDL-C and 235 mg/dL for total cholesterol (TC). FH should be considered in any child with an LDL-C or TC above these cut points. Finally, the percentage of FH children in the first decade

Unfortunately, Dr. Kwiterovich passed away on August 15, 2014 at the age of 74. He was an esteemed colleague and one of the world's foremost authorities on pediatric dyslipidemias. The scientific community will miss him dearly. The Editor really appreciates his seminal contribution to this book.

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(52%) significantly exceeded that in the second (39%) ($P<0.01$) [8]. We now know that the dearth of affected FH adolescents here were due to a 10–20% reduction that occurs in LDL-C in both FH and normal children during adolescence that can cause a false-negative result. The mechanism of lowering of LDL-C during adolescence is not elucidated [9].

Children are usually screened because a parent has hypercholesterolemia, or there is a family member with premature CVD. Such selective screening identifies an unacceptably low number of children with FH. In one study, a positive family history for coronary artery disease (CAD) was detected in 18.3% of 71 children with TC >95th percentile, and only 11.8% of 34 children with presumed heterozygous FH [10]. In contrast, universal lipid screening will detect 96% of FH children aged 1–9 years with a 1% false-positive rate [11]. Screening for FH should occur before adolescence to avoid the false negatives that can occur during this time of life.

FH heterozygotes usually are healthy children with no physical findings. About 5–10% manifest Achilles tendon xanthomas in the second decade. Increased carotid intima media thickness (cIMT) and decreased vascular reactivity start in FH children around 8–10 years of age [12, 13]. Children with FH are at a high risk of premature CVD as adults without treatment; 25% of males and 12% of females develop CVD by 40 years of age, and 50% of males and 25% of females do so at 50 years of age [14]. Coronary plaque burden was assessed by noninvasive computed tomography coronary angiography (CTCA) in 140 asymptomatic statin- treated middle-aged adult patients with FH. The extent of CAD was related to gender and TC levels and ranged from absence of plaque in one out of six patients to extensive CAD with plaque causing >50% lumen obstruction in almost a quarter of patients with FH [14].

FH Homozygotes If two parents are FH heterozygotes, there is a one in four chance that their child may inherit two faulty LDLR genes. These children may be true homozygous or compound heterozygous for two mutant alleles of *LDLR*. FH homozygotes are rare with a prevalence of about one in a million (1/500×1/500×1/4.) Thus, it is unlikely that most physicians will ever see an FH homozygote child. FH homozygotes usually have TC levels between 600 and 1000 mg/dL; planar xanthomas by the age of 5 years, notably in the webs of fingers and toes, the knees, and buttocks; and often develop life-threatening supravalvular aortic stenosis and CAD in the second decade [15]. FH homozygotes may require CTCA at baseline to exclude or investigate coronary atherosclerotic lesions. If a previous child has homozygous FH, prenatal diagnosis can be performed in future pregnancies to detect FH homozygotes.

Treatment of Youth with Heterozygous FH A diet low in total fat, saturated fat, *trans* fat, and cholesterol in youth with FH can be safely used to lower LDL-C about 5–10% [1–4]. The diet can be supplemented with plant sterols or stanols (usually purchased as commercially available margarines) to decrease cholesterol absorption and lower LDL-C another 5–10%. Most FH heterozygous children require high doses of more potent statins, or the addition of a bile acid sequestrant (BAS) or ezetimibe to a statin, to lower LDL-C sufficiently, i.e., to below the mean of normal children (<110 mg/dL) [1]. Decreased LDLR activity can also lead to moderate hypertriglyceridemia due to decreased uptake of intermediate-density lipoprotein (IDL; see above). High-density lipoprotein-cholesterol (HDL-C) levels can be normal, borderline, or low.

Treatment of FH Homozygotes FH homozygotes respond somewhat to high doses of statins (with a fall in LDL-C of between 100 and 200 mg/dL) [1]. Niacin can lower LDL-C in FH homozygotes about another 25%. Both statins and niacin decrease production of hepatic very low-density lipoprotein (VLDL), leading to decreased production of LDL (Fig. 5.1). Ezetimibe, a cholesterol absorption inhibitor (CAI), lowers LDL-C another 25% in FH homozygotes and has a Food and Drug Administration (FDA) approval [1]. Such triple-lipid-altering therapy in FH homozygotes may lower LDL-C to a range closer to that found in FH heterozygotes. FH homozygotes

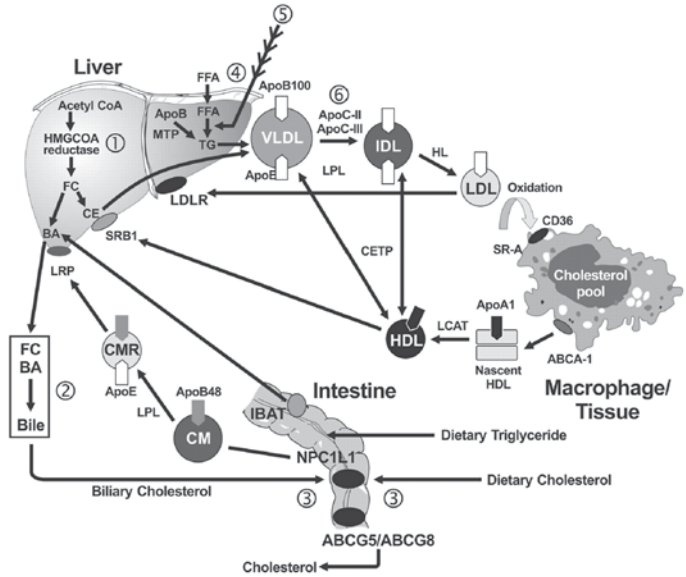

Fig. 5.1 Three major pathways of plasma lipoprotein metabolism are shown: (*1*) transport of dietary (exogenous) fat (*left*), (*2*) transport of hepatic (endogenous) fat (*center*), and (*3*) reverse cholesterol transport (*bottom*). Sites of action of the six major lipid-altering drugs on exogenous and endogenous pathways of lipoprotein metabolism are: (*1*) inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase by statins; (*2*) binding of bile acids by sequestrants, interfering with their reabsorption by the ileal bile acid transporter (IBAT); (*3*) binding of a cholesterol absorption inhibitor to the Niemann–Pick C1L1, decreasing the absorption of dietary and biliary cholesterol; (*4*) decreased mobilization of free fatty acids (FFA) by niacin, leading to decreased uptake of FFA by liver and reduced very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) production; (*5*) inhibition of TG synthesis by ω-3 fatty acids; (*6*) upregulation of lipoprotein lipase (LPL) and decreased production of apoC-III, an inhibitor of LPL, by a fibric acid derivative, leading to decreased VLDL-TG. The hepatic cholesterol pool is decreased by the agents at steps 1, 2, and 3, each leading to an upregulation of the LDLR. *LCAT* lecithin–cholesterol acyl transferase, *ABCA-I* ATP-binding cassette protein A-I, *ABCG* ATP-binding cassette protein G, *BA* bile acids, *CE* cholesteryl esters, *CM* chylomicrons, *CMR* chylomicron remnants, *SR-A* class A scavenger receptor, *SRB1* class B scavenger receptor. (Reproduced with permission from P. O. Kwiterovich, Jr.)

often require weekly LDL apheresis to lower LDL-C to a less atherogenic range. There is evidence that drastic lowering of LDL-C by LDL apheresis increases longevity in FH homozygotes and decreases cardiovascular morbidity in FH heterozygotes refractory to or intolerant of statins [15]. If LDL apheresis cannot be performed, then hepatic transplantation may be considered. The results of liver transplant are inconsistent. In one report, a 5-year-old FH child homozygous for the p.W577R *LDLR* defect had significant progression of coronary atherosclerosis despite intensive treatment with diet, statins, colestipol, and LDL-apheresis; at 16 years of age, liver transplantation was performed leading to normalization of LDL-C, and regression of symptomatic coronary atherosclerosis and xanthomas 9 years later [16]. In another report, a 14-year-old girl presented with severe bilateral coronary ostial stenosis and tight supravalvular aortic narrowing 10 years *after* liver transplantation, despite normalization of the lipids and apparently died of sepsis [17].

Newer Pharmacologic Treatment of FH Homozygotes The safety and efficacy of mipomersen, an antisense inhibitor of apolipoprotein B (apoB), was studied in 51 FH homozygotes, aged 12 years or older, on maximum lipid-altering therapy, in a randomized, placebo-controlled 26-week study, in which 34 patients were assigned to mipomersen, 200 mg/week, and 17 to placebo [18]. Of 46 patients who completed the study, the

mean fall in LDL-C of 24.7% in the mipomersen group versus 3.3% in the placebo was observed. Side effects included injection site reactions in 76% of the patients in the mipomersen group versus 24% in the placebo group. Four patients in the mipomersen group had increases in liver function tests exceeding three times the upper limit of normal with none in the placebo group. The drug, which was developed by Isis Pharmaceuticals, was approved in 2012 in the USA as an orphan drug and is marketed under the brand name of Kynamro by *Genzyme*. Kynamro was approved with a *Risk* Evaluation and Mitigation Strategy (REMS) which requires certification of pharmacies and prescribers, as well as documentation that the drug is being properly used with each new prescription. Possible liver toxicity and fatty liver will be monitored along with a variety of general medical side effects.

Inhibitor of Microsomal Triglyceride Transfer Protein in FH Homozygotes In a dose escalation study, the safety, efficacy and tolerability of a microsomal triglyceride transfer protein (MTP) inhibitor, lomitapide, was studied for 26 weeks or longer in six FH homozygotes aged 18 years or older and off all lipid-altering therapy for 4 weeks [19]. At a maximum dose of 1 mg/kg, inhibition of MTP caused a 50% reduction in the synthesis of LDL leading to close to 50% reduction in LDL-C. At the maximum therapy, accumulation of hepatic fat ranged from less than 10% to more than 40%. Cuchel et al. [20] did a single-arm, open-label, phase 3 study of lomitapide for 26 weeks in 29 adult FH homozygotes on current lipid-altering therapy. The lomitapide dose was escalated on the basis of safety and tolerability from 5 mg to a maximum of 60 mg a day. The median dose of lomitapide was 40 mg/day, which produced a 50% decrease in LDL-C. Five patients had moderately elevated LFTs which fell to normal after the study was completed; hepatic fat increased to 10%. In December 2012, the FDA in the USA approved lomitapide marketed as Juxtapid by Aegerion Pharmaceuticals. Juxtapid is also available only through a restricted REMS program.

Monoclonal antibodies that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9) activity have been developed and lower LDL-C up to 50%, by blocking PCSK9 and its effect on decreasing LDLR. This new agent can be used either in combination with a statin or alone in those with statin intolerance (see below). However, no data are available on the use of these PCSK9 inhibitors in children with heterozygous FH or FH3 due to PCSK9 mutations.

Ex vivo gene therapy Novel long-term persisting vectors derived from adeno-associated viruses and lentiviruses, are now available and investigations are under way to determine their safety and efficacy in preparation for clinical application for a variety of diseases including homozygous FH [21].

Phenocopies of FH Homozygotes Other primary disorders affecting LDLR activity (see below) can also present with planar, tendon, or tuberous xanthomas similar to FH homozygous children, and so can adolescents with the dominant form of dysbetalipoproteinemia (see below). Patients with secondary disorders of dyslipidemia accompanied by xanthomas include biliary cirrhosis, congenital biliary atresia, Alagille syndrome, myelomas, and Wolman disease [3, 4]. These disorders have other clinically salient findings to distinguish them from FH homozygotes.

Familial Ligand-Defective apoB-100

Heterozygotes with familial ligand-defective apoB-100 (FDB) may present with normal, moderately elevated, or markedly increased LDL-C [14, 18]. Hypercholesterolemia is usually not as severe in FDB as in FH heterozygotes. About 1 in 20 of affected patients with FDB has tendon xanthomas and more extreme hypercholesterolemia. FDB represents a small fraction of patients with premature CAD, i.e. no more than 1%. In FDB patients, there is delayed removal of defective apoB-100 LDL from blood despite normal LDLR activity but the clearance of triglyceride (TG)-enriched particles, VLDL remnants and IDL, is not affected. The most commonly recognized mutation in FDB is a missense mutation (p.R3500Q)

in the LDLR-binding domain of apoB-100 [22]. The frequency of FDB heterozygotes is about 1 in 1000 in central Europe [5], but appears less common in other populations. Dietary and drug treatment of FDB is similar to that used for FH heterozygotes.

Heterozygous FH3

The clinical presentation of heterozygous FH3 is indistinguishable from FH heterozygotes [5, 6]. FH3 results from mutations in PCSK9 [5, 6]. PCSK9 facilitates the degradation of LDLR and more recent data expand the potential pathways that may be involved in the molecular effect of PCSK9 on degradation of LDLR [6]. Gain-of-function mutations that increase PCSK9 activity decrease LDLR activity, producing marked hypercholesterolemia. Conversely, loss-of-function mutations that decrease PCSK9 activity increase LDLR and produce levels of LDL-C < 80 mg/dL and decreased CAD.

Autosomal Recessive Hypercholesterolemia

Autosomal recessive hypercholesterolemia (ARH) is a rare autosomal recessive disorder that usually presents with LDL-C in between those in FH heterozygotes and FH homozygotes [5]. Their onset of CAD often occurs later than that in FH homozygotes. Children with ARH often have large tuberous xanthomas. ARH is more prevalent in Sardinian families. Parents are consanguineous and most often have normal LDL-C levels. LDLR activity in ARH fibroblasts is normal, but it is defective in lymphocytes where LDL are not internalized normally. Recessive null mutations in a novel gene called LDL receptor adaptor protein 1 (LDLRAP1; also known as ARH) cosegregate with hypercholesterolemia in families with ARH [5]. LDLRAP1 protein contains a conserved phosphotyrosine-binding domain, and functions as an accessory adaptor protein that interacts with the LDLR via its cytoplasmic domain, enabling LDLR to engage with the clathrin-coated pit machinery for endocytosis. Fortunately, youth with ARH respond quite dramatically to treatment with statins and ezetimibe. A BAS may also be added to the statin for further reduction in LDL-C. Despite this therapy, some ARH patients, especially those with CAD, may also require LDL apheresis. [23].

Sitosterolemia

Children and adolescents with this rare, autosomal recessive disorder can present with normal to markedly elevated TC and LDL-C levels, tendon and tuberous xanthomas, premature CAD, and aortic stenosis [5]. Homozygotes manifest abnormal intestinal hyperabsorption of plant sterols (sitosterol, campesterol, and stigmasterol), shellfish sterols, and cholesterol. In normal humans, very little plant sterols are absorbed and plasma plant sterol levels are low (0.3–1.7 mg/dL) constituting < 1% of plasma total sterols. The levels of total plant sterols (13 to 37 mg/dL) in patients with sitosterolemia are very elevated and represent 7–16% of the total plasma sterols. Sitosterolemia patients often present in childhood with striking tuberous and tendon xanthomas, despite normal or FH heterozygote-like LDL-C levels. The diagnosis is made by documenting elevated plant sterols using high-performance liquid chromatography. The parents and obligate heterozygous siblings usually have normal LDL-C and only slightly higher plant sterol levels. Two ATP-binding cassette (ABC) half-transporters, ABCG5 and ABCG8 [5], normally limit the intestinal absorption of plant sterols and cholesterol and promote their excretion (Fig. 5.1). Sitosterolemia is caused by two mutations in either of the two adjacent genes that encode ABCG5 or ABCG8, thereby enhancing absorption of dietary sterols and reducing hepatic excretion of sterol into bile (Fig. 5.1). This leads to an increased hepatic content of cholesterol and plant sterols, suppression of LDLR, inhibition of LDLR synthesis, and elevated LDL-C.

Dietary treatment is *paramount* in sitosterolemia. In addition to the standard low-cholesterol, low-saturated-fat diet, plant foods with a high plant sterol content, such as oils and margarines

[1, 2], must be avoided. BAS are particularly effective in lowering plant sterol levels. Ezetimibe is also quite effective and approved by FDA for use in patients with sitosterolemia [24]. These patients respond less well to statins because 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase is already inhibited from the increased hepatic sterol content.

Cholesterol 7α-Hydroxylase Deficiency

Several patients have been described with a deficiency in the rate-limiting enzyme of bile acid synthesis, cholesterol 7α-hydroxylase, which converts cholesterol into 7α-hydroxycholesterol (Fig. 5.1, upper left). Thus, hepatic cholesterol can increase, decreasing LDLR and increasing levels of LDL-C and TG-enriched remnants, leading to both hypercholesterolemia and hypertriglyceridemia [25]. As with patients with sitosterolemia, these subjects are relatively resistant to statin therapy.

Lysosomal Acid Lipase Deficiency

Wolman Disease and Cholesteryl Ester Storage Disease

Human lysosomal acid lipase (LAL) is essential for the intralysosomal hydrolysis of LDL-derived cholesteryl ester (CE) into free cholesterol (FC). There are two general presentations of LAL deficiency, Wolman disease and CE storage disease (CESD), both autosomal recessive traits at the LAL locus. Wolman disease, associated with deficient LAL activity, leads to massive intralysosomal accumulation and is always fatal in early infancy. In contrast, CESD is characterized by very low levels of LAL activity that is sufficient to allow survival of the affected patients into adulthood. In one report, the splice defect in Wolman, which affects one of the invariable nucleotides of the splice consensus sequences (position +1), does not permit any correct splicing, whereas the defect observed in CESD (position −1) allows some correct splicing (3% of total LAL messenger RNA (mRNA)) and therefore the synthesis of some functional enzyme [26].

Metabolic Derangement There is an abnormal responsiveness of the LAL-deficient cells to the regulatory actions of LDL, namely decreased formation of FC from CE leading to decreased LDL-mediated suppression of the activity of HMGCoA reductase and to decreased LDL-mediated activation of cellular CE formation [27]. The enhanced synthesis of cholesterol contributes to increased VLDL production, increased apoB synthesis, leading to increased secretion of VLDL, elevated LDL, and low HDL (in CESD) [28].

Clinical Presentation Wolman disease is fatal with a very short life span, usually under 1 year [29]. Marked abdominal distension, persistent and forceful vomiting, watery stools, severe anemia, and failure to thrive start in the first weeks of life. Hepatosplenomegaly is invariably present and may be massive. The most striking feature is calcification of the adrenal glands. Circulating vacuolated lymphocytes and foam cells in bone marrow are almost constant findings.

In contrast to Wolman disease, CESD is characterized by a mild and relatively variable phenotype [29]. The principal and sometimes only sign, hepatomegaly, is evident at birth or in early childhood, increases with time, and eventually leads to hepatic fibrosis. Acute or chronic liver failure and jaundice have been observed and may require liver transplantation. Recurrent abdominal pain occurs frequently. Children and adults with CESD can present with a combined hyperlipidemia. Patients with CESD can survive for longer periods of time [29], and some adults with CESD develop premature atherosclerosis.

Treatment Infants with Wolman disease may respond either to transplantation of unrelated human leukocyte antigen (HLA)-mismatched umbilical cord-blood-derived stem cells, which restored normal LAL activity before permanent end-organ damage, [30], or to hematopoietic cell transplantation [31]. In a 9-year-old child with CESD, a notable combined dyslipidemia was shown to be due to increased formation of VLDL, increased biosynthesis of apoB and cholesterol, and low HDL-C [28]. Lovastatin (maximum dose 20 mg twice daily) reduced both the rate of cholesterol and apoB synthesis and the secretion of

Table 5.1 Levels of lipids, lipoproteins, and apoB in children with the most common genetic lipoprotein abnormalities. (Data are from Cortner et al. [51])

Lipoprotein disorder	Age (years)	Plasma concentrations (mg/dL)					
		TC	TG	HDL-C	LDL-C	APOB	LDL-C/APOB
Heterozygous, FH (n=20)	8.0±4.7	323±44	86±36	44±8	262±45	219±42	1.22±0.22
FCHL (n=65)	9.3±4.7	220±51	120±91	45±11	149±48	153±39	0.98±0.19
HyperapoB (n=11)	7.8±4.6	200±20	91±35	52±7	130±16	138±21	0.95±0.10
Normals (n=110)	8.7±1.8	162±31	70±39	51±10	97±27	85±20	1.15±0.20

TC total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *APOB* apolipoprotein ß, *FH* familial hypercholesterolemia, *FCHL*, familial combined hyperlipidemia

VLDL, leading to significant reductions in TC, LDL-C, and TG in CESD [28]. Clinical trials are ongoing to evaluate enzyme replacement therapy with recombinant human LAL enzyme.

Disorders of LDL Metabolism in Children and Adolescents Due to Overproduction of VLDL/IDL/LDL

Familial Combined Hyperlipidemia

Goldstein and colleagues [32] ascertained families in Seattle in whom the proband had premature myocardial infarction before 60 years of age. A family was considered to have familial combined hyperlipidemia (FCHL) if the proband had one of three lipid patterns, i.e., elevated TC (increased LDL alone), elevated TG (increased VLDL alone), or both TC and TG (increased LDL and VLDL) were elevated *and* a first-degree relative was affected with a *different* lipid pattern than the proband [32]. The FCHL families suggested this lipoprotein disorder was an autosomal dominant with delayed expression into adulthood.

Metabolic Abnormalities in FCHL The hypertriglyceridemia so common in FCHL is due to overproduction of hepatic TG-rich VLDL [33]. In plasma, there is increased activity of CETP, that facilitates the exchange of TG in VLDL for CE in LDL and HDL, leading to a CE-enriched VLDL and TG-enriched LDL and HDL. As the TG in LDL and HDL are hydrolyzed by hepatic lipase (HL), increased small dense LDL and cholesterol-depleted HDL are produced. Thus, elevated small LDL often accompanies the significant hypertriglyceridemia in FCHL. This phenotype is also referred to as hyperTG hyperapoB and is strongly associated with CVD, type 2 diabetes, visceral obesity, and the metabolic syndrome [34].

Insulin resistance is often an important etiologic component and results in increased delivery of free fatty acids (FFA) to the liver due to increased peripheral lipolysis of TG in adipocytes. Increased hepatic VLDL production occurs due to increased substrate availability of FFA, increased lipogenesis, and decreased apoB-100 degradation (leading to increased apoB synthesis).

Other abnormalities of TG metabolism in FCHL include the CE enrichment in the IDL remnants from VLDL, and postprandial hypertriglyceridemia.

Relevance of FCHL and hyperTG hyperapoB to Youth As the field of pediatric hyperlipidemia evolved, it became clear from observations from a number of pediatric lipid clinics, such as those reported by Cortner et al. [35] (Table 5.1), that FCHL was more prevalent than FH in their dyslipidemic families. hyperapoB was also expressed in this age group. As judged by the low LDL-C/apoB ratio, those youth with FCHL had increased numbers of small dense particles (Table 5.1). Those with FH had elevated LDL-C/apoB ratio indicating increased numbers of larger, more cholesterol-enriched particles. These results reflected the metabolic defects of increased production of VLDL/IDL/LDL in FCHL and decreased catabolism of IDL and LDL in FH. Finally, as judged by the extent of their apoB elevations, some youth judged to have FCHL had as many atherogenic LDL particles as those found in FH children. However, the LDL-C level was much lower in those with FCHL (Table 5.1).

The question of delayed expression of FCHL is not completely resolved. The simplest explanation is that differences are related to: mode of ascertainment, i.e., proband with premature myocardial infarction [33] or angiographically documented coronary atherosclerosis [36], versus referral of youth with known combined hyperlipidemia to lipid clinics [35, 37]; false negatives due to significant decrement of LDL during adolescence; and genetic heterogeneity especially given the apparent oligogenic nature of the etiology of FCHL [38].

Regardless of the multiple factors that may influence the expression of dyslipidemia in the pediatric age group, results of universal lipid screening indicate that there are a significant number of children with elevated LDL-C (>130 mg/dL) [39]. Of 20,226 10-year-old fifth-grade preadolescent students in West Virginia, a total of 71.4% of children met National Cholesterol Education Program (NCEP) guidelines for cholesterol screening on the basis of positive family history. Of those, 1204 (8.3%) had an elevated LDL-C >130 mg/dL, and 1.2% of these children had a dyslipidemia that warranted possible pharmacologic treatment (LDL-C >160 mg/dL) [39]. Of the 28.6% who did not have a positive family history (using NCEP guidelines), 548 (9.5%) had an LDL-C >130 mg/dL, 1.7% of whom warranted pharmacologic treatment. The panoply of fundamental defects causing LDL-C >160 mg/dL are not known at this time. Those with FH and defects in the LDLR can be distinguished from those with FCHL and hyperTG hyperapoB in whom the fundamental defects are not known. Clearly, a comprehensive screening assessment is important to identify those with elevated LDL-C, so appropriate hygienic measures or, in some cases, drug therapy may be instituted.

Genetics of FCHL FCHL accounts for up to 20% of premature CAD. Despite this, the identification of single gene defects underlying FCHL has remained elusive [38]. Two major strategies have been used to dissect the complex genetic background of FCHL, the candidate-gene and the linkage approach. A rather extensive list of genes has been associated with FCHL or its phenotypic traits. Some genes affect the FCHL phenotype in many pedigrees, while others are expressed in only several kindreds. One approach is to integrate these individual genes into common metabolic pathways such as adipocytes, production of hepatic fat and lipoproteins, and clearance of apoB-containing lipoproteins [38]. The adaptation of new traits beyond the lipid traits may identify novel pathways in FCHL. For example, variations of the activity or the expression of various nuclear factors (upstream stimulatory factor 1, USF1; transcription factor 7-like 2, TCF7L2; Hepatocyte nuclear factor 4 alpha, HNF4 alpha) [40], which regulate the expression of multiple genes involved in the metabolism of lipids or carbohydrates, may have a major role in the pathophysiology of FCHL.

Acylation-stimulating protein (C3adesArg/ASP) is an adipokine that acts on its receptor C5a anaphylatoxin chemotactic receptor (C5L2) to stimulate TG synthesis in adipose tissue [41]. A defect in ASP-mediated TG synthesis was previously described in a subset of hyperapoB/FCHL subjects. One of the 61 unrelated proband had a heterozygous variant (c.G968T) in C5L2, resulting in p.Ser323lle substitution in the carboxyl terminal region [42]. Eight family members of the proband were identified with one altered (±) C5L2 allele. Nine other family members had the wild-type (+/+) C5L2 sequence. The abnormal allele was associated with increased plasma TG, TC, LDL-C, apoB, and ASP. In cell-based ASP bioactivity assays, those with C5L2 (±) variant ($n=2$) had a 50% reduction in ASP-stimulated TG synthesis, glucose transport, and marked reduction in maximal binding (B(max)) [42]. By contrast, those with normal C5L2 alleles (+/+) responded normally. The p.Ser323Ile variant may alter C5L2 function and might be one molecular defect contributing to FCHL.

Treatment of Disorders of VLDL Overproduction Youth with the VLDL overproduction syndrome are likely to be insulin resistant, and a low-fat, high-carbohydrate diet typically has an adverse effect on the combined dyslipidemia lipoprotein profiles, often increasing TG, decreasing HDL-C, and increasing the total num-

ber of LDL particles. Consequently, a moderate low-fat diet in which simple carbohydrates are significantly decreased (low glycemic index) and unsaturated fatty acids replace saturated fatty acids is recommended [1, 2]. Affected children are often overweight or obese presenting a difficult problem to treat. Without some form of regular supervised aerobic exercise, at least every other day for 1 h, it is usually a significant challenge to optimize the lipoprotein profile.

The goal is to avoid the use of medications in youth with elevated TG, low HDL-C, and increased LDL particles. Patients with serum TG exceeding 500 mg/dL deserve more attention. Some children and adolescents will respond significantly to diet, aerobic exercise, and weight loss or weight control. Many will not. ω-3 Fish oils can be used in a dose of two 1-g capsules with breakfast and dinner (4 g per day). A 50% reduction in TG may be achieved but the response is pleiotrophic, especially if the diet is not strict [1, 2].

In regard to LDL, a child with FCHL 10 years of age or older may have an LDL-C >160 mg/dL (Table 5.1) and an elevated apoB, indicating a sufficiently elevated number of LDL particles to warrant treatment with a statin in those with a family history of premature CAD [1, 2].

Disorders of LDL Metabolism in Children and Adolescents Due to *Decreased* Production of VLDL/IDL/LDL: Disorders of *Reduced* LDL-C Levels

Abetalipoproteinemia

Abetalipoproteinemia is a rare, autosomal recessive disorder characterized by fat malabsorption, acanthocytes, and hypocholestrolemia in *infancy* [3, 43, 44]. Later in life, deficiency of fat-soluble vitamins leads to atypical retinitis pigmentosa, posterior column neuropathy, myopathy, and coagulopathy [3, 59, 60]. Fat malabsorption in infancy is associated with symptoms of failure to thrive (poor weight gain and steatorrhea) and lipid vacuoles invading enterocytes, which are visible on intestinal biopsy. Fat malabsorption is due to the inability to assemble and secrete chylomicrons from enterocytes. Symptoms of neurological problems begin during adolescence and include: dysmetria, cerebellar ataxia, spastic gait, and axonal peripheral neuropathy mimicking vitamin E malabsorption or Friedreich ataxia [3, 43, 44]. Anemia and arrhythmias may also present.

TC levels are exceedingly low (20–50 mg/dL). Total plasma apoB (apoB-48 and apoB-100) is undetectable, and thus the apoB-containing lipoproteins, i.e., chylomicrons, VLDL, IDL, and LDL, are absent. HDL levels are measurable but low. Vitamin E levels are extremely low. Parents of affected children have normal lipid levels.

The absence of plasma apoB was initially believed to be due to defects in *APOB*. However, the defect in synthesis and secretion of apoB-containing lipoproteins was found to be secondary to absent MTP, which normally permits the transfer of lipid to both apoB-48 and apoB-100 [3, 43, 44]. MTP is a heterodimer composed of the ubiquitous multifunctional protein, protein disulfide isomerase, and a unique 97-kDa subunit. Abetalipoproteinemia is caused by mutations that lead to the absence of a functional 97-kDa subunit.

Treatment of Abetalipoproteinemia The intake of fat is first reduced to 5–20 g/day to control steatorrhea, a step that results in marked clinical improvement and growth acceleration. The diet should also be supplemented with linoleic acid (e.g., 5 g corn oil or safflower oil/day). MCT as a caloric substitute for long-chain fatty acids may produce hepatic fibrosis, and thus MCT should be used with caution [3, 44, 45]. However, some children respond to MCT oil and/or fish oils [45]. Fat-soluble vitamins should be added to the diet. High-dose oral vitamin E (150–200 IU/kg/day) is essential to prevent or ameliorate neurologic and retinal complications. Vitamin E levels increase on treatment but remain low. Rickets can be prevented by normal quantities of vitamin D, but high doses of vitamin A (200–400 IU/kg/day) may be required to raise the level of vitamin A in plasma to normal. Enough vitamin K (5–10 mg/day) should be given to maintain a normal prothrombin time.

Table 5.2 Acceptable, borderline, and high plasma lipid, lipoprotein, and apolipoprotein concentrations for children and adolescents

Category	Acceptable	Borderline	High[1]	Low[1]
TC	<170	170–199	≥200	
LDL-C	<110	110–129	≥130	
Non-HDL-C	<123	123–143	≥144	
apoB	<90	90–109	≥110	
TG				
– 0–9 years	<75	75–99	≥100	
– 10–19 years	<90	90–129	≥130	
HDL-C	>45	35–45		<35
apoA-I	>120	110–120		<110

apoA apolipoprotein A, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TC* total cholesterol, *TG* triglycerides

All values are in mg/dL. Values for plasma lipid and lipoprotein levels are from the National Cholesterol Education Program (NCEP) Expert Panel on Cholesterol Levels in Children [93]. Non-HDL-C values from Bogalusa are equivalent to NCEP Pediatric Panel cutoff points for LDL-C [124]. Values for plasma apoB and apoA-I are from the National Health and Nutrition Examination Survey III (NHANES III) [126]. [1]The cutoff points for a high or low value represent approximately the 95th and 5th percentiles, respectively [93, 124, 126]. For HDL-C and apoA-I, the tenth percentiles are 40 and 115 mg/dL.

Hypobetalipoproteinemia

The phenotype of hypobetalipoproteinemia (hypobeta) is characterized by notably low levels of LDL-C and apoB, usually defined as less than the lower fifth percentile (Table 5.2). TC is low; VLDL-C and TG are low or normal. Hypobetalipoproteinemia can be primary, or secondary to anemia, dysproteinemias, hyperthyroidism, and intestinal lymphangiectasia with malabsorption, myocardial infarction, severe infections, and trauma.

Familial Hypobetalipoproteinemia Familial hypobetalipoproteinemia is inherited as an autosomal dominant disorder. The mutations occur in *APOB* but not exclusively so. Affected individuals are usually asymptomatic, the prevalence of CVD is quite low, and longevity is often found. Those with a defect in *APOB* have decreased synthesis of apoB and reduced secretion of VLDL from liver, which can lead to about a threefold increase in hepatic fat. A relatively large number of mutations in *APOB* cause familial hypobetalipoproteinemia [46]. Almost all of the mutations are either nonsense or frameshift mutations that create a premature stop codon and a truncated apoB. Familial hypobetalipoproteinemia has also been linked to a susceptibility locus on chromosome 3p21, and in some families is linked neither to APOB nor to chromosome 3p21 [46].

Familial Combined Hypolipidemia Musunuru and colleagues [47] reported that two nonsense mutations in the angiopoietin-like 3 gene (*ANGPTL3*) on chromosome 4 resulted in markedly decreased LDL-C that was accompanied by notably low TG and HDL-C, a phenotype they termed familial combined *hypolipidemia*. LDL-C and TG levels were inherited as codominant traits while the low HDL-C was only present in the compound heterozygous patients. This novel finding in this large family suggests a new mechanism for decreasing LDL-C in patients.

Loss-of-Function Mutations in PCSK9 The phenotype of hypobetalipoproteinemia is also found in those with a loss-of-function mutation in the PCSK9 gene [5, 6]. In this case, the low LDL results not from decreased production of VLDL but from enhanced LDLR activity due to the decreased PCSK9 function [5, 6] (see also above). Patients with this cause of familial hypobeta also have a considerable lifelong reduction in CVD [48].

Homozygous Hypobetalipoproteinemia

The clinical phenotype of children with homozygous hypobetalipoproteinemia depends upon whether they are homozygous for null alleles in *APOB* (i.e., make no detectable apoB) or homozygous (or compound heterozygotes) for other alleles, which produce lipoproteins containing small amounts of apoB or a truncated apoB [49]. Null-allele homozygotes are similar phenotypically to those with abetalipoproteinemia including fat malabsorption, neurologic disease, and hematologic abnormalities as their prominent clinical presentation; they require similar treatment. However, the parents of these children have low LDL-C levels in contrast to parents of children with abetalipoproteinemia, who are normolipidemic.

Chylomicron Retention Disease

Chylomicron retention disease (CRD) or Anderson's disease is a rare genetic condition that causes malnutrition, failure to thrive, growth failure, and vitamin E deficiency, among other complications [50, 51]. The diagnosis is suspected based on a phenotype of chronic diarrhea with fat malabsorption and very low, but not absent LDL-C and apoB. In contrast to abetalipoproteinemia and homozygous hypobetalipoproteinemia, TG are normal in CRD [51]. Fat-laden enterocytes and vitamin E deficiency are invariably present and hepatic steatosis is common. Muscular complications include increased creatine kinase (CK) levels and cardiomyopathy. Ophthalmologic and neurological complications in CRD are less severe than in other types of familial hypobetalipoproteinemia; for example, there is little acanthocytosis and no retinitis pigmentosa. CRD is due to mutations in *SAR1B*, leading to a defective Sar1b protein, which prevents the transport of prechylomicrons from the endoplasmic reticulum to the Golgi apparatus [51]. No postprandial chylomicrons or apoB-48 are detected. Dietary treatment is critical because when a low-fat diet supplemented with lipid soluble vitamins (A and E) and essential fatty acids, is implemented normal growth resumes with reduction of gastrointestinal symptoms. Departure from a low-fat diet produces a rapid relapse and recurrence of symptoms. Essential fatty acid deficiency is especially severe early in life and requires especially large amounts of vitamin E to prevent neurological complications.

Disorders of TG Metabolism in Children and Adolescents Due to *Decreased* Catabolism of TG-Rich Lipoproteins.

Disorders of Exogenous Hypertriglyceridemia

In patients with severe hypertriglyceridemia in lipid clinics, most have severely deficient LPL activity [52]. Mutations in *APOC2,* glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), and *APOA5* are rare but the associated clinical phenotype is severe [52]. LPL and GPIHBP1 defects present in childhood while apoC-II and apoA-V defects usually present in adults.

Defective or Missing Lipoprotein Lipase LPL deficiency is a rare autosomal recessive disorder that causes profound hypertriglyceridemia (as high as 10,000 mg/dL), due to massive increases in chylomicrons and the inability to clear dietary fat [3, 43]. Because CM replace water (volume) in plasma, sodium levels artifactually decrease between 2 and 4 meq/L for each 1000-mg/dL increase of plasma TG [3]. Marked hypercholesterolemia, e.g., 300–1000 mg/dL, is usually also present, secondary to the hyperchylomicronemia; the child will have a ratio of TG to TC of at least 5 and usually 10. VLDL-C is normal, and HDL-C and LDL-C are low.

Obligate heterozygous parents of affected children are often consanguineous and have normal lipid levels, or a moderate hypertriglyceridemia. To date, more than 80 mutations in the LPL gene have been reported [43]. Missense mutations predominate in the LPL gene, with a preferential location in exons 3, 4, and 5, and in the catalytic triad, Asp_{156}, His_{241}, and Ser_{132}.

The diagnosis of LPL deficiency requires a determination of postheparin lipolytic activity (PHLA) [3]. The intravenous injection of heparin (60 U/kg) releases the membrane-bound lipases into the bloodstream. Total and HL lipolytic activity are determined and the LPL activity calculated as the difference. HL activity is normal and LPL activity markedly decreased. The level of apoC-II is normal, as judged by immunochemical methods.

The disorder usually presents early in the first year of life. Creamy blood is often noted in a hematocrit tube or when blood is drawn. Abdominal pain is common, presenting as colic in the infant or as an acute abdominal condition later in childhood. Other clinical features may include eruptive xanthomas, hepatosplenomegaly, and lipemia retinalis. Premature atherosclerosis does not occur in LPL deficiency since the chylomicrons are too large to enter the vascular wall to be atherogenic.

Defects in apoC-ll Hypertriglyceridemia can range from 800 to almost 10,000 mg/dL in a patient with a deficiency of apoC-II. Elevated chylomicrons may be expressed alone or accompanied by increased VLDL [3, 43]. TC can also be normal or increased (about 150 to 1000 mg/dL). The LDL and HDL-C levels are below the fifth percentile of normal persons (Table 5.2). This autosomal recessive disorder is rare. Abnormalities of the apoC-II gene are caused by either small deletions or splice-site mutations [3, 43].

LPL activity is absent or very low. apoC-II is present in only trace amounts. Addition of apoC-II to plasma of these patients in vitro, or by blood or plasma transfusion in vivo, restores normal PHLA activity. The problem usually presents in adulthood with pancreatitis, although one homozygote developed pancreatitis at the age of 6 years.

Defective GPIHBP1 GPIHBP1 is anchored in endothelial cells and "picks up" LPL from interstitial spaces and shuttles it across endothelial cells to the capillary lumen [53]. When GPIHBP1 is absent, hypertriglyceridemia can be severe. In addition, mutations in either GPIHBP1 or LPL that affect the ability of either to bind to *each other* can cause hypertriglyceridemia [53]. PHLA is very low.

Reduced apoA-V Levels of apoA-V are negatively correlated with TG. apoA-V may normally stimulate proteoglycan-bound LPL at the endothelium of capillaries [54]. Despite its low concentration in plasma (~150 ng/ml), apoA-V modulates lipoprotein metabolism by binding to GPIHBP1, an interaction that effectively localizes TG-rich lipoproteins in the vicinity of GPIHBP1's other ligand, LPL [55]. A number of molecular variants in apoA-V are associated with low apoA-V and higher TG levels; the aggregation of five variants can be associated with TG of >1000 mg/dL [43].

Treatment of Profound Exogenous Hypertriglyceridemia Treatment of profound exogenous hypertriglyceridemia requires a stringent restriction in fat to 10–15 g/day. (See also above.) Intake of linoleic acid must be maintained as 1% of the calories. With severe hyperchylomicronemia, medium-chain triglycerides (MCT), which are absorbed directly through the portal vein, can be added to the diet as 15% of calories. MCT can increase compliance to the strict low-fat diet and often lower TG to a greater extent than expected. A subset of LPL-deficient patients with unique, possibly posttranscriptional genetic defects respond to therapy with MCT oil and ω-3-fatty acids by normalizing fasting plasma TG; a therapeutic trial with MCT oil and fish oils should, therefore, be considered in patients with LPL deficiency [3, 4]. Standard lipid-altering drugs such as statins, fibrates, and niacin are ineffective in LPL, apoC-II and GPIHBP1 deficiency.

Disorders of Endogenous Hypertriglyceridemia

Together, more than 20% of the susceptibility to hypertriglyceridemia now is accounted for by common and rare genetic variants [55]. Previously, the classical Fredrickson hypertriglyceridemic phenotypes (I, IIb, III, IV, and V), once considered to be distinct based on biochemical features, now

have a shared genetic architecture. Thus, the use of the phenotypes has been avoided since they are nonspecific for genotypes.

Familial Hypertriglyceridemia (FHT) In some families, plasma TC, LDL-C, and apoB levels are normal, and chylomicrons are absent but VLDL-C and TG levels are elevated (>95th percentile, Table 5.2). This phenotype can occur in dyslipidemic children from such families. FHT is distinguished from FCHL by showing that the affected parent and siblings of the proband have *both* normal LDL-C and apoB levels, in contrast to FCHL, where LDL-C is borderline high or elevated and apoB or LDL-P is significantly increased. VLDL particles in FHT and FCHL are both TG-enriched; however, VLDL and apoB are being overproduced in FCHL, while in FHT VLDL are not being overproduced but the hydrolysis of their TG are decreased abnormally. The basis for such slower hydrolysis of TG may be related to common genetic variants in LPL while the rarer genetic variant is not present. Adults with FHT manifest glucose intolerance, obesity, hyperuricemia, peripheral vascular disease (PVD), and to a lesser extent CVD. FHT may be inherited as an autosomal dominant trait with delayed expression [55].

Disorders of Endogenous and Exogenous Lipoprotein Transport Dysbetalipoproteinemia (Type III Hyperlipoproteinemia)

Adults with dysbetalipoproteinemia present with elevations in both TC and TG, usually but not always, above 300 mg/dL. The hallmark of the disorder is the presence of VLDL that migrate as beta lipoproteins (β-VLDL), rather than prebeta lipoproteins (dysbetalipoproteinemia). β-VLDL reflects the accumulation of cholesterol-enriched remnants of both hepatic VLDL and intestinal chylomicrons (Fig. 5.1) [56]. These remnants result from the presence of a dysfunctional apoE, the ligand for the receptor-mediated removal of both chylomicron and VLDL remnants by the liver.

Premature atherosclerosis of the coronary, cerebral, and peripheral arteries in adults is often present. Xanthomas are common, especially planar lesions in the creases of the palms, and tuberoeruptive xanthomas over the knees or buttocks. Occasionally, tuberous and tendon xanthomas are found. Hyperuricemia and glucose intolerance occur in up to half the patients with this syndrome.

Human apoE exists as three major isoforms (E2, E3, and E4), each of which is specified by an independent allele at the locus for the apoE gene [56]. One in 100 persons is homozygous for the apoE2 allele, which results in decreased affinity of the TG-enriched remnants to their hepatic receptors; however, because the prevalence of this disorder is only 1:10,000, other modifying factors such as hypothyroidism, low-estrogen state, obesity, or diabetes are necessary for full-blown clinical expression. This recessive form of dysbetalipoproteinemia has a delayed expression beyond childhood.

In summary, the diagnosis of dysbetalipoproteinemia is based on: (1) demonstration of E2E2 genotype, (2) the presence of β-VLDL, and (3) a cholesterol-enriched VLDL (VLDL-C/TG ratio >0.30). LDL-C and HDL-C levels are low or normal [56].

Dysbetalipoproteinemia in Children and Adolescents A dominant form of dysbetalipoproteinemia is caused by one of several rare variants of apoE that usually involve the substitution of neutral or acidic amino acids for basic ones in the region of apoE that interacts directly with the LDLR [56]. The dominant form can be expressed in childhood and does *not* require the presence of modifying factors. Affected adolescents often present with yellow creases in their palms (planar palmar xanthomas). The diagnosis of this rarer form of dysbetalipoproteinemia will require partial sequencing of the apoE gene.

Treatment Children or adults with dysbetalipoproteinemia are very responsive to a low-fat, low-cholesterol diet that decreases the burden of TG-enriched remnants. Fibric acid derivatives

have traditionally been the treatment of choice, which normalizes both the TC and TG levels. Niacin and statins are also quite effective.

HL Deficiency Patients with HL deficiency can present with features *similar* to type III dyslipoproteinemia, including hypercholesterolemia, hypertriglyceridemia, accumulation of TG-rich remnants (including β-VLDL), planar xanthomas, and premature CVD [57]. Recurrent bouts of pancreatitis have been described. HL is homologous to LPL and pancreatic lipase. HL hydrolyzes TG and phospholipids in lipoproteins and normally converts IDL to LDL and large HDL_2 to HDL_3. In HL deficiency, therefore, LDL-C is usually low and HDL-C is often quite *high* (despite the hypertriglyceridemia).

HL deficiency is rare and inherited as an autosomal recessive trait. Obligate heterozygotes are normal. The diagnosis is made by a PHLA test to determine that HL activity is absent but LPL activity is normal. The molecular defect leading to severe HL deficiency has been reported in a Québec-based kindred. In the proband and two of her brothers, very low to undetectable HL activity resulted from compound heterozygosity for two rare HL gene (*LIPC*) mutations, a previously unknown missense mutation in exon 5 designated p.A174T and the previously reported p.T383M mutation in exon 8 of the HL gene [58].

Treatment includes a low-total-fat diet. In one report, the hypercholesterolemia and hypertriglyceridemia in HL deficiency improved dramatically on treatment with lovastatin, while gemfibrozil reduced TG but elevated LDL-C [57].

Treatment of More Severe Combined Exogenous and Endogenous Hypertriglyceridemia Treatment of the combined exogenous/endogenous TG disorders, including HL deficiency, starts with a fat-restricted diet, reduction to ideal weight, and, when necessary, drug therapy including the fibrates, ω-3 fatty acids, niacin, and the statins (see steps 1, 4, 5, 6; Fig. 5.1). Unlike the disorders of exogenous TG metabolism, the combined TG disorders of both exogenous and endogenous TG metabolism will respond to combined treatment with fibrates, fish oils, or niacin, which can often lower TG about 50%.

Familial Disorders of HDL Metabolism

The most common cause of the phenotype of low HDL-C levels (hypoHDL) is arguably secondary to VLDL overproduction, and the subsequent expression of hypertriglyceridemia, increased small LDL particles, and low HDL-C [59]. A number of *primary* HDL disorders include: familial hypoalphalipoproteinemia (hypoalpha) [60, 61]; homozygous gene deletions or nonsense mutations in apoA-I [60, 62]; missense mutations in apoA-I [60, 63]; more than 100 common and rare variants in ABCA1, including the prototype Tangier disease [64, 65]; and lecithin–cholesterol acyl transferase (LCAT) deficiency [66].

At the other end of the spectrum, there are several familial disorders of HDL metabolism that present with *elevated* HDL-C levels (>95th percentile; Table 5.2) and reduced CVD. These include a deficiency in CETP [67, 68], and familial hyperalphalipoproteinemia (hyperalpha) [60, 62].

Apolipoprotein A-I Mutations

APOA1 exists on chromosome 11 as part of a gene cluster with *APOC3* and *APOA*4. Molecular defects in *APOA1* include gene inversions, gene deletions, and nonsense and missense mutations [60, 62]. Homozygous gene deletions or nonsense mutations are rare and exhibit little if any biosynthesis of apoA-I by the liver and intestine. The virtual absence of apoA-I is accompanied by marked decreases in HDL-C. Obligate heterozygotes, as well as the homozygotes, develop premature CVD. In addition to precocious CVD, homozygous children can manifest other clinical findings of peripheral cholesterol deposition, e.g., retinopathy, cataracts, and xanthomas. Missense mutations in *APOA1* have been described in kindreds with low HDL-C levels. However, the relationship to premature CAD is less clear [60, 62].

Tangier Disease Tangier disease is an autosomal recessive disorder in which HDL-C levels are extremely low and of an abnormal composition (HDL Tangier or T). HDL_T are chylomicron-like particles, which disappear when a patient consumes a low-fat diet [64, 65].

The classic findings in children with Tangier disease include enlarged orange yellow tonsils, splenomegaly, and a relapsing peripheral neuropathy. The orange tonsils reflect the deposition of beta carotene-rich CE in foam cells in the lymphatic tissue. Foam cells can also occur in skin, peripheral nerves, bone marrow, and the rectum. Mild hepatomegaly, lymphadenopathy, and corneal infiltration (in adulthood) may also occur.

APOA1 in Tangier patients is normal. The underlying defect is a deficiency in *ABCA1* [64, 65] (Fig. 5.1). The very low HDL-C is due to the lack of cholesterol efflux by the deficient ABCA1 to nascent HDL; this deficiency can be measured in fibroblasts from Tangier patients [64, 65]. Some but not all patients with Tangier disease have premature CAD in adulthood [64, 65]. Treatment with a low-fat diet diminishes the abnormal lipoprotein species.

LCAT Deficiency

LCAT is located on the surface of HDL particles, and transfers fatty acids from the sn-2 position of phosphatidylcholine (lecithin) to the 3-β-OH group on cholesterol. In this process, lysolecithin and esterified cholesterol are generated (α-LCAT). Esterification can also occur on VLDL/LDL particles (β-LCAT).

Both α- and β-LCAT activities are missing in patients with classic LCAT deficiency [66], a rare, autosomal recessive disorder. More than several dozen mutations have been described in *LCAT,* which is located on chromosome 16. The diagnosis is suspected in patients presenting with low HDL-C, corneal opacifications, and renal disease (proteinuria, hematuria). The ratio of plasma UC to TC is measured, with a result >0.7 diagnostic of LCAT deficiency.

In *fish eye disease,* only α-LCAT activity is absent. Patients present with corneal opacifications, but do not develop renal disease [66]. Variability in clinical presentations of fish eye disease, compared to LCAT deficiency, may be due to differences in total LCAT activity.

To date, there is no treatment of the primary defects. Patients usually die from renal disease, and atherosclerosis may be accelerated by the underlying nephrosis. Patients with LCAT deficiency, and other lipid metabolic disorders associated with renal disease, should be aggressively treated, including a low-fat diet. The secondary dyslipidemia associated with the nephrotic syndrome responds to statin therapy.

Cholesteryl Ester Transfer Protein Deficiency

The role of cholesteryl ester transfer protein (CETP) in atherosclerosis is not completely understood. CETP is upregulated in liver and peripheral tissues in response to either dietary or endogenous hypercholesterolemia. Elevated HDL-C due to complete deficiency of CETP was initially described in Japanese families [67]. Several mutations in *CETP* are known. The prevalence of CAD in CETP deficiency is not straightforward. Some patients develop CHD in spite of lower levels of apoB in CETP deficiency [67]. Thus, it has not been resolved whether a genetic CETP deficiency is an independent risk factor for CAD.

Due to its important role in modulating HDL levels, CETP inhibitors were developed to raise plasma HDL-C. However, many side effects, including increased death from CAD, attributed to interference with aldosterone metabolism, were found with the first CETP inhibitor (CP529, 414: torcetrapib) [68]. Another CETP inhibitor (dalcetrapib) produced a modest increase in HDL-C, but did not produce any decrease in CAD; no marked side effects were observed. Anacetrapib produced a significant increase in HDL-C and a decrease in LDL-C and lipoprotein (a) (Lp (a)). A clinical trial is ongoing to determine the effect of anacetrapib on reduction of CAD.

Scavenger Receptor Class B Type I Receptor Deficiency

Scavenger receptor class B type I (SR-BI) is a functional lipoprotein receptor that participates in the selective uptake of CE from HDL [69], LDL [70] and VLDL [71] and is regulated by a number of factors. One of its major functions is to mediate the uptake of CE from the core of these lipoproteins. Single-nucleotide polymorphisms (SNPs) in the *SCARB1* gene are significantly associated with HDL-C levels [72]. Certain SNPs in *SCARB1* are significantly associated with subclinical carotid atherosclerosis [73].

Deficiency of Endothelial Lipase

Endothelial lipase (EL) is a member of the TG lipase family of proteins that includes LPL and HL. EL is a product of *LIPG* and primarily hydrolyzes phospholipids with little TG lipase activity. EL hydrolyzes the lipids in HDL the most efficiently of all the lipoproteins, converting HDL from a larger to a smaller particle. Rare loss-of-function EL variants produce a higher HDL-C [74]. However, these variants did not mediate any decrease in CAD.

Elevated Lp (a)

Lp (a) consists of a glycoprotein, apo (a), covalently linked to apoB-100 of LDL through a disulfide bond [75]. Apo (a) is highly homologous to plasminogen but has no protease activity. Lp (a) levels are highly heritable and are almost entirely related to the apo (a) gene on chromosome 6q27. Lp (a) enters the vascular wall and promotes atherosclerosis through its CE content, and thrombosis through its inhibition of plasminogen activity on the surface of endothelial cells. Lp (a) also appears to bind oxidized phospholipids, which may promote inflammation [75]. The precise physiological function of Lp (a) is unknown but it is a causative risk factor for CVD [76]. Lp (a) is significantly elevated in a small but definite group of *children* who develop *either* hemorrhagic or thrombotic stroke, often without any other lipid abnormality, but who may also have other thrombotic risk factors [77].

Diagnosis and Treatment of elevated Lp (a) The best method for diagnosis of elevated Lp (a) is an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody. The upper limit of normal is 75 nmol/L. Niacin and estrogen can effectively lower Lp (a) levels, while the statins and fibrates do not. Although clinical trial evidence is lacking regarding the benefit of specifically lowering Lp (a) on the prevalence of CVD, the recommended approach is to treat LDL-C more aggressively in patients with CVD who also have elevated Lp (a). A statin is used to reduce LDL-C to <100 mg/dL, at a minimum. Niacin can be added to reduce Lp (a) and to increase HDL-C. Treatment of children with elevated Lp (a) and stroke is a clinical judgment. My own approach is to use aspirin 81 mg/day and consider a statin if the child has a LDL-C >110 mg/dL, the 75th percentile.

Guidelines for the Clinical Evaluation and Treatment of Dyslipidemia in Children and Adolescents

Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents

The National Heart, Lung, and Blood Institute initiated development of cardiovascular health guidelines for pediatric care providers based on a formal evidence review of the science with an integrated format addressing all the major cardiovascular risk factors simultaneously [1, 2]. Evaluated risk factors included family history, age, nutrition/diet, physical inactivity, tobacco exposure, blood pressure, lipid levels, overweight/obesity, diabetes mellitus, predisposing conditions, metabolic syndrome, inflammatory markers, and prenatal factors. These guidelines are reviewed here but with a focus on dyslipoproteinemia and atherosclerosis. The Expert Panel's

Report [1, 2] may be consulted for more details about other evaluated risk factors.

The Expert Panel reviewed the evidence for lipid screening [1, 2], and considered selected lipid screening versus universal lipid screening. A primary objective for universal lipid screening was to detect youth with heterozygous FH who were at high risk for precocious CVD as adults if untreated.

Evidence Supporting Universal Screening for Heterozygous FH FH remains the clearest biologic and genetic model linking elevated levels of LDL in children to premature atherosclerotic events in adults. The first expert pediatric panel from the National Cholesterol Education Program (NCEP) [78] in 1992 recommended selective lipid screening of children with a family history of premature CVD or known hypercholesterolemia in a parent to detect children with FH at markedly increased risk of adult premature CAD. However, selective screening misses 30–60 % of children with FH who are at the highest risk of developing premature CVD [10, 79].

Wald et al. [80] performed a meta-analysis of 13 studies using 1907 FH cases and 16,221 controls to examine detection rates of FH (sensitivity) for specified false-positive rates (0.1, 0.5, and 1 %) in newborns and in five age groups 1–9, 10–19, 20–39, 40–59, and > or = 60 years. TC discriminated best between those with and without FH when they were between ages 1 and 9, when the detection rates with TC were 88 %, 94 %, and 96 % for false-positive rates of 0.1, 0.5, and 1 %. The results were similar with LDL-C. Screening newborns was much less effective. Once an affected child was identified, measurement of TC detected about 96 % of parents with the disorder.

Cascade screening is the process of searching for relatives with FH once an individual is diagnosed with FH [81]. Cascade testing is not a suitable method of population screening for FH, because a separate method of systematically identifying new FH index cases is required to achieve a reasonable level of FH detection in the population [81]. Such an alternative systematic method of identifying new cases could itself be the method of population screening [81].

If screening for children with FH is to be efficacious, there must be a safe and beneficial treatment. Because children with FH only respond to a stringent diet low in total fat, saturated fat, and cholesterol with an average 10 % fall in LDL-C, the effect of statins was examined in a number of randomized, placebo-controlled, clinical trials of 10- to 17-year-old FH children [82]. The statins lowered LDL-C impressively from 21 to 39 % in the different studies [82]. There were no serious side effects. Furthermore, Wiegman et al. [83] demonstrated that treatment of FH children, aged 8–17 years, randomized to 20 or 40 mg of pravastatin/day ($n = 104$) for 2 years, had regression of cIMT compared to those in the placebo ($n = 107$) group. This was the first evidence that treatment of children with FH with a statin produces a decrease in early, subclinical lesions of atherosclerosis. Further, "earlier is better" since those younger Dutch children treated at 8–11 years of age at baseline had significantly lower cIMT 5 years later than those treated at 12–18 years of age [84].

Additional Beneficial Effects of Universal Lipid Screening In addition to detecting 90 % or more of children with FH at 9–11 years of age, universal lipid screening has the potential to identify a larger group of children who have less marked oligogenic dyslipidemia, or dyslipidemia associated with obesity and the metabolic syndrome, and increased cIMT.

Prevalence of Abnormal Lipid Levels in US Children The National Health and Nutrition Examination Survey (NHANES) for 1999–2006 reported that the prevalence of abnormal lipid levels among youths aged 12–19 years was 20.3 % [85]. This prevalence varied by BMI; 14.2 % of normal-weight youths, 22.3 % of overweight youths, and *42.9 % of obese* youths had at least one abnormal lipid level. Among all youths, 32 % had a high BMI and therefore would be candidates for lipid screening under American Academy of Pediatrics (AAP) recommendations [86]. "Given the high prevalence of abnormal lipid levels among youths who are overweight and obese in this study, clinicians should be aware of

lipid screening guidelines, especially recommendations for screening youths who are overweight or obese" [85].

Clinical Implications of Obesity and Dyslipidemia in Youth Juonala and colleagues [87] pooled 4380 subjects from the CVD in Young Finns Study, the Childhood Determinants of Adult Health Study, the Bogalusa Heart Study, and the Muscatine Study, and showed that obesity during childhood strongly and significantly *predicted* the following outcomes in adults in their fourth decade: type 2 diabetes, hypertension, high LDL-C, low HDL-C, high TG, and high-risk cIMT. Analyses were adjusted for age, sex, height, length of follow-up, and cohort. Those who had a normal BMI ($N=2794$) in childhood and remained nonobese as adults, or those who were obese ($N=274$) in childhood but nonobese as adults did *not* have increased risk for lipid- and nonlipid CVD risk factors or elevated cIMT. Conversely, as adults, both those ($n=500$) who were overweight or obese in both childhood and adulthood and those ($n=812$) who had normal BMI in childhood but were obese had increased risk for CVD [87]. These data are impressive and further emphasize the importance of prevention since if an obese child became a nonobese adult, they did not have increased risk of CVD outcomes.

Evidence that Universal Lipid Screening Will Detect "High-Risk" Dyslipoproteinemic Children who Develop Significant Subclinical Atherosclerotic Lesions as Adults The lipid and nonlipid risk factors for cardiovascular (CVD) disease in adults are expressed in childhood [88]. Strong and internally consistent relationships exist between baseline lipid and nonlipid CVD risk factors in free-living populations of children and adolescents and the development in young adults of: (1) atherosclerotic postmortem lesions [89, 90], (2) cIMT (90^{th} percentile) [91], and (3) coronary calcium [92, 93]. It is now known that the lipid and nonlipid CVD risk factors predict adult carotid IMT beginning at 9 years of age [88]. Predictors of these lesions include higher LDL-C, lower levels of HDL-C, obesity, higher blood pressure levels, and cigarette smoking. Non-HDL-C (total cholesterol minus HDL-C), an index of the cholesterol carried by all the atherogenic apoB-containing lipoproteins, was also found to predict early atherosclerotic lesions in youth and young adulthood [94].

Evidence that Selective Lipid Screening Will Miss Dyslipoproteinemic Children who May Qualify for Drug Treatment of Elevated LDL-C Lipid screening for FH will also provide the opportunity of detecting those with less severe but nevertheless significant dyslipoproteinemia that benefit from modification of lifestyle and may warrant drug therapy. Ritchie et al. [95] assessed *selective* versus *universal* lipid screening in a general population of 20,266 fasting fifth-grade students in West Virginia. A total of 14,470 (71.4%) met NCEP 1992 Guidelines [78] for lipid screening based on a family history of CAD. Of those, 1204 (8.3%) had elevated LDL-C (≥130 mg/dL) and 170 (1.2%) of these 14,470 children warranted possible pharmacologic treatment (LDL-C ≥160 mg/dL). Of the 5798 (28.6%) who did *not* have a positive family history, 548 (9.5%) had elevated LDL-C and 98 (1.7%) had LDL-C ≥160 mg/dL, indicating consideration of pharmacologic treatment. Universal lipid screening identifies children with either a modest or more marked LDL-C, who are undetected by selective screening, missing the opportunity for hygienic treatment and pharmacologic therapy in those 2–3% who have more extreme LDL-C elevations.

Concerns About the Efficacy, Safety, and Cost of Universal Lipid Screening Opponents of universal lipid screening point out that there are no randomized placebo-controlled clinical trials of either intensive hygienic intervention or pharmacologic LDL lowering starting in youth at high risk for future CVD that demonstrate that treatment for *three or more decades* decreases *clinical CVD events in adulthood* [96]. Such a study would be of such magnitude and expense that it is unlikely to ever be accomplished. Some initial data from the UK are available, indicating that treatment of young adults with FH, age 20–39

years, with statins led to a substantial reduction in coronary mortality [97].

Estimates of the cost-effectiveness of identifying and treating patients with FH are highly favorable in health-care systems that can implement cascade screening based on index case identification of middle-age adults and genetic testing; for example, in the UK, the cost is about US$ 7000/quality-adjusted life year [98]. Although the additional costs of universal screening are not known, the benefits of earlier CVD prevention in high-risk individuals would be considerable as will cost savings, as statin costs are reduced as those medications go off patent [96].

Finally, there has been concern about the adverse effects of "labeling" a child as having a cholesterol problem or being obese [96]. In this regard, there are few if any data from a clinical trial. In The Dietary Intervention Study of Children, thousands of children aged 8–10 years were first screened in schools to detect those with a higher LDL-C (average 90th percentile). After being randomized into either a behaviorally based intervention group, or a usual care group, 3 years later both groups had extensive psychological testing. There were no adverse effects for children in the intervention group in terms of academic functioning, psychological symptoms, or family functioning [99]. There was no evidence for adverse effects of being labeled as having high LDL-C or for obtaining dietary advice.

Pediatric "Metabolic Syndrome"

There is no current consensus regarding the definition of the metabolic syndrome in youth. Cook et al. [100] proposed a definition from the third NHANES survey in those aged 12–17 years. The metabolic syndrome was considered present if three or more of these factors are present: (1) TG of 110 mg/dL or higher; (2) HDL-C of 40 mg/dL or lower; (3) waist circumference, at the 90th percentile or higher; (4) fasting glucose, 110 mg/dL or higher; and (5) blood pressure, at the 90th percentile or higher for age, sex, and height (the percentiles are those derived from NHANES) [100]. A BMI higher than the 95th percentile for age and gender has been proposed as an alternative to waist circumference [96].

Obesity, Dyslipidemia, and the Metabolic Syndrome Obesity plays a critical role in the development of dyslipidemia and the metabolic syndrome [102–106]. The severity of obesity and insulin resistance fuels the development of the metabolic syndrome. Acanthosis nigricans is often a sign of underlying insulin resistance. Elevated highly sensitive C-reactive protein and decreased adiponectin [102] are often present. Koskinen et al. [107] found that apoB, but not oxidized LDL or small LDL, was associated with metabolic syndrome in youth. Metabolic syndrome variables cluster from childhood to adulthood [103]. The metabolic syndrome in youth predicts adult metabolic syndrome, brachial artery distensibility, subclinical atherosclerosis, diabetes, and CVD two to three decades later but is no better than body mass index alone [104–106, 108].

Screening for Dyslipidemia in Pediatrics

For the reasons outlined above, each child 9–11 years of age optimally should have a TC, HDL-C, and non-HDL-C performed around their 10-year-old visit to their pediatrician or family practitioner. Each of these lipid parameters can be measured accurately in a *nonfasting state*. If the TC or non-HDL-C is elevated above the 95th percentile or the HDL-C is low below the 5th percentile, (Table 5.2), an appointment is made to obtain a follow-up fasted sample. A lipid profile including a TC, LDL-C, HDL-C, and TG is ordered. A normal result is a TC, LDL-C, TG, or non-HDL-C < 75th percentile, or a HDL-C > 25th percentile (Table 5.2).

The patient may have an elevated or borderline-elevated TC, LDL-C, and TG, or a low or borderline-low HDL-C (Table 5.2) [78], or some combination. The non-HDL-C is calculated as (TC − HDL-C = non-HDL-C). Similar percentiles and definitions are available for non-HDL-C (Table 5.2) [109]. Youth who present with one

Table 5.3 Causes of secondary dyslipidemia in children and adolescents

Exogenous	*Storage disease*
Alcohol	Cystine storage disease
Oral contraceptives	Gaucher disease
Prednisone	Glycogen storage disease
Anabolic steroids	Juvenile Tay–Sachs disease
13-*cis*-retinoic acid	Niemann–Pick disease
Endocrine and metabolic	Tay–Sachs disease
Acute intermittent porphyria	*Acute and transient*
Type I and type II diabetes	Burns
Hypopituitarism	Hepatitis
Hypothyroidism	*Others*
Lipodystrophy	Anorexia nervosa
Pregnancy	Cancer survivor
Renal	Heart transplantation
Chronic renal failure	Idiopathic hypercalcemia
Hemolytic–uremic syndrome	Kawasaki disease
Nephrotic syndrome	Klinefelter syndrome
Hepatic	Progeria (Hutchinson–Gilford syndrome)
Benign recurrent intrahepatic cholestasis	Rheumatoid arthritis
Congenital biliary atresia	Systemic lupus erythematosus
Alagille syndrome	Werner syndrome

or more elevated apoB-containing lipoproteins and/or or low apoA-I-containing lipoproteins will require closer follow-up. Measurement of thyroid, liver, renal tests, and urinalysis (to rule out common secondary causes of dyslipidemia) is essential (Table 5.3). As many as 40 % of obese children may have a dyslipidemia usually characterized by high TG and low HDL-C [100]. In addition, those considered to have the metabolic syndrome will often have elevated or borderline non-HDL-C [110] (Table 5.2).

Well-standardized immunochemical methods are available for apoB and apoA-I measurements [111]. Cutoff points for apoB and apoA-I from the National Health and Nutrition Education Survey (NHANES) are used [111] (Table 5.2). Those with a low HDL-C and elevated TG but normal or borderline LDL-C (Table 5.1) should have an apoB measured. Or, the number of LDL particles (LDL-P) can be assessed by nuclear magnetic resonance (NMR) spectroscopy. If the apoB or LDL-P are elevated in such children (Table 5.2), then the child probably has FCHL. The complete dyslipidemic expression of FCHL is often delayed until adulthood, although elevated apoB or LDL-P may be the first expression of FCHL in adolescents and young adults [112]. apoA-I can be measured in a child with a low HDL-C to determine the severity of the phenotype.

Advanced lipoprotein testing has been used in clinical research studies to determine the subclasses of VLDL, LDL, and HDL in children and adolescents using NMR spectroscopy [113–116]. Guidelines derived from such methods for the diagnosis and treatment of dyslipidemia in youth are currently being developed.

Guidelines for Treatment of Dyslipidemia in Children and Adolescents

Dietary Therapy

Treatment and Follow-Up with Dietary Treatment

It is highly recommended that the following web site (http://www.nhlbi.nih.gov/guidelines/cvd_ped/index.htm) [2] be visited to review the nutritional information synthesized by the recent Pediatric Panel. The following overall summary

of the recommendations for a Child 1 diet are as follows.

Long-term follow-up studies demonstrate that subjects who were breast-fed have sustained CV health benefits, including lower cholesterol levels, lower BMI, reduced prevalence of type 2 diabetes, and lower cIMT in adulthood [1, 2].

Ongoing nutrition counseling has been effective in assisting children and families to adopt and sustain recommended diets for both nutrient adequacy and reducing CV risk [1, 2].

Within appropriate age- and gender-based requirements for growth and nutrition, in normal children and in children with dyslipidemia, intake of total fat can be safely limited to 30% of total calories, saturated fat intake limited to 7–10% of calories, and dietary cholesterol limited to 300 mg/d. Under the guidance of qualified nutritionists, this dietary composition has been shown to result in lower TC and LDL-C levels, less obesity, and less insulin resistance [1, 2].

Under similar conditions and with ongoing follow-up, these levels of fat intake may have similar effects starting in infancy. However, fats are important to infant diets due to their role in brain and cognitive development. Fat intake in infants less than 12 months of age should not be restricted without medical indication [1, 2].

The remaining 20% of fat intake should comprise a combination of *cis*-monounsaturated and polyunsaturated fats. Intake of *trans* fats should be limited as much as possible.

Between ages 1 and 2 years as children transition from breast milk or formula, milk reduced in fat (ranging from 2% milk to fat-free milk) can be used based on the child's growth, appetite, intake of other nutrient-dense foods, intake of other sources of fat, and risk for obesity and CVD.

Optimal intakes of total protein and total carbohydrate in children were not specifically addressed, but with a recommended total fat intake of 30% of energy, the Expert Panel recommends that the remaining 70% of calories include 15–20% from protein and 50–55% from carbohydrate sources [1, 2].

Plant-based foods are important low-calorie sources of nutrients including vitamins and fiber in the diets of children; increasing access to fruits and vegetables has been shown to increase their intake.

Reduced intake of sugar-sweetened beverages is associated with reduced obesity measures [1, 2].

Safety and Efficacy of Dietary Therapy in Infants, Children, and Adolescents Overall, the Child 1 diet in children appears safe and efficacious when performed under supervision. Medical and nutritional support is necessary to reinforce good dietary behaviors and ensure nutritional adequacy. The Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) [117, 118] is a randomized, prospective low-saturated-fat dietary counseling program, starting at 7 months of age. Beneficial effects mediated in part by the diet-induced reduction in TC include improved insulin sensitivity at 9 years of age [119], enhanced endothelial function in 11-year-old boys, but not in girls [120], decreased obesity in girls [121], and reduction of overweight-related cardiometabolic risk factors in adolescents [122].

In the Dietary Intervention Study in Children (DISC), starting at ages of 8–10 years, healthy children with high LDL-C levels (average 130 mg/dL) were ascertained through cholesterol screening in six schools and randomized into an intervention group and a usual care group. The intervention group received an intense behavior-based dietary intervention while the usual care group ate a normal diet. At the end of 3 years the intervention group had small (3.2 mg/dL) but significantly lower mean LDL-C levels than the usual care group [123], despite a rather notable fall in LDL-C levels during adolescence in both study groups [124]. The low-fat, low-cholesterol diet in the intervention group was associated with normal growth and development [125]. The intake of calcium, zinc, vitamin E, and phosphorus were below average but adequate [125]. The Intervention diet was associated with lower blood pressure levels [126].

Dietary Supplement with Plant Sterols. The use of margarines (about two-three servings daily) high in either plant stanol esters [127, 128] or plant sterol esters [129] can reduce LDL-C an additional 10–15%, when added to a low-fat diet.

Table 5.4 Guidelines for use of pharmacologic agents to lower LDL-C in children and adolescents [93]

Risk factors for CVD	Post-dietary LDL-C level	LDL-C treatment goal
None	> or = 190 mg/dL	Minimum < 130 mg/dL
		Desirable < 110 mg/dL
	> or = 160 mg/dL	
(1) Positive family history for premature CVD, or		
		Minimum < 130 mg/dL
(2) Two or more other CVD risk factors		Desirable < 110 mg/dL

Water-soluble fiber [130] such as psyllium [131, 132] may also provide an additional 5–10 % lowering of LDL-C.

Effect of a Low-Fat Diet in Childhood on Future CVD in Adulthood That a low fat-diet in childhood will prevent CVD in adulthood has been inferred from epidemiologic and clinical trial studies in adults [1, 2].

For higher-risk children and adolescents, a more stringent diet, Child 2, is recommended.

Guidelines for Treatment of Dyslipidemia in Children and Adolescents

Pharmacological Therapy

When to Start Drug Therapy The primary use of lipid-altering drugs in pediatrics is to lower significantly elevated LDL-C [1–4] (Table 5.4). Drug treatment to lower LDL-C can be initiated at 10 years of age. The AAP [86] indicated that onset of treatment might be lowered to 8 years of age in children with marked elevations in LDL-C and a striking family history of premature CVD. This is related to the recent evidence that "earlier is better" in regard to producing the greatest regression of cIMT [84]. A more conservative approach is to wait to treat with drugs until Tanner stage II in males and after menstruation in girls but there is no evidence that statins affect sexual development.

Criteria for Instituting Drug Therapy Pharmacologic treatment of elevated LDL-C in youth can be considered if the post-dietary LDL-C is: (1) ≥ 190 mg/dL and there is a negative or unobtainable family history of premature CVD and no major CVD risk factors; or (2) ≥ 160 mg/dL and there is a family history of premature CVD or two or more risk factors for CVD are present [1–4, 78] (Table 5.4).

LDL-C Goals for Drug Treatment The minimum goal after drug treatment is a LDL-C < 130 mg/dL (Table 5.4). A desirable goal is a LDL-C < 110 mg/dL, which is below a borderline-elevated LDL-C of 110–129 mg/dL (Table 5.2).

HMGCoA Reductase Inhibitors (Statins)

The statins are generally the first class of drugs that are used to treat children 10–17 years of age with autosomal dominant hypercholesterolemia (FH, Defective apoB-100; FH3) or significant FCHL.

A meta-analysis of a number of randomized, placebo-controlled trials of the statins [82–84] showed good efficacy for lowering LDL-C and apoB from about 20 to 40 %, depending on the statin used. There was no major side effect in the children treated with the statins, compared with placebo [82–84]. Atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, and rosuvastatin are approved by the FDA for use in children with FH 10–17 years of age. The equivalent potencies are about: 5 mg rosuvastatin = 10 mg atorvastatin = 20 mg simvastatin = 40 mg lovastatin = 40 mg pravastatin = 80 mg fluvastatin. Both atorvastatin

and rosuvastatin have long half-lives of about 17 h. Thus, they can be taken in the morning or evening, in contrast to the other statins, which should be taken at bedtime because of their short, half-lives. All statins except fluvastatin and rosuvastatin are available generically.

Efficacy of Statins on LDL-C Reduction: Consideration of Combined LDL-C-Lowering Agents The ability of the statins to achieve LDL-C goals (Table 5.4) will be related to how high the baseline LDL-C is elevated. In one study, the average baseline post-dietary LDL-C in FH heterozygotes was 232 mg/dL. When a higher dose of 20 mg of the most potent statin, rosuvastatin, was used, the mean LDL-C fell 50%, but still 40% of these FH children and adolescents did not achieve an optimal LDL-C goal of <110 mg/dL (a normal LDL-C level, Table 5.2) [133]. A second drug can be considered to take advantage of the complementary action on LDL-C reduction when either a BAS or ezetimibe is added to a statin. All three of these agents reduce hepatic cholesterol leading to an upregulation of LDLR activity. The effect of BAS and ezetimibe on increasing HMGCoA reductase activity is obviated by the concurrent use of a statin (Fig. 5.1). One can also avoid the use of the highest dose of one of the more potent statins. For example, ezetimibe was combined with simvastatin to affect an additional 15–25% decrease in LDL-C in FH heterozygous children [134]. There is also a nonlinear dose–response relation when a BAS or niacin is added to statin [135]. Combination of another LDL-C-lowering agent to a statin should be undertaken in consultation with a lipid specialist.

Effect of Statins on cIMT Wiegman et al. [83] found that a 24% reduction in LDL-C with pravastatin in FH heterozygotes 8–15 years of age significantly decreased cIMT compared with placebo. Younger age at statin initiation was an independent predictor of effect of treatment on cIMT in this Dutch study [84]. In an open label study in FH heterozygotes 10–16 years of age using fluvastatin 80 mg/day, median LDL-C fell 34% but there was no significant change in carotid IMT [136]. Early intervention with statins appears likely to reduce the risk of future atherosclerosis and CVD in those with FH.

Side Effects of the Statins in Children and Adolescents Statins are generally well tolerated, especially in youth, and have an excellent safety profile with minimal side effects. In a meta-analysis [82], the prevalence of elevated alanine amino transferase three times above the upper limit of normal (ULN) in the statin group was 0.66% (3 per 454). Instances of asymptomatic increases (>10-fold) in creatine kinase (CK), although unusual, have been reported in adolescents receiving statin therapy [82]. CK can also increase notably following several days of vigorous physical activity, which resolves spontaneously within a week. No cases of rhabdomyolysis have been reported [82, 133, 134].

Liver function tests (aspartate aminotransferase, AST; alanine transaminase, ALT) should be monitored at baseline, following 6–8 weeks after initiating treatment and every 4 months for the first year. After that, youth on a stable dose of a statin can have their liver function tests monitored every 6 months. Consideration should be given to reducing the dosage of drug, or its discontinuation, should the liver function tests exceed three times the upper limit of normal and remain there. CK should be measured at baseline and repeated if the patient develops muscle aches and cramps. The statin is discontinued if the CK is >5 times the upper limit of normal in those with symptoms of myositis, or >10 times the upper limit of normal in asymptomatic patients. In adults, 1/500 to 1/1000 patients may develop myositis on a statin, which can lead to life-threatening rhabdomyolysis [137]. Rhabdomyolysis is a rare event, occurring at an incidence of 1.2 per 10,000 patient-years [137]. Three statins, lovastatin, simvastatin, and atorvastatin, are metabolized by the CYP3A4 isozyme of the cytochrome P450 microsomal enzyme system, and consequently have drug interactions with other agents metabolized by CYP3A4. Examples include erythromycin, verapamil, cyclosporine, HIV protease inhibitors, sertraline, and the fibric acid derivative gemfibrozil. Larger intakes of grapefruit juice with these agents can also inhibit CYP3A4.

Special Issues in Young Females

The statins are effective and safe in adolescent girls, with no significant adverse effect on growth and development or on gonadal and adrenal hormones [82].

Because of the potential risk to a developing fetus, statins are contraindicated during pregnancy. Birth control is mandatory for those who are sexually active. Because of this concern, the long-term commitment to therapy, and the fact that risk of CAD increases after menopause, some specialists believe that statins should not be used to treat adolescent FH females. Others do recommend treatment of adolescent FH females, especially those with a striking family history of premature CAD. Additional studies are needed to document the long-term safety of statins and to determine their effects on future CVD.

Bile Acid Sequestrants (BAS)

BAS were the only class of drugs originally recommended by NCEP [78] for pharmacologic lipid-lowering therapy because of their long track record of safety over three decades. BAS do not enter the bloodstream, but bind bile acids in the intestine [138], preventing their reabsorption through the ileal bile acid transporter (IBAT) (Fig. 5.1). The decreased return of bile acids stimulates the conversion of cholesterol to bile acids through 7α-hydroxylase, lowering the hepatic cholesterol content and inducing LDL receptors (Fig. 5.1). The BAS produce a modest LDL-C reduction of about 10–15% [139–142]. The first-generation BAS, cholestyramine and colestipol, suffered from significant tolerability issues including constipation, heartburn, bloating, decreased serum folate levels, and interference with the absorption of other drugs [138]. In one study, over 80% of FH heterozygous children discontinued BAS after an average of 22 months, secondary to gritty taste and gastrointestinal complaints [141]. The second-generation sequestrant, colesevelam (625-mg tablets, three twice daily or six once a day), has a greater affinity for bile salts and can be used in a lower dose. Colesevelam is associated with less annoying side effects than cholestyramine, such as constipation and gritty taste. Colesevelam, alone or combined with a statin, is approved by the FDA as an adjunct to diet and exercise to reduce LDL-C in boys and post-menarchal girls, aged 10–17 years with heterozygous FH. Colesevelam can be administered as 625-mg tablets or as an oral suspension [142].

Safety of BAS In randomized clinical trials, cholestyramine did not affect height velocity [138–140]. Levels of fat-soluble vitamins were maintained, except that the BAS group had significantly lower 25-hydroxyvitamin D than the placebo group. Low-folate and high-homocysteine levels have been reported on BAS [139].

Cholesterol Absorption Inhibitor (CAI)

The CAI ezetimibe decreases the intestinal absorption of cholesterol derived from diet and from bile by about 50% through its high-affinity inhibition of NPC1L1 [143] (Fig. 5.1). This leads to a decrease in hepatic cholesterol, increased LDL receptor activity, and decreased LDL-C levels. NPC1L1 is localized at the brush border of enterocytes that normally moves cholesterol from mixed micelles into the cells of the jejunum [143] (Fig. 5.1). Ezetimibe lowers LDL-C by about 15–20% either alone or when combined with a statin [24, 139, 145]. Ezetimibe is not yet approved by the FDA for use in children, except in very rare cases of sitosterolemia [24] or homozygous FH [145]. While there have been isolated case reports of possible ezetimibe-associated myopathy, there is no evidence from randomized clinical trials of increased myopathy or rhabdomyolysis with ezetimibe [138]. Other side effects include gastrointestinal upset, headache, and increased incidence (about 3%) of elevated liver function tests when combined with a statin. Ezetimibe is administered in one dose only, 10 mg/day.

Niacin (Nicotinic Acid)

Niacin is not routinely used in pediatrics since there are few published data on its safety and efficacy of niacin in youth. The single exception is the FH homozygous patient (see above).

Fibrates

Fibric acid derivatives (fibrates) are agonists for the peroxisome proliferator-activated receptor alpha (PPAR alpha), which upregulate the gene for LPL and apoA-V, and downregulate the gene for apoC-III [147] (Fig. 5.1). Fibrates also upregulate the gene for apoA-I, which increases HDL-C levels. Use of a fibrate is usually reserved for that adolescent with fasting TG over 500 mg/dL, who may be at an increased risk of pancreatitis. The most common side effects of fibrates are upset stomach, nausea, or vomiting. Abdominal pain is the second most common side effect. There is a slightly increased risk of gallstones. Gemfibrozil can potentiate drugs that prevent blood clotting (anticoagulants), causing bleeding. Use of gemfibrozil with statins can potentiate myositis, myalgias, and rhabdomyolysis. Such combined therapy is used only in consultation with a lipidologist.

Fish Oils

Fish oils are enriched in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These ω-3 fatty acids inhibit the production of TG in liver by several postulated mechanisms, including interfering with the incorporation of FFA into TG (Fig. 5.1). Fish oil preparations concentrated with EPA and DHA can be used as prescription formulations, Lovaza and Vascepa. The prescription versions of ω-3 fatty acids are not yet approved by the FDA for use in children. TG can be significantly lowered in youth up to 25–50% at a dose of two 1-g capsules taken twice daily (4 g per day). Side effects are mostly gastrointestinal upset and a "fishy taste or smell" [1–4]. Fish oils should be kept refrigerated to minimize the fishy taste and odor.

Treatment of Dyslipidemia Secondary to Other Diseases [1–4]

Insulin Resistance Metformin has been used in several studies of obese adolescents with the metabolic syndrome and hyperinsulinemia to lower fasting plasma glucose and insulin [148, 149].

Type I Diabetes Youth with type I diabetes are at high risk for CVD as adults and already have increased cIMT [150]. After dietary therapy and the best achievable diabetic control, the American Diabetes Association strongly recommends the use of statins in those with LDL-C > 160 mg/dL and to consider it in those with a LDL-C > 130 mg/dL [150].

Nephrotic Syndrome The dyslipidemia in children with the nephrotic syndrome can be marked, with both TC and TG that approach 300 mg/dL or higher [151]. Those patients who are *unresponsive* to steroids and have a post-dietary intervention LDL-C of more than 160 mg/dL may be at an increased risk for CVD [151] and may warrant treatment with a statin, which is effective in this condition.

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) presents in adolescent females with menstrual disorders, acne, and hirsutism [152, 153]. Insulin resistance and dyslipidemia are often present. After diet and weight control, an estrogen/progesterone combination is often used [152]. Metformin can be considered, especially in those who are obese. Increased cIMT is present in young adults with PCOS [152, 153], and treatment with a statin can be considered in those with LDL-C > 160 mg/dL.

Summary

This chapter covers the fundamental and practical aspects of dyslipoproteinemia in children and adolescents. Plasma lipoprotein metabolism germane to youth is examined. The inherited dyslipoproteinemias are discussed in sufficient detail to serve as a resource to support further diagnostic evaluations and treatment strategies in such children and adolescents. Finally, most youths seen by practitioners do not have an inherited dyslipoproteinemia but one that is influenced by oligogenic factors and affected by environmental contributors such as diet, physical inactivity, and obesity. A strong case is made for universal lipid screening between 9 and 11 years of age. Thus, there is a challenge to detect and treat children with both genetic and common dyslipoproteinemias as well. The practical section on diagnosis and treatment provides an integrated approach to dyslipoproteinemia.

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Type 2 Diabetes Mellitus and Dyslipidemia

6

Henna Cederberg and Markku Laakso

Introduction

Type 2 diabetes, a state of chronic hyperglycemia characterized by insulin resistance and a pancreatic β cell dysfunction, is associated with microvascular complications in the eyes, kidney, and nervous system [1] and macrovascular complications affecting coronary, peripheral and carotid arteries [2]. Cardiovascular disease (CVD) accounts for more than 70 % of mortality in individuals with type 2 diabetes [3, 4], and individuals with type 2 diabetes are at two- to fourfold increased risk of coronary artery disease (CAD) [2, 5, 6]. The risk of cerebrovascular disease in type 2 diabetes is increased by two- to threefold [7–10], and the risk of peripheral arterial disease by three- to fivefold compared to nondiabetic individuals [11–15].

The etiology of CVD in type 2 diabetes is multifactorial, and originates from several abnormal CVD risk factors, including dyslipidemia, elevated blood pressure, smoking, chronic hyperglycemia, insulin resistance, and hypercoagulability [16]. Among these risk factors for CVD, LDL cholesterol level has shown to be the strongest predictor of nonfatal or fatal myocardial infarction (MI) in the UK Prospective Diabetes Study (UKPDS) [17]. Therefore, elevated level of serum low-density lipoprotein (LDL) is the most important treatment target in the prevention of CVD in patients with type 2 diabetes [18].

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Epidemiology

Dyslipidemia in Type 2 Diabetes

Type 2 diabetes is characterized by profound abnormalities in fatty acid (FA) metabolism, which result in an abnormal lipoprotein cascade [19], including elevated levels of total and very-low-density lipoprotein (VLDL) triglycerides and postprandial triglyceride-rich lipoprotein remnants and apolipoprotein B [20–23], and low levels of high-density lipoprotein (HDL) cholesterol [19, 20]. The composition of lipoprotein particles is altered in type 2 diabetes, and characterized by increased numbers of atherogenic apoB lipoproteins and reduced numbers of HDL particles [21].

Prevalence of Dyslipidemia in Type 2 Diabetes

Among 10,197 Finnish men from the Metabolic Syndrome in Men (METSIM) Study, a randomly selected population-based cohort, compared to nondiabetic individuals, those with type 2 diabetes had markedly higher levels of total triglycerides (1.38 vs. 1.87 mmol/L, respectively;

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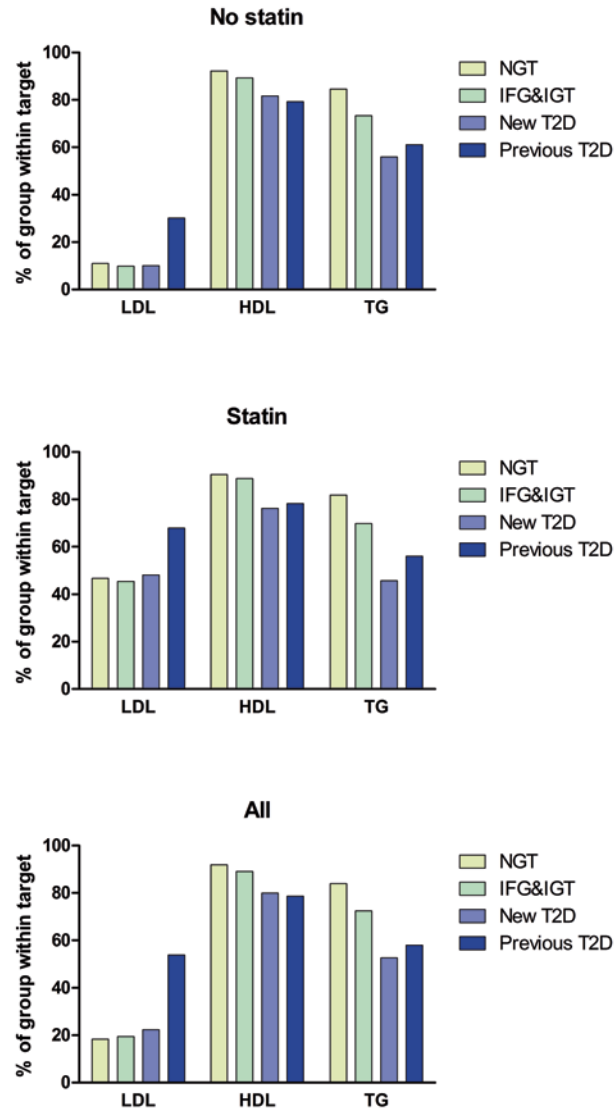

Fig. 6.1 Percentage of individuals within target lipid levels by glucose tolerance status and statin medication in the Metabolic Syndrome in Men (METSIM) Study. Target levels: *LDL* cholesterol < 2.6 mmol/L (< 100 mg/dL), *HDL* cholesterol > 1.0 mmol/L (> 39 mg/dL), and total triglycerides < 1.7 mmol/L (< 150 mg/dL). *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *NGT* normal glucose tolerance, *TG* triglycerides, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *T2D* type 2 diabetes

$P < 0.001$) and lower levels of HDL cholesterol (1.47 vs. 1.34 mmol/L, respectively; $P < 0.001$; individuals on statin treatment were excluded from these analyses). In contrast, total cholesterol (5.58 vs. 5.47 mmol/L) and LDL cholesterol (3.57 vs. 3.45 mmol/L) levels were quite similar in these two groups (Fig. 6.1, Table 6.1). Participants with previously diagnosed type 2 diabetes had lower levels of HDL cholesterol (1.28 vs. 1.37 mmol/L, $P = 0.005$) than individuals with newly diagnosed type 2 diabetes, whereas no significant difference between these groups was observed in the levels of total triglycerides.

Similar findings for lipids and lipoproteins in type 2 diabetes have been reported in previous studies [6, 24–26] (Table 6.2). Comparison of lipid and lipoprotein levels in individuals with type 2 diabetes and in nondiabetic control subjects in different populations, including the Framingham Heart Study [25], San Antonio Family Heart Study [26], Australian Diabetes, Obesity and Lifestyle Study [26], and Diabetes

Table 6.1 Lipid and lipoprotein levels in individuals with type 2 diabetes as compared to nondiabetic participants in the Metabolic Syndrome in Men (METSIM) Study (individuals on statin treatment were excluded)

Variable	NGT		IFG and IGT		Newly diagnosed T2D		Previously diagnosed T2D			
	N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*P*	*P*†
Total cholesterol (mmol/L)	2411	5.49±0.88	4196	5.62±0.97	438	5.76±1.13	285	5.04±1.03	<0.001	<0.001
LDL cholesterol (mmol/L)	2411	3.52±0.79	4193	3.60±0.83	438	3.68±0.91	285	3.09±0.86	<0.001	<0.001
HDL cholesterol (mmol/L)	2411	1.51±0.39	4194	1.44±0.39	438	1.37±0.43	285	1.28±0.37	<0.001	0.005
Total triglycerides (mmol/L)	2411	1.21±0.66	4196	1.48±1.13	438	1.93±1.32	285	1.77±1.05	<0.001	0.070
ApoA1 (g/L)	2411	1.42±0.24	4194	1.42±0.24	438	1.42±0.27	285	1.34±0.25	<0.001	<0.001
ApoB (g/L)	2411	1.03±0.25	4194	1.10±0.28	438	1.20±0.33	285	1.04±0.31	<0.001	<0.001

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *NGT* normal glucose tolerance, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *T2D* type 2 diabetes, *SD* standard deviation. ApoA1, apolipoprotein A1; apoB, apolipoprotein B. P, oneway ANOVA. P†, Newly detected T2D vs. previously diagnosed T2D, t-test. Glucose tolerance status is defined according to the American Diabetes Association criteria [88]. Conversion from SI to conventional units: divide total, LDL and HDL cholesterol by 0.0259, triglycerides by 0.0113, and apolipoproteins by 0.01.

Table 6.2 Comparison of lipid and lipoprotein levels in individuals with type 2 diabetes and in nondiabetic controls in different populations [24–26]

Population	Nondiabetic individuals (mmol/L)	Individuals with type 2 diabetes[c](mmol/L)
Total cholesterol		
Framingham Heart Study (mean, SD)	5.3 (1.0)	5.8 (1.2)*
San Antonio Family Heart Study (median, IQR)	4.7 (4.1–5.4)[a]	5.2 (4.6–6.1)*
Australian Diabetes, Obesity and Lifestyle Study (median, IQR)	5.7 (5.1–6.4)[a]	5.8 (5.3–6.6)
DECODE Study (mean, SE)	5.93 (0.01)[b]	5.96 (0.04)
LDL cholesterol		
Framingham Heart Study (mean, SD)	3.3 (0.9)	3.6 (1.0)*
San Antonio Family Heart Study (median, IQR)	2.82 (2.29–3.37)[a]	3.06 (2.53–3.71)*
Australian Diabetes, Obesity and Lifestyle Study (median, IQR)	3.67 (3.00–4.23)[a]	3.70 (3.15–4.25)
DECODE Study (mean, SE)	–	–
HDL cholesterol		
Framingham Heart Study (mean, SD)	1.5 (0.4)	1.1 (0.4)*
San Antonio Family Heart Study (median, IQR)	1.24 (1.09–1.50)[a]	1.20 (1.03–1.45)
Australian Diabetes, Obesity and Lifestyle Study (median, IQR)	1.42 (1.20–1.67)[a]	1.22 (1.04–1.53)*
DECODE Study (mean, SE)	1.45 (0.01)[b]	1.28 (0.02)*
Total triglycerides		
Framingham Heart Study (mean, SD)	1.2 (0.8)	2.9 (3.7)*
San Antonio Family Heart Study (median, IQR)	1.22 (0.87–1.66)[a]	1.94 (1.46–2.55)*
Australian Diabetes, Obesity and Lifestyle Study (median, IQR)	1.20 (0.90–1.55)[a]	1.94 (1.29–2.89)*
DECODE Study (mean, SE)	1.36 (0.01)[b]	1.97 (0.03)*

[a]NGT (normal glucose tolerance) group
[b]NFG (normal fasting glucose) and NGT group
[c]Diabetes type not defined in the Framingham Heart Study
*$P < 0.05$ as compared to the nondiabetic controls expressed in this table
SD standard deviation, *IQR* interquartile range, *SE* standard error, *DECODE Study* Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe Study. Conversion from SI to conventional units: divide values of total, LDL and HDL cholesterol by 0.0259 and triglycerides by 0.0113

Epidemiology: Collaborative analysis of Diagnostic criteria in Europe (DECODE) Study [24], have consistently shown higher levels of total triglycerides and lower levels of HDL cholesterol in individuals with type 2 diabetes as compared to nondiabetic controls [24–26]. In the Framingham Heart Study and in the San Antonio Family Heart Study, individuals with diabetes also had significantly higher total and LDL cholesterol levels as compared to nondiabetic individuals [24–26].

Gender differences exist in the lipid profile of individuals with type 2 diabetes. In a Finnish study, both HDL cholesterol and total triglycerides were higher in women with type 2 diabetes than in men with type 2 diabetes [27]. In the UKPDS including 2693 patients with newly diagnosed type 2 diabetes, lipid and lipoprotein levels were higher in women than in men (mean total cholesterol 5.7 vs. 5.2 mmol/L, LDL cholesterol 3.8 vs. 3.3 mmol/L, HDL cholesterol

1.10 vs. 1.04 mmol/L, and total triglycerides 1.6 vs. 1.5 mmol/L) [17]. Similarly in the Framingham Heart Study, significantly higher percentage of diabetic women, compared to nondiabetic women, exceeded the NCEP cutoff points for elevated total cholesterol (>6.2 mmol/L or 240 mg/dL; 35 vs. 18%), elevated LDL cholesterol (>3.4 mmol/L or 130 mg/dL; 60 vs. 45%), low HDL cholesterol (<1.0 mmol/L or 39 mg/dL; 49 vs. 9%), and elevated total triglycerides (>2.25 mmol/L or 200 mg/dL; 41 vs. 6%) ($P \leq 0.02$) [25]. Corresponding percentages in diabetic versus nondiabetic men for elevated total triglycerides were 36 versus 16% and for low HDL cholesterol were 55 versus 36% ($P < 0.001$ for both), but no differences in total and LDL cholesterol were observed [25].

Lipid abnormalities are observed already in children and adolescents with type 2 diabetes [28–31]. Pooled data from the USA show that at the time of diagnosis of type 2 diabetes in adolescents, 33–54% have an elevated total cholesterol (>5.17 mmol/L or 200 mg/dL), 24–46% have elevated LDL cholesterol (>3.36 mmol/L or 130 mg/dL), 29–61% have elevated triglycerides (>1.69 mmol/L or 150 mg/dL), and 44% have low HDL cholesterol (≤1.0 mmol/L or 39 mg/dL) [29].

Dyslipidemia as a Predictor of CVD in Individuals with Type 2 Diabetes

The UKPDS, including 2693 patients with type 2 diabetes, showed that among all CVD risk factors measured, high level of LDL cholesterol was the most important predictor of CAD and MI [17]. Low level of HDL cholesterol was the second most important predictor for CAD, followed by high hemoglobin $A1_c$, elevated levels of systolic blood pressure, and smoking. This translated to a 36% risk reduction in the incidence of CAD per 1 mmol/L (39 mg/dL) reduction in LDL cholesterol and a 15% decrease in CAD per 0.1 mmol/L (4 mg/dL) increase in HDL cholesterol. In contrast, total triglyceride level was not an independent risk factor for CAD.

Mechanisms Underlying the Association Between Dyslipidemia and Type 2 Diabetes

Diabetic dyslipidemia frequently coexists with a cluster of cardiometabolic risk factors, including central obesity and deposition of fat in the liver, muscle, and myocardium, as well as insulin resistance, endothelial dysfunction, and low-grade inflammation [22]. Overweight and obesity, particularly visceral and hepatic deposition of fat are key characteristics underlying the link between disturbances in glucose and lipid metabolism.

Alterations in Triglyceride-Rich Lipoprotein Particles in Type 2 Diabetes

The underlying mechanisms giving rise to diabetic dyslipidemia are illustrated in Fig. 6.2 [22, 32–37]. Impaired storage of free fatty acids (FFA) in the adipose tissue together with increased lipolysis attributable to insulin resistance causes spillover and flux of FFAs into the liver [34]. Hepatic de novo lipogenesis is enhanced as lipogenic enzymes, regulated by the transcription factor, sterol regulatory element-binding protein 1-c, are activated by a defective insulin signaling [22, 32]. Subsequently, there is a significant overproduction of VLDL particles from the liver resulting in hypertriglyceridemia [32]. A vicious cycle occurs as increased FFA levels together with enhanced de novo lipogenesis further promote VLDL synthesis and secretion from the liver [22]. Hepatic insulin resistance has recently been associated with elevated VLDL concentrations already in nondiabetic individuals [38]. In insulin-resistant individuals, an excess production of chylomicrons from lipids and VLDL overproduction due to carbohydrates of dietary origin lead to elevated levels of total triglycerides and postprandial triglyceride-rich lipoprotein remnants [33].

Hepatic VLDL overproduction together with increased apoCIII production [39] yields particles enriched with apoCIII which inhibits

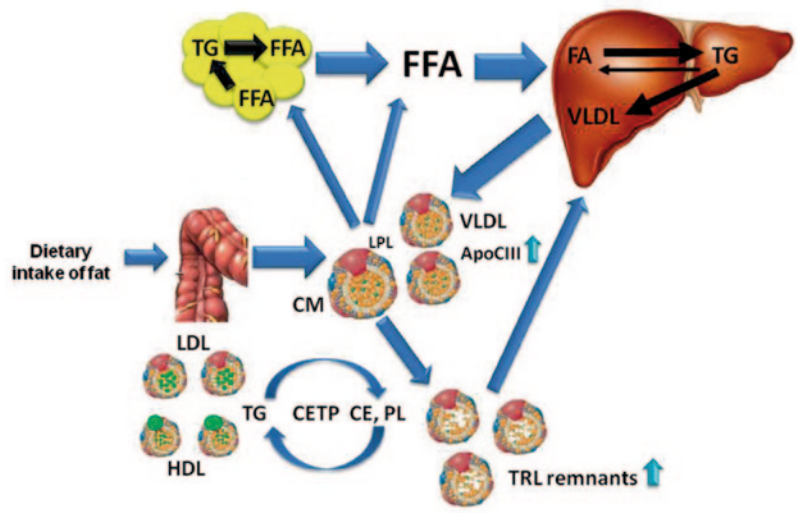

Fig. 6.2 A schematic representation of the mechanisms underlying diabetic dyslipidemia. Modified from [22]. *TG* triglyceride, *FFA* free fatty acid, *FA* fatty acid, *VLDL* very-low-density lipoprotein, *CM* chylomicron, *LPL* lipoprotein lipase, *LDL* low-density lipoprotein, *HDL* high density lipoprotein, *CETP* cholesterylester transfer protein, *CE* cholesteryl ester, *PL* phospholipid, *TRL remnants* triglyceride-rich lipoprotein remnants

intravascular lipolysis. Removal of circulating postprandial triglyceride-rich lipoprotein remnants is impaired attributable to impaired activity of heparin sulfate proteoglycans [40]. Prolonged residence time and compositional changes in postprandial triglyceride-rich lipoprotein remnant particles promoted by cholesteryl ester transfer protein (CETP) yield subsequent triglyceride enrichment of LDL and HDL particles and the production of small dense LDL and HDL particles [22]. Approximately half of individuals with type 2 diabetes have preponderance of small dense LDL particles, with a stepwise decrease in LDL particle size present already in impaired glucose tolerant subjects [41]. Gender difference also seems to exist, with a greater decrease in LDL size in women than in men with diabetes [21].

Alterations in Lipoprotein Particles in Prediabetes

Our recent study suggests that changes in lipoprotein particle concentrations are present already in the prediabetic stage [42]. We demonstrated that concentrations of all lipid components in the VLDL subclasses were increased as a function of worsening glucose tolerance in 9399 men of the METSIM cohort. Concentrations of HDL subclass particles, particular larger HDL particles, were reduced with the worsening of glucose tolerance, largely attributable to insulin resistance. Individuals with isolated impaired glucose tolerance seem to have more adverse changes in lipoprotein subclasses than individuals with impaired fasting glucose, including increased concentrations of larger-sized VLDL subclass particles and their lipid particles, and decreased concentrations of large HDL particles and their lipid components.

Type 2 Diabetes and Postprandial Lipemia

Postprandial lipemia has been associated with endothelial dysfunction, oxidative stress, and atherogenic effects on the arterial wall in patients with type 2 diabetes [43, 44]. Concentrations

of plasma triglycerides peak at 4–6 h after the ingestion of a fat load, and a significantly greater response of plasma triglycerides to a standard fat load has been reported in individuals with type 2 diabetes as compared to nondiabetic controls [45]. The prolonged exposure to triglycerides in type 2 diabetes affects the metabolism of LDL and HDL subclasses [21]. Type III hyperlipoproteinemia (familial dysbetalipoproteinemia), characterized by homozygosity for the apolipoprotein E2 isoform and markedly elevated plasma levels of cholesterol and triglycerides mainly in VLDL remnants and IDL, is triggered by poorly controlled type 2 diabetes and hyperinsulinemia [46–48]. Type V hyperlipoproteinemia (severe hypertriglyceridemia), characterized by grossly elevated plasma triglyceride levels (usually >1000 mg/dL (>11.3 mmol/L)) derived from both dietary sources (chylomicrons) and hepatic triglyceride synthesis (VLDL), is also associated with poorly controlled diabetes [47]. The severe hypertriglyceridemia associated with these disorders increases the risk of acute pancreatitis [49].

Efficacy of Lipid-Lowering Medication in Prevention of CVD in Patients with Type 2 Diabetes: Evidence from Randomized Controlled Clinical Trials

Robust evidence from randomized controlled clinical trials has demonstrated the efficacy of lipid-lowering treatment in the primary and secondary preventions of CVD mortality and major CVD events [50, 51]. Many studies, but not all, have included patients with type 2 diabetes.

Statins

Statins are specific inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, an enzyme with a key role in the synthesis of cholesterol in the liver. The efficacy of statins has been evaluated in several trials. The trials focusing solely on patients with type 2 diabetes are discussed in more detail below.

Heart Protection Study

In the Heart Protection Study, a total of 2912 patients with type 2 diabetes and without previously diagnosed occlusive arterial disease, aged from 40 to 80 years, were randomized to receive simvastatin 40 mg once daily or placebo for a mean follow-up of 4.8 years. First major coronary events including nonfatal MI or death from CAD were significantly reduced by 33% (95% confidence interval (CI) 17–46), $P=0.0003$) in the group receiving simvastatin treatment [52].

CARDS Study

In the Collaborative Atorvastatin Diabetes Study (CARDS), a total of 2838 individuals with type 2 diabetes and without previously documented CVD, aged 40–75 years, were randomized to receive treatment with either atorvastatin 10 mg once daily or placebo for a mean follow-up of 3.9 years [53]. Treatment with atorvastatin resulted in a significant reduction in first major coronary events (odds ratio (OR) 0.64, 95% CI 0.45–0.91), major cerebrovascular events (OR 0.52, 95% CI 0.31–0.89), and nonfatal MI (OR 0.59, 95% CI 0.36–0.98) [53]. Reductions in all cause mortality (OR 0.72, 95% CI 0.51–1.02) and CAD mortality (OR 0.74, 95% CI 0.40–1.36) were not significant [54].

ASPEN Study

The Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in noninsulin-dependent diabetes mellitus (ASPEN) included 1905 patients with type 2 diabetes and without prior MI or interventional procedure, aged from 40 to 75 years, who were randomized to receive atorvastatin 10 mg once daily or placebo for a mean follow-up of 4.0 years [55]. No significant changes in revascularization procedures (OR 0.92, 95% CI 0.60–1.40), major cerebrovascular events (OR 0.92, 95% CI 0.54–2.56), or all-cause mortality (OR 1.06, 0.54–1.64) were observed in the treatment group compared to the control group [54].

Meta-analyses of CVD Risk Reduction with Statins/ Lipid-Lowering Treatment in Patients with Type 2 Diabetes Versus Patients Without Diabetes

Meta-analyses of trials including participants with type 2 diabetes have consistently shown significant benefit in the secondary and primary prevention of CVD events with statins

A meta-analysis of the Cholesterol Treatment Trialists' [50] Collaborations in 2008 included 18,686 individuals with diabetes (of which 17,220 with type 2 diabetes) and 71,370 individuals without diabetes from altogether 14 randomized controlled trials evaluating statin therapy [50]. Statin therapy was concluded to safely reduce the 5-year incidence of major coronary events, coronary revascularization, and stroke by approximately 20% per 1 mmol/L (39 mg/dL) reduction in LDL cholesterol [50]. Proportional reduction in all-cause mortality of 9% per 1 mmol/L (39 mg/dL) reduction in LDL cholesterol was observed (relative risk, RR, 0.91, 99% CI 0.82–1.01, $P=0.02$), which reflected a reduction in vascular mortality (RR 0.87, 99% CI 0.76–1.00, $P=0.008$). In individuals with diabetes, reductions were observed in major vascular events (0.79, 0.72–0.86, $P<0.001$), MI or coronary death (0.78, 0.69–0.87, $P<0.0001$), coronary revascularization (0.75, 0.64–0.88, $P<0.0001$), and stroke (0.79, 0.67–0.93, $P=0.0002$). Observed reductions in event rates were similar in diabetic and nondiabetic individuals (the latter with a 13% reduction in all-cause mortality (0.87, 0.82–0.92, $P<0.0001$) and major vascular events (0.79, 0.76–0.82, $P<0.0001$)).

The Diabetogenic Potential of Statin Treatment

Statin therapy is generally safe and well tolerated [51]. However, concerns have recently arisen regarding the diabetogenic effect of statin treatment. Emerging evidence suggests a slightly elevated risk of incident type 2 diabetes with statin treatment [52, 56–60]. Statin therapy was associated with a 9% increased risk for incident diabetes in a meta-analysis of 91,140 participants from 13 statin trials [61]. The effect was both ageand dose dependent [61, 62]. The mechanisms underlying the association of statin therapy with incident diabetes are unclear [63–65] and further studies are required to reveal the mechanisms underlying the diabetogenic potential of statins. However, the risk–benefit ratio favors the use of statin in CVD prevention since the risk of incident diabetes is small in relation to the reduction of CVD events [61].

Fibrates

Statins effectively lower plasma LDL cholesterol and apoB concentrations. However, in diabetic dyslipidemia, residual risk attributable to particularly elevated levels of plasma total and VLDL triglycerides and low levels of HDL cholesterol often persists [22]. Largely for this reason, fibrates have been evaluated as monotherapy and add-on therapy to statins in diabetic dyslipidemia.

FIELD

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) is the largest study ($N=9795$) to date to evaluate CVD outcomes in patients with type 2 diabetes without statin treatment at study entry. The participants, of whom 7664 did not have a previous history of CVD, were randomized to receive treatment with 200 mg fenofibrate or placebo for a mean follow-up of 5 years [66]. Fenofibrate did not significantly reduce the risk of primary outcome of CAD events. It reduced, however, total CVD events (hazard ratio (HR) 0.89 (95% CI 0.80–0.99), $P=0.035$) mainly due to fewer nonfatal MIs and revascularizations. No difference was observed in total mortality between the groups. Fenofibrate was also associated with less albuminuria progression ($P=0.002$) and less retinopathy needing laser treatment ($P=0.003$) [67, 68]. However, there was a high drop-in rate for statin treatment in this trial (33.8% of participants started statin medication in the placebo arm and 18.1% in the fenofibrate arm), which has very likely reduced the event rate, and may have had an impact on the observed treatment effects with fenofibrate [66].

ACCORD

The effect of fenofibrate as an add-on therapy to simvastatin was evaluated in 5518 patients with type 2 diabetes in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Study [69]. During a mean follow-up of 4.7 years, as compared to simvastatin therapy, the combination of fenofibrate and simvastatin did not reduce the first occurrence of fatal or nonfatal CVD endpoints [HR 0.92 (95% CI 0.79–1.08), $P=0.32$]. A possible benefit for patients with both high baseline triglyceride level and low HDL cholesterol level was suggestive, but not statistically significant ($P=0.057$ for interaction).

Meta-analyses of Fibrate Trials

A systematic review and meta-analysis of 18 fibrate trials in 45,058 diabetic and nondiabetic individuals showed an overall reduction in major CVD events (RR 0.90 (95% CI 0.82–1.00), $P=0.048$) and in coronary events (0.87 (0.81–0.93), $P<0.0001$), but no effect on stroke or overall mortality among all participants both with and without type 2 diabetes [70]. Fibrates were noticed to slow the progression of microvascular complications (0.86 (0.75–0.98), $P=0.028$) and the progression of microalbuminuria and retinopathy (0.63 (0.49–0.81), $P<0.0001$), but data for these complications were available only for a limited number of trials. Subgroup analysis showed only a nonsignificant reduction in coronary events among the participants with diabetes (0.89 (0.78–1.02)) as compared to the significant reduction among those without diabetes (0.88 (0.91–0.94)) (Table 6.2).

In another meta-analysis of six trials (including ACCORD, FIELD, Bezafibrate Infarction Prevention (BIP) study, Helsinki Heart Study (HHS), Lopid Coronary Angiography Trial (LOCAT) Study, and Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT)), fibrates significantly reduced the risk of vascular events (RR 0.75 (95% CI 0.65–0.86), $P<0.001$) in the total population of 6 atherogenic dyslipidemia cohorts, including individuals both with and without type 2 diabetes, and in a subgroup analysis of 5068 subjects with both high triglycerides and low HDL cholesterol levels (0.71 (0.62–0.82), $P<0.001$), as well as among 15,303 subjects with low HDL cholesterol (0.84 (0.77–0.91), $P<0.001$), but not among those with neither high triglycerides nor low HDL cholesterol [71]. A similar subgroup effect was observed also in another meta-analysis of five trials including individuals both with and without type 2 diabetes (ACCORD, FIELD, BIP, VA-HIT, and HHS) [72]. No subgroup analysis for comparison of effect of treatment with fibrates on CVD outcomes in those with type 2 diabetes as compared to nondiabetic controls is available for the latter two meta-analyses.

Other Lipid Lowering Treatments

Ezetimibe Ezetimibe reduces LDL cholesterol by inhibition of intestinal cholesterol absorption via Nieman-Pick-C1-like-1 enterocyte receptor [73]. It results in an additional 20% reduction in plasma LDL cholesterol levels when combined with statin therapy [74] and an approximately 10% decrease in triglyceride and apoB levels [75]. Data on cardiovascular endpoints in patients with type 2 diabetes are lacking for ezetimibe. In the Stop atherosclerosis in Native Diabetes Study (SANDS) carotid intima media thickness regressed similarly in the group treated with ezetimibe combined with statin, as compared to the group treated with statin alone, but progressed in the standard treatment arm ($P<0.0001$ for intergroup difference) [73].

Niacin Niacin (nicotinic acid or vitamin B_3) lowers plasma levels of all atherogenic apoB-containing lipoproteins and increases HDL cholesterol levels [76]. Early studies involving niacin suggested cardiovascular benefit and a reduction in mortality in different populations, but also poor tolerability due to adverse effects [77]. Several smaller studies suggesting a regression in atherosclerosis with niacin treatment have involved small numbers of patients, and therefore they have limited power to dem-

onstrate beneficial effects on CVD endpoints [77]. Two multicenter randomized clinical trials, the "Atherothrombosis Intervention on Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes Trial" (AIM-HIGH) and "'Heart Protection Study—Treatment of HDL to Reduce the Incidence of Vascular Events" (HPS2-THRIVE) trial have recently investigated the role of niacin in the secondary prevention of CVD events [78, 79]. Data from the AIM-HIGH trial comparing a combination treatment with niacin and simvastatin with simvastatin alone in 3414 participants (of whom 34% with diabetes) with atherosclerotic CVD and low LDL cholesterol levels showed no incremental clinical benefit of niacin therapy in the prevention of CVD [78]. The HPS2-THRIVE trial including 25,673 patients with preexisting CVD (32% with diabetes), randomized to treatment with extended release niacin and antiflushing agent laropiprant or placebo as add-on to simvastatin with/without ezetimibe, was terminated early due to the lack of efficacy at 3-years follow-up and a concern for an increased risk of side effects [79].

Omega 3 FAs Omega 3 FAs, such as eicosapentaenoic acid and docosahexaenoic acid reduce serum triglyceride levels by inhibition of hepatic VLDL triglyceride secretion via reduced hepatic triglyceride synthesis [80]. Overall, the findings from different randomized clinical trials have been inconsistent [22] and a meta-analysis of trials of secondary prevention including 20,485 patients showed no reduction in total CVD events [81]. The evidence for omega 3 FAs in type 2 diabetes is inconclusive, as no effect on secondary CVD end points or CVD mortality was observed in the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial among 12,537 patients with dysglycemia or recently diagnosed type 2 diabetes [82]. Of note, however, the ORIGIN Trial tested a dose of 1 g per day of omega-3 FAs (docosahexaenoic acid/eicosapentaenoic acid), whereas doses of up to 4.8 g have been previously used to lower the levels of triglycerides.

Conclusions from Trial Evidence

In summary, statin treatment has the most robust evidence especially for secondary prevention of CVD events in individuals with type 2 diabetes, but also for primary prevention. Considering the high risk of CVD events in individuals with type 2 diabetes, the absolute risk reduction of CVD with statin-based lipid-lowering treatment is approximately threefold larger in individuals with type 2 diabetes than in nondiabetic individuals in both primary and secondary preventions.

Guidelines for Lipid-Lowering Treatment

Both American and European guidelines for the treatment of lipid disorders in individuals with type 2 diabetes recognize the compelling evidence between dyslipidemia and incident CVD in this high-risk group (Table 6.3). LDL cholesterol is the main treatment target in all guidelines and a treatment target of < 100 mg/dL (< 2.6 mmol/L) is recommended in the updated National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines [83], and an optional treatment target of < 70 mg/dL (< 1.8 mmol/l) for individuals with CVD or considered very high risk has been recommended [84, 85]. Lifestyle and dietary changes are the basis of treatment. Statin treatment is evidence based, especially in secondary prevention of CVD mortality and mortality, but also in primary prevention in individuals with high risk of CVD events. American Diabetes Association also recommends an HDL cholesterol target of > 1.0 mmol/L (> 40 mg/dL) for men and > 1.3 mmol/L (> 50 mg/dL) for women, and a total triglyceride target of < 1.7 mmol/L (< 150 mg/dL) [84]. However, the role of HDL cholesterol in the etiology of CVD is controversial based on a recent genetic study showing that it may not be causally associated with CVD events, in contrast to LDL cholesterol which has a causal association with CVD events [86]. A recent study has also suggested that total triglycerides are causally associated with an increase in the risk of CAD [87]. Combination therapy with

Table 6.3 Treatment guidelines for lipid management in type 2 diabetes

Expert organization	LDL cholesterol target	HDL cholesterol target	Triglyceride target	Primary treatment target	Therapeutic agent of choice
NCEP ATP III updated (2004) [83]	<100 mg/dL (<2.6 mmol/L) [Optional goal: <70 mg/dL (<1.8 mmol/L)]			LDL cholesterol levels	Statin or bile acid sequestrant or nicotinic acid. Therapeutic lifestyle changes
ADA (2011) [84]	<100 mg/dL (<2.6 mmol/L) without overt CVD <70 mg/dL (<1.8 mmol/L) with overt CVD	>40 mg/dL (>1.0 mmol/L) in men >50 mg/dL (>1.4 mmol/L) in women	<150 mg/dL (<1.7 mmol/L)	LDL cholesterol levels	Statin Combination therapy[b] may be considered if targets not achieved with maximal statin dose therapy
Joint ESC 2012[a] [85]	<2.5 mmol/L (97 mg/dL) without CVD risk factors or target organ damage <1.8 mmol/L (70 mg/dL) if CVD or CKD or ≥1 CVD risk factors			LDL cholesterol levels	Statin Inconsistent data available from studies examining the benefits of fibrates

[a] Includes European Association for the Study of Diabetes (EASD) and International Diabetes Federation (IDF-Europe)
[b] With statin and other lipid lowering therapeutic agent
NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; *ADA* American Diabetes Association; *ESC* European Society of Cardiology; *CVD* cardiovascular disease; *CKD* chronic kidney disease. Reference numbers are indicated in brackets

statin and another lipid-lowering agent may be considered if treatment targets are not achieved with a maximal dose of statins but the evidence for efficacy of combination therapy is currently lacking.

Concluding Remarks

Type 2 diabetes is associated with significantly elevated risk of CVD. High level of LDL cholesterol is the most important modifiable risk factor for CVD in type 2 diabetes. Diabetic dyslipidemia is characterized by elevated total and VLDL triglycerides and postprandial triglyceride-rich lipoprotein remnants, and low levels of HDL cholesterol as well as elevated levels of apolipoprotein B and small dense LDL particles. Lifestyle changes with exercise and dietary modification are the basis of treatment of dyslipidemia in type 2 diabetes, and very often should be combined with lipid-lowering treatment. Statin treatment is evidence based, especially in secondary prevention of CVD in individuals with type 2 diabetes, and also in primary prevention in individuals with high risk of CD events. Combination therapy with statin and another lipid-lowering agent may be considered if treatment targets are not achieved with a maximal dose of statins.

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Type 1 Diabetes Mellitus and Dyslipidemia

7

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease resulting in destruction of the pancreatic beta cells leading to absolute insulin deficiency [1] in contrast to type 2 diabetes (T2D) characterized by insulin resistance—frequently related to obesity—and insufficient compensatory insulin secretion. T1D is the predominant form of diabetes in youth occurring in approximately 1/300 youth in the USA by 18 years of age, but it is also diagnosed in adulthood, and accounts for 5–10 % of all cases of diabetes worldwide [2]. Differences in the underlying pathophysiology (autoimmune destruction of beta cells compared to obesity with insulin resistance and resultant beta-cell dysfunction) are important points to consider in the context of dyslipidemia in T1D and the contrasts with that of dyslipidemia in T2D (as outlined in detail in Chap. 6). Another important consideration is that many people with T1D are under the age of 21 years and the screening and treatment of dyslipidemia in children and adolescents with T1D are less evidence-based and different from those of adults. In this chapter, the history, epidemiology, etiology and pathogenesis, classification, clinical findings, differential diagnosis, complications, prognosis and clinical course, and treatment of dyslipidemia in T1D are reviewed.

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History

Prior to 1921 and the discovery of insulin by Banting and Best [3], T1D was a uniformly fatal disease. Since the discovery of insulin, T1D has been transformed from a subacute and fatal disease with no effective medical treatment to a chronic disease with a high burden of daily individual care with important acute (severe hypoglycemia and diabetic ketoacidosis, DKA) and chronic (retinopathy, neuropathy, nephropathy, and cardiovascular disease (CVD)) complications.

Historically, achieving near-normal glycemic control was extremely difficult due to limitations in medical care including technology, and patients with T1D were characterized by underinsulinization and a thin body habitus. Fasting lipid profiles in patients with T1D were then characteristic of insufficient insulin and poor glycemic control [4] (see later section on etiologies of dyslipidemia in T1D).

In contrast, since the landmark Diabetes Control and Complications Trial (DCCT) publication in 1993 on the beneficial effects of intensive diabetes management on reduction of microvascular complications [5], and in 2005 from the Epidemiology of Diabetes Interventions

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Table 7.1 Clinical characteristics of the DCCT/EDIC and EDC cohorts

Characteristic	Conventional			EDC			Intensive		
	DCCT		EDIC				DCCT		EDIC
	Baseline (1983–1989) (*n*=730)	Closeout (1983–1993) (*n*=723)	Year 12 (2005) (*n*=606)	Baseline (1986–1988) (*n*=161)	Year 10 (1996) (*n*=105)	Year 18 (2006) (*n*=88)	Baseline (1983–1989) (*n*=711)	Closeout (1993) (*n*=698)	Year 12 (2005) (*n*=620)
Age, mean(SD),y	27(7)	33(7)	46(7)	20(4)	31(4)	40(4)	27(7)	34(7)	46(7)
Duration, mean (SD),y	5(4)	12(5)	24(5)	11(2)	21(2)	30(2)	6(4)	12(5)	25(5)
BMI, mean(SD)	24(3)	25(3)	28(5)	24(3)	26(4)	28(5)	23(3)	27(4)	28(5)
BMI ≥ 30,%	2	6	28	3	1	27	1	19	31
Current smoker,%	18	20	12	20	17	15	19	20	15
HbA_{1c}, %(SD)	8.9(1.9)	9.1(1.5)	7.7(1.2)	9.0(1.7)	8.5(1.4)	8.3(1.4)	8.9(1.6)	7.4(1.1)	7.8(1.2)

BMI body mass index, *DCCT* Diabetes Control and Complications Trial, *EDC* epidemiology of diabetes complications, *EDIC* epidemiology of diabetes interventions and complications, *SD* standard deviation

and Complications (EDIC) on macrovascular disease [6], increased emphasis has been placed on achieving near-normal glycemia to prevent long-term microvascular and macrovascular vascular complications. Diabetes care has improved as a result of these efforts and improvements in technology [7] such as self-monitoring of blood glucose using home glucose monitors and continuous glucose monitors [8], continuous subcutaneous insulin infusions (insulin pumps) [9], insulin analogues with pharmacokinetic properties for basal and bolus administrations [10], as well as emerging artificial pancreas technology [11, 12].

With these advances in care, glycemic control has improved, but unfortunately rates of obesity in people with T1D in the USA have become similar to the increased rates of obesity in the general population in the USA. For example, body mass index (BMI) has increased over time in the DCCT-EDIC and the Pittsburgh Epidemiology of Diabetes Complications (EDC) studies, in part with the aging of the study population and increasing obesity in the USA, but also with more intensive glycemic control (Table 7.1). Increased rates of obesity in diabetes in children have been reported over the last decade [13]. The Search for Diabetes in Youth (SEARCH) study reported that 37% of females and 32% of males with T1D were either overweight or obese [14]. Intensive control of T1D typically improves plasma lipid and lipoprotein concentrations in people with T1D but at times may unfavorably affect lipoprotein composition, an effect that may have deleterious effects on CVD risk [15]. However, intensive glycemic control remains the cornerstone of T1D care and intensive control reduced CVD events by 57% in the DCCT-EDIC study [6]. Still, increased weight gain with intensive glycemic control can be an impediment to reaching A1c goals and could worsen some CVD risk factors. Obesity also can result in insulin resistance both in people without diabetes [16, 17] and in those with T1D both historically [18, 19] and in the post-DCCT era with achievement of tighter glycemic control [20, 21]. More specifically, insulin resistance has pro-atherogenic effects on the fasting lipid profile and lipoprotein subfraction cholesterol distribution in adults and adolescents with T1D [22–24].

Epidemiology

T1D has been increasing at 2–5% annually worldwide based on numerous multicenter epidemiologic studies such as the SEARCH [25, 26], EURODIAB [27, 28], and the DIAMOND (World Health Organization Multinational Project for Childhood Diabetes) studies [29, 30]. The SEARCH study estimated the prevalence of T1D was 2.28/1000 youth less than 20 years of age in the USA or more than 150,000 youth with diabetes in the USA in 2001, the majority with

T1D [25]. It has been estimated that there are now approximately 1.4 million people with T1D in the USA and 30 million globally [31]. Rates of T1D vary worldwide as would be expected, given the variation in genetics of the autoimmune system, exposure to environmental triggers, and differences in health-care infrastructure resulting in differences in survival from diagnosis of T1D and life span post diagnosis. The DIAMOND study reported a 2.8% annual increase in incidence of T1D from 1990 to 1999 in 114 populations from 57 countries (43,013 cases of T1D from a study population of 84 million children ≤14 years old) with similarly increased rates worldwide. Such sustained and rapid increases argue for an environmental or gene–environment interaction instead of genetic shifts in such a short time period. Multiple studies are ongoing to investigate the etiology of T1D with the goal of identifying targets for prevention [32, 33]. Barring dramatic scientific breakthroughs, such studies are likely long-term projects highlighting the need to improve care for people with T1D such as lipid health.

As with many chronic diseases of childhood, due to advances in clinical care, people with T1D are living longer and healthier lives, emphasizing the need for refinement of care for chronic comorbidities such as CVD for which dyslipidemia is a key risk factor. For example, historically in the 1970s and 1980s, the development of renal disease (proteinuria) was associated with rapid progression to death, frequently from cardiovascular causes [34, 35]. More recently, due to advances in diabetes care such as improved glycemic and blood pressure control, patients with T1D without evidence of diabetic kidney disease (either estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 or microalbuminuria) at baseline had similar mortality outcomes to the nondiabetic population over 7 years in the FinnDiane study [36] and over 20 years in the Pittsburgh EDC study [37]. More recent data from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study indicate that coronary artery calcification progresses in a step-wise manner with increasing levels of urinary albumin excretion and decreasing levels of GFR. However, even in the absence of these early indications of diabetic kidney disease, people with T1D have increased odds of progression of coronary artery calcification over 6 years as compared to nondiabetic controls [38]. Whether increases in coronary artery calcification predict an earlier development of CVD events and/or related mortality in people with T1D remains to be shown.

Although clinical care and outcomes for patients with T1D continue to improve, improvements in outcomes are urgently needed [39, 40]. Data from 28,887 children followed by EURODIAB in 12 European countries found a standardized mortality rate (SMR) of 2.0 [41]. The importance of CVD is emphasized as the predominant cause for premature mortality in people with T1D in a report from the UK with a hazard ratio of 3.7 for annual mortality for people with T1D compared to the general population (8.0 vs. 2.4/100,000 person-years) [42]. These data underscore the need for improved CVD health in patients with T1D; however, given that these data are based on historic outcomes prior to the widespread adoption of many of the current methods of care for T1D, there is reason to believe health outcomes, for those more recently diagnosed with T1D will be superior. For example, the Pittsburgh EDC cohort reported that life expectancy for people with T1D diagnosed between 1965 and 1980 was 15 years greater than those diagnosed between 1950 and 1964 [43].

Since the 2003 American Diabetes Association (ADA) statement on Management of Dyslipidemia in Children and Adolescents with Diabetes [44], data have accumulated indicating dyslipidemia is common in youth with T1D. A retrospective cross-sectional analysis of 682 youth with T1D < 21 years of age reported 18.6% had total cholesterol (TC) > 200 mg/dl or high-density lipoprotein (HDL)-cholesterol < 35 mg/dl [45] with longitudinal analysis in the same cohort indicating sustained abnormalities over time with only 6% being treated with a lipid-lowering medication [46]. The prospective Diabetes Follow-up Registry (DPV) study ($n = 27{,}358$) in Germany and Austria reported dyslipidemia (defined as TC > 200 mg/dl, low-density lipoprotein cholesterol (LDL-C) > 130 mg/dl, or

Table 7.2 Lipid abnormalities in pediatric T1D epidemiologic reports [2–5]

Study	High TC (mg/dl) (>200)	Low HDL-C (mg/dl) (<35) (<40)	High LDL-C (mg/dl) (>100) (>130)	High TG (mg/dl) (>150)
BDC (%)	15	3.5	NA	NA
DPV				NA
<11 years (%)	19	5	8	
12–16 years (%)	28	3	11	
17–26 years (%)	31	5	15	
SEARCH				
<10 years (%)	12	7	11	0
≥10 years (%)	19	12	15	9
T1D Exchange (%)				
6≤13 years (%)		3	31	6
13≤20 years (%)		9	38	11

DPV Diabetes Follow-up Registry, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *T1D* type 1 diabetes, *BDC* Barbara Davis Center

HDL-C < 35 mg/dl) in 29% of the participants under 26 years of age with increasing rates in older age categories [47]. Similarly, only 0.4% in this study received lipid-lowering medications. In the SEARCH study among youth with T1D ($n = 2165$), the prevalence of LDL-C > 160, > 130, and > 100 mg/dl was 3, 14, and 48%, respectively [48]. Among these participants, only 1% were on lipid-lowering medications indicating that in the years after the 2003 ADA statement [44], few pediatric endocrinologists were treating elevated LDL-C pharmacologically as recommended by the ADA. However, these data may not reflect more current practice in pediatrics. More recently, data from the T1D exchange report similar rates of dyslipidemia among participants with available data, 95 and 86% met ADA (HDL-C ≥ 35 mg/dl) and International Society for Pediatric and Adolescent Diabetes (ISPAD; HDL-C > 1.1 mmol/L or 41 mg/dl) HDL-C targets and 35% and 10% exceeded LDL-C (< 100 mg/dl) and TG (< 150 mg/dl) targets, respectively [49] (Table 7.2).

Data from the DCCT [50] and others such as the CACTI study [51] indicate that adults with well-controlled T1D have a fasting lipid profile similar to or even less atherogenic than nondiabetic controls. Additionally, rates of statin treatment have increased in adults with T1D over time. For example, the CACTI study reported statin use increased from 17 to 32 to 46% of the cohort over three study visits from 2000 to 2006 [52]. Longitudinal data from the DCCT-EDIC, the EDC, and Scottish Care Information-Diabetes Collaboration database studies also show a similar increase in the use of statins [53, 54]. However, despite this increase in statin use, 39% of people with T1D > 40 years of age in the Scottish study were not on a statin. Moreover, the trials with statins have included too few patients with T1D to be informative but the outcomes suggest benefit on major CVD events [55]. One of the potential effects of more intensive glycemic control is increased weight gain with associated deleterious effects on the lipid panel [15, 56]; however, the increased use of statins in T1D may explain improved lipid profiles over time.

Risk factors for elevated lipids in T1D include male sex, older age, waist circumference and visceral fat, and hemoglobin A1c [52]. In longitudinal analyses of epidemiologic data in both children and adults, change in A1c is associated with change in lipids; however, these associations are relatively modest with, for example, a 4-mg/dl change in LDL-C for every 1% change in A1c [52, 57], a much weaker effect than would be expected from a statin. This suggests that while glycemic control is the cornerstone of care, it may be insufficient to achieve lipid targets in most people with T1D (Fig. 7.1).

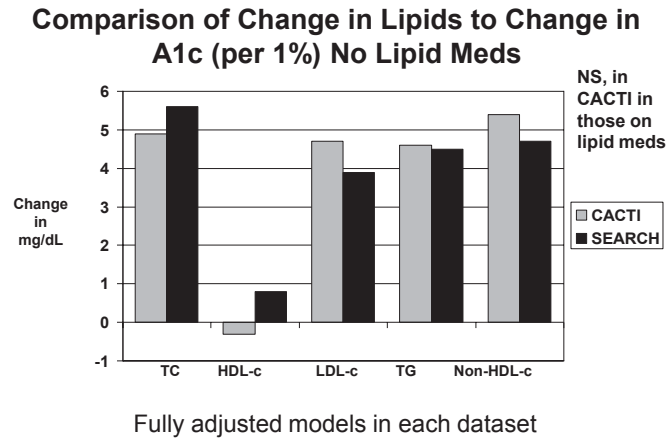

Fig. 7.1 Association of change in lipids per 1% change in A1c in CACTI and SEARCH. (Modified from [6, 7]). *CACTI* Coronary Artery Calcification in Type 1 Diabetes, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TC* total cholesterol, *TG* triglyceride

Etiology and Pathogenesis

Excellent reviews of the pathophysiology of lipid disorders in T1D have been published historically [4] and more recently by Verges which is summarized below [58]. In general, abnormalities of lipoproteins in T1D can be classified in the context of the underlying glycemic control which is a function of matching exogenous insulin delivery to maintain near euglycemia (or the failure to do so) and how this alters normal lipid and lipoprotein physiology (Fig. 7.2). T1D lipid pathophysiology can be considered in the categories of untreated T1D with extreme insulin deficiency such as seen in DKA (Table 7.3) in contrast to treated T1D with varying degrees of glycemic control and insulinization such as insulin resistance (Fig. 7.3). Basic lipid and lipoprotein physiology has been reviewed in detail in Chap. 1 as has the pathophysiology of dyslipidemia in T2D in Chap. 6 to which the reader may refer for contrast and comparison. Dyslipidemia in T2D is characterized by the effects of insulin resistance and obesity commonly resulting in decreased HDL-C and elevated TG.

With extreme insulin deficiency such as seen in untreated T1D (i.e., DKA), there is a marked increase in lipolysis of stored triglycerides (TG) from adipose tissue that are delivered to the liver with an increase in very-low-density lipoprotein (VLDL) TG synthesis and secretion that progressively diminishes as insulin deficiency becomes more severe [59]. In addition, lipoprotein lipase (LPL) activity is decreased [60, 61] with subsequent decreases in catabolism [58] of TG-rich lipoproteins and subsequent increases in TG-rich lipoproteins such as VLDL and variable chylomicrons in fasting plasma. LDL-C and HDL-C are also reduced as a consequence of decreased TG-rich lipoprotein metabolism and elevated plasma TG, respectively [62]. Cholesteryl ester transfer protein (CETP) mediates the transfer of TG from the increased TG-rich lipoproteins to HDL-C which is a substrate for hepatic lipase, which also contributes to its catabolism and a decrease in HDL-C. Initiation of insulin therapy leads to rapid improvement of the dyslipidemia seen acutely in DKA [63].

Patients with T1D and poor glycemic control have a relative insulin deficiency with elevated free fatty acids (FFA) and an increased production of VLDL, leading to hypertriglyceridemia [61, 64]. In this setting, the excess are preferentially partitioned to TG synthesis rather than oxidative metabolism and ketone body formation. Using isotope dilution in a small number of patients with T1D and average HbA1c of 8.8% demonstrated normal apo B secretion and metabolic clearance rates, but the VLDL-TG/VLDL apoB and the VLDL-C/VLDL apoB ratios were increased in those with diabetes [65]. In contrast, people with T1D and well-controlled glycemia have TG and LDL-C concentrations that are normal or slightly decreased and HDL-C

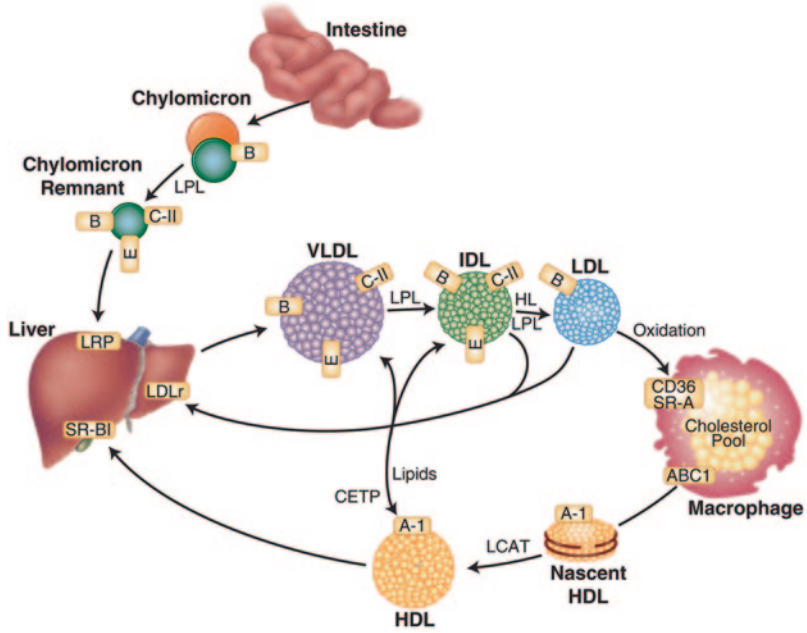

Fig. 7.2 Normal lipid and lipoprotein physiology. *CETP* cholesteryl ester transfer protein, *HDL* high-density lipoprotein, *IDL* intermediate-density lipoprotein, *LCAT* lecithin–cholesterol acyltransferase, *LDL* low-density lipoprotein, *LPL* lipoprotein lipase, *VLDL* very-low-density lipoprotein

Table 7.3 Effects of insulin deficiency on lipid and lipoprotein pathophysiology

Insulin state	Hormone-sensitive lipase	Lipoprotein lipase	Hepatic VLDL production	LDL apoB/apoE receptor expression	LCAT	Hepatic lipase
Deficient	↑	↓	↑	↓	↓	↓
Increased peripherally	↓	↑	↓	↑	↑	↑

LCAT lecithin–cholesterol acyltransferase, *LDL* low-density lipoprotein, *VLDL* very-low-density lipoprotein

plasma concentrations that are normal or slightly increased [51, 61, 64].

With current methods of intensive glycemic control, insulin is delivered subcutaneously and leads to peripheral hyperinsulinemia [66]. Consequences of this include increased downregulation of VLDL production [67, 68] and increased LPL activity, another possible mechanism for lower TG [69]. Mechanistic explanations for the often-reported increases in HDL-C in well-controlled T1D are less certain and include increased LPL activity and decreased CETP activity due to peripheral hyperinsulinemia [70]. However, when patients with T1D and excellent glycemic control (HbA1c 6.9 ± 1.7%) were infused with insulin subcutaneously, cholesteryl ester transfer was accelerated and both systemic insulin levels and LPL specific activity were increased [71]. Following intraperitoneal delivery basal systemic insulin levels declined by more than one half and both LPL and cholesteryl ester transfer returned to normal. Increases in HDL2 [70, 72] are consistent with the increases in LPL; however, increases in HDL3 [73] subfractions have also been reported and may be secondary to an increase in apolipoprotein A-I-only HDL particles [70].

Data are needed on whether differences exist in lipoprotein metabolism in people with T1D

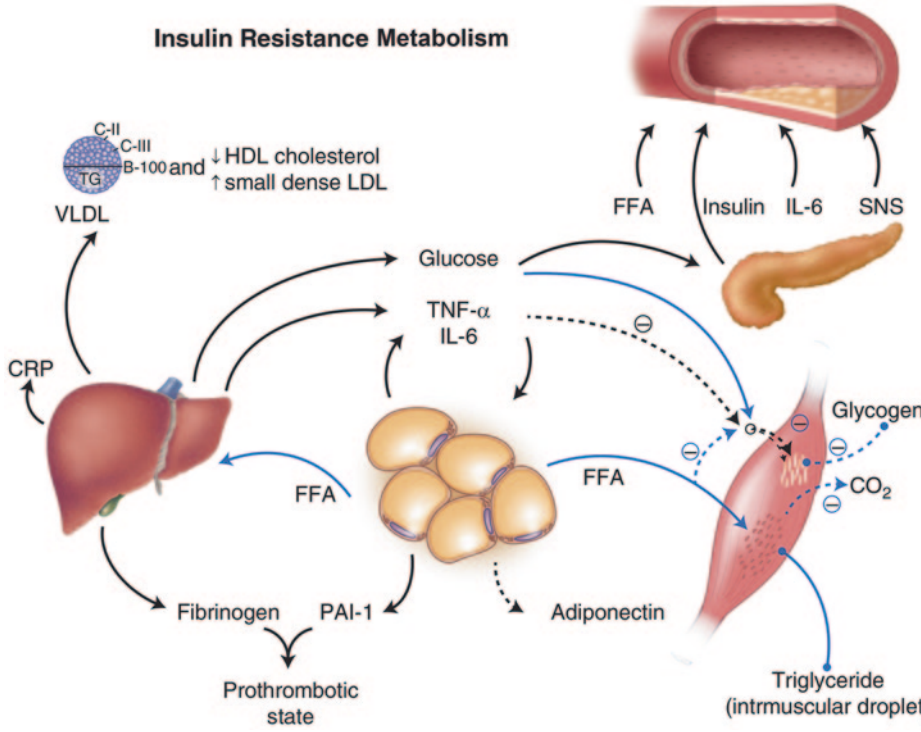

Fig. 7.3 Lipid and lipoprotein metabolism in insulin resistance. (Modified from [8]). *CRP* C-reactive protein, *FFA* free fatty acids, *HDL* high-density lipoprotein, *IL* interleukin, *LDL* low-density lipoprotein, *TNF* tumor necrosis factor, *VLDL* very-low-density lipoprotein

with additional diagnoses such as celiac disease. Comorbidities such as nephrotic syndrome will have a stronger effect on lipoproteins than T1D. For women with T1D of childbearing age, emphasis is placed on achieving tight glucose control prior to becoming pregnant. However, data on use of oral contraceptives in women with T1D and effects on lipids is limited. One small clinical trial reported no adverse effects of oral contraceptives on lipoprotein metabolism in women with T1D [74].

While the current clinical method of insulin delivery in T1D is subcutaneous, research is ongoing on intraperitoneal insulin infusion with implantable insulin pumps that more closely mimic physiologic insulin delivery. Such devices have the potential to achieve more physiologic control of T1D [75, 76]. Reported effects on plasma lipids include both increases in [77] and no effect on [78–80] TG, no effect on TC and apolipoprotein B [77–80], and no effect on [78–80] or decrease in [77] HDL-C.

In addition to the effects of glycemic control and peripheral hyperinsulinemia, kidney disease in patients with diabetes is also associated with pro-atherogenic effects on plasma lipids, although the mechanisms responsible for these changes are not clearly defined. Advances have been made in the prevention and treatment of diabetic nephropathy in the past decades [34]; however, kidney disease continues to be a major cause of morbidity and mortality in T1D and mostly associated with CVD [36, 37]. Overt proteinuria in T1D was associated with increases of TC, TG, and LDL-C and decreases in HDL-C [61, 68, 81]. Macroalbuminuria was associated with increased TG, TC, LDL-C in men and women and a decreased HDL-C in women in the EURODIAB insulin-dependent diabetes mellitus (IDDM) complications study [82]. Even

Table 7.4 Pros and cons of pharmacologic treatment

Pros	Cons
Dyslipidemia tracks into adulthood and likely will remain abnormal	Wait until adulthood to treat dyslipidemia
	The 10-year risk of a CVD event is unknown at the present time
	Refer patient to an adult endocrinologist once the patient is 18 years for treatment at that time
Adolescent risk factors predict surrogate markers of cardiovascular disease (cIMT) in adults (Bogalusa, Young Finns)	Some data suggest that regression or, at least, slowing of progression of atherosclerosis with aggressive treatment is possible in adults
Dyslipidemia is associated with atherosclerosis in childhood	There are no data to show that treatment in youth will reduce long-term CVD complications
Dyslipidemia is an important microvascular and macrovascular risk factor	*Primum non nocere*
	There are potential adverse events from dyslipidemia
	There is potential teratogenicity for adolescent females
DM is considered a CVD risk factor equivalent in adults. Earlier DM onset results in a longer DM disease burden and potential adverse "vasculo-metabolic memory" and an increased "area under the curve" for CVD risk factors	Cost: (1) There number needed to treat to prevent CVD events is unable to be calculated; (2) many years of treatment are required with the potential for lifetime treatment
There is a long-term elevated risk of CVD in youth with dyslipidemia (PDAY , Young Finns, Bogalusa)	There is some measurement variability with regression to the mean of lipid measures, although they tend to track as high or normal
There is a preponderance of data on lowering CHD risk in adults, why wait?	There are no outcome data, no safety data in youth with diabetes

cIMT carotid intima-media thickness, *CHD* coronary heart disease, *CVD* cardiovascular disease, *DM* diabetes mellitus, *PDAY* pathobiological determinants of atherosclerosis in youth

microalbuminuria, the earliest stage of diabetic nephropathy screened for clinically, is associated with more atherogenic lipid changes. Microalbuminuria was associated with increased TG in the EURODIAB study [82] and in other studies with increased apo B [83–85] and LDL-C [83, 84] and an increased apoB to apoA1 ratio [84, 85].

As T1D is frequently diagnosed in childhood, the pediatric population and the physiologic changes seen in puberty in addition to the pathophysiologic differences of T1D are a consideration. For example, the ADA [86], the American Heart Association (AHA) [87], the American Academy of Pediatrics [88], and the ISPAD [89] all have thresholds for pharmacologic treatment of dyslipidemia and goals for lipids. Age- and sex-specific normal and abnormal values linked to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP:ATP III) lipoprotein thresholds have also been calculated using National Health and Nutrition Examination Survey (NHANES) data [90] that recognize physiologic variations seen with pubertal development, although these data were not generated in children and adolescents with T1D. Additional considerations for dyslipidemia in the pediatric diabetes population have been reviewed and include costs, lack of outcome data, potential lifelong treatment, and adverse effects while argument to treat includes tracking of lipids from childhood into adulthood, a preponderance of data in adults on the benefits of lowering lipids to prevent CVD in adults, and association of lipids with surrogate CVD markers among others (Table 7.4 and Fig. 7.4) [91].

In addition to these quantitative differences in lipids in T1D, qualitative differences are also evident as reviewed by Verges [63]. The VLDL-C/TG ratio is increased due to enriched esterified cholesterol in contrast to TG [92, 93], perhaps due to increased cholesterol ester transfer between lipoproteins [93]. We in fact found less VLDL-C and more HDL-C in men with T1D versus control men ($P < 0.05$), but among T1D women, there was a shift in cholesterol to denser LDL, despite more statin use [94].

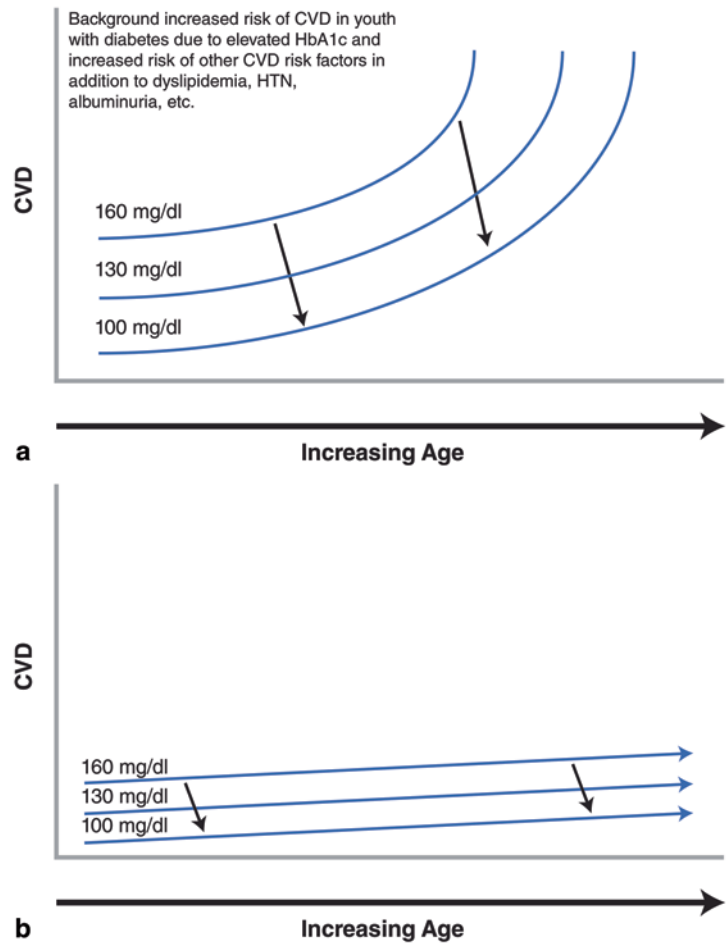

Fig. 7.4 Hypothetical relationship between LDL-C, LDL-C lowering, and future CVD in youth with diabetes. (Modified from [9]). *CVD* cardiovascular disease, *HTN* hypertension, *LDL* low-density lipoprotein

Among control subjects, men had more cholesterol distributed in VLDL and LDL but less in HDL than women; however, among those with T1D, there was no sex difference. Within sex and diabetes strata, a more atherogenic cholesterol distribution by insulin resistance was seen in men with and without diabetes, but only in women with T1D [94].

In vitro, the VLDL isolated from patients with T1D stimulated more cholesteryl ester synthesis in macrophages than did VLDL isolated from control subjects [95]. Of note, the outer layer of VLDL and LDL particles in patients with T1D has increased free cholesterol to lecithin ratio [61, 93], possibly reducing the stability and fluidity of lipoproteins and increasing CVD risk [96]. Enrichment of LDL-C with TG and increased small, dense LDL particles has also been reported [97–99] which are associated with increased CVD risk [100], although reduction of the proportion of small, dense LDL particles has also been reported with intensification of glycemic control [101]. Glycation of apo B within LDL, in relationship with hyperglycemia, reduces LDL binding to apoB/apoE receptors [102, 103] and glycated LDL increases formation of foam cells in the arterial walls due to preferential uptake by macrophages [63]. Increased oxidation of LDL is associated with the glycemic excursions common in T1D [104], resulting in rapid uptake by macrophages and foam cell formation and promotion of monocyte chemotaxis by increasing synthesis of adhesion molecules by endothelial cells and cytokines by macrophages, with resultant increases in the in-

flammatory atherosclerotic process seen in T1D which is associated with CVD [105].

HDL in people with T1D is often enriched with TG [61, 93], attributed to increased cholesterol ester transfer between lipoproteins [93]. The outer layer of HDL has increased sphingomyelin to lecithin ratio, increasing HDL rigidity [106], which may not be reversible with improved glycemic control [107]. With glycation of apo A-I within HDL, HDL-mediated reverse cholesterol pathway is also impaired in T1D with less effective cholesterol efflux [108]. The antioxidant, anti-inflammatory, antithrombotic, and vasoreactant properties of HDL that are anti-atherogenic may also be reduced in patients with T1D. The decrease in paraoxanase activity is an example with resultant decreases in erythrocyte membrane protection and LDL particle oxidation [109, 110] with decreased ability to prevent oxidized LDL-induced endothelium-dependent vasoconstriction in vitro [111].

Classification and Goals

In children with diabetes, goals for lipids include: LDL-C < 100 mg/dl, HDL-C > 35 mg/dl, TG < 150 mg/dl. Similar cut points are used for AHA and ISPAD [89, 112]. These goals for lipids in youth with T1D were new in the last decade and the topic of ongoing revision is based on emerging data. In pediatrics, the goal of preventing future cardiovascular disease must be balanced with the potential adverse effects of dyslipidemia medications, even though rare. For adults, NCEP:ATP III considers diabetes to be a CHD risk equivalent and therefore uses goals for LDL-C and non-HDL-C of < 100 and < 130 mg/dl, respectively. The most recent joint position statement from the ADA and AHA does not distinguish CVD risk between T1D and T2D, citing a lack of evidence to do so [113]. Further data are needed to determine whether goals and therapies for dyslipidemia in people with T1D should differ from those with T2D.

It is important to recognize that patients with T1D can have other forms of dyslipidemia that are genetic or acquired. Genetic forms of hypertriglyceridemia or hypercholesterolemia can be discerned by assessing a family history of early-onset CHD in first-degree relatives and by their fasting lipid profiles. Acquired causes can be coexisting diseases, drugs, and/or lifestyle. Because people with T1D may have other autoimmune disorders, a history for symptoms of thyroid and adrenal or celiac disease is important and a general set of laboratory tests which include a comprehensive metabolic panel and urinalysis for protein and thyroid stimulating hormone (TSH). Routine screening for elevations of lipoprotein (a) is not indicated; however, it is important to recognize in patients with nephropathy that increases in lipoprotein (a) are often seen and may contribute to atherosclerotic risk [114].

Clinical Findings

Approach to the Patient

Routine screening for dyslipidemia is recommended for people with T1D with the timing of initial testing dependent on a patient's age and their family history of dyslipidemia and CVD. Due to the profound insulinopenia associated with the typical presentation of T1D, a lipid panel measured prior to metabolic stabilization reflects the pathophysiologic effects of insulin deficiency, i.e., elevated TG and decreased LDL-C and HDL-C. Although there are clinical circumstances in which investigation of more severe hypertriglyceridemia at presentation is indicated, for most patients with T1D, evaluation for dyslipidemia should be delayed until after initial levels of glycemic control have been achieved 6–8 weeks after presentation.

The ADA recommends screening for dyslipidemia in children with T1D after glycemic control has been established in children > 2 years of age if there is a family history of hypercholesterolemia or a CVD event before age 55 years of age or if the history is unknown (Table 7.5). If family history is not a concern, then screening is recommended at puberty (≥ 10 years of age). If normal, then repeat measurements every 5 years are

Table 7.5 ADA recommendation on lipid screening and management in youth with diabetes [115]

	T1D	T2D
Initial screening age (after glycemic control is obtained)	More than 2 years at diagnosis if unknown or with a positive family history; otherwise at 12 years(puberty)	At diagnosis
Rescreening if lipid levels are normal	5 years	2 years
Optimal concentrations	LDL-C: <100 mg/dl HDL-C: >35 mg/dl Triglycerides: <150 mg/dl	LDL-C: <100 mg/dl HDL-C: >35 mg/dl Triglycerides: <150 mg/dl
Management of elevated LDL-C Initial therapy	Glycemic control, MNT, physical activity, weight control, tobacco cessation	Glycemic control, MNT, physical activity, weight control, tobacco cessation
After 3–6 months	LDL-C >160 mg/dl: begin medication LDL-C 130–159 mg/dl: recommended after MNT failure based on other CVD risk factors	LDL-C >160 mg/dl: begin medication LDL-C 130–159 mg/dl: recommended after MNT failure based on other CVD risk factors
	Pregnancy counseling if statin is started	Pregnancy counseling if statin is started

ADA American Diabetes Association, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *MNT* medical nutrition therapy, *T1D* type 1 diabetes, *T2D* type 2 diabetes

recommended [115]. The AHA has similar risk stratification (Fig. 7.5) [112].

Additional considerations in a patient with T1D include the risk of hypoglycemia while the patient is in the fasted state. Herein, if the exogenous insulin exceeds a patient's needs, hypoglycemia may be consequential. For example, asking an insulin-dependent patient to fast and then drive to a laboratory to provide a sample could result in severe hypoglycemia with tragic consequences. Recently, it has been suggested that a nonfasting sample may be an effective screening tool for most people with T1D [91]. Data from the large DPV registry ($n=29{,}979$) suggests that fasting status had a minimal effect on TC, LDL-C, and HDL-C [116]. Therefore, it seems reasonable to screen for dyslipidemia in people with T1D with a nonfasting sample with the caveat that a repeat evaluation may be required to better delineate lipid health.

Physical Findings

The physical findings of dyslipidemia in T1D share the same features as in patients without diabetes and exist based on the specific underlying lipid abnormality, i.e., a patient with T1D could also have a genetic form of dyslipidemia as described in other chapters in this text. Historically, people with T1D had normal or lower BMIs, although now rates of overweight and obesity in patients with T1D are similar to those of the general population [14]. Increased BMI and waist circumference may be indicators of increased risk for dyslipidemia. A thorough physical examination in people with T1D includes vital signs, weight, waist circumference, BMI, examination for arcus cornealis, xanthomatosis, edema, carotid bruits, heart murmurs, abdominal aneurysm, thyromegaly, foot examination, and deep tendon reflex relaxation time.

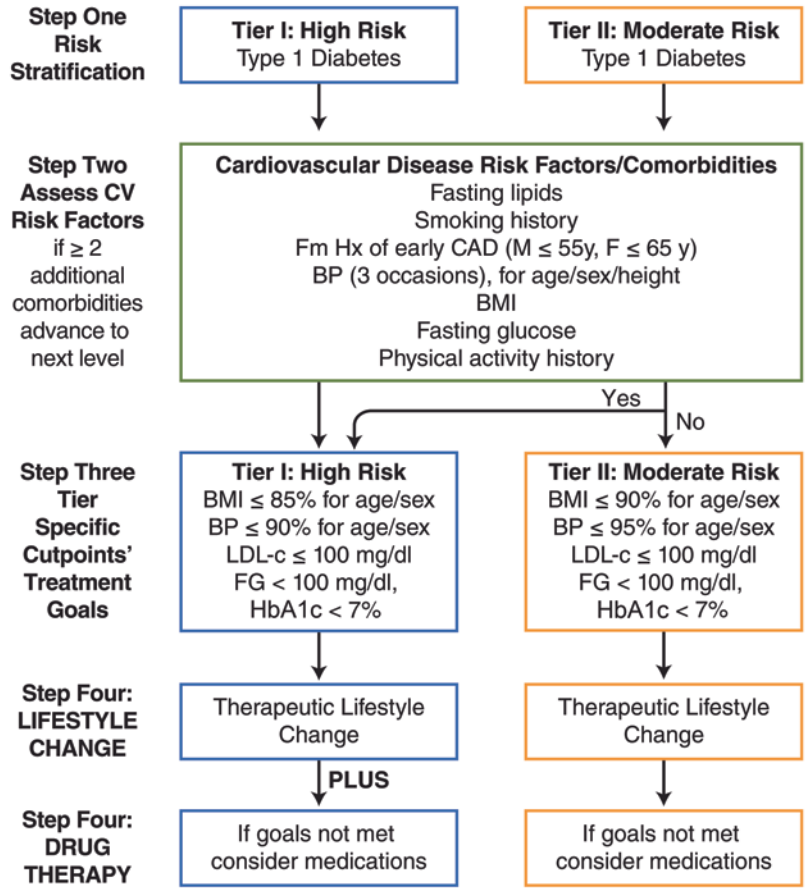

Fig. 7.5 AHA guidelines for risk stratification and treatment in youth with diabetes. (Modified from [9]). *AHA* American Heart Association, *BMI* body mass index, *BP* blood pressure, *FG* fasting glucose, *LDL-C* low-density lipoprotein

Laboratory Tests

As noted, current screening recommendations for dyslipidemia in T1D start with a fasting lipid profile, although a nonfasting lipid profile may have an increasing role as a screening tool. Additionally, tests to consider the influence of lipid levels include thyroid function tests (autoimmune hypothyroidism is seen in approximately 10% of people with T1D and, if untreated, has a negative effect on lipids), kidney disease including urine microalbumin, and if >300 mg/dl, a 24-h urine for protein (and creatinine) analysis, blood urea nitrogen (BUN) analysis, and liver function tests. Although some would consider the need for additional lipoprotein quantification, e.g., by fast protein liquid chromatography, nuclear magnetic resonance (NMR), or a vertical auto profile, this ancillary approach remains to be researched and is not needed for routine clinical care.

Differential Diagnosis

The differential diagnosis for dyslipidemia in T1D should start with an assessment of glycemic control. As noted in the section on pathophysiology, poorly controlled T1D with underinsulinization will result in increased TG (rarely to levels associated with pancreatitis) that requires urgent therapeutic attention. The dyslipidemia seen in DKA and uncontrolled T1D improves quickly with initiation of insulin therapy, although the

challenges of daily self-care make adherence to therapy difficult in a subset of people with T1D. In addition to poor glycemic control, insulin resistance due to increasing adiposity and associated peripheral hyperinsulinemia can result in a lipid profile similar to that seen in the metabolic syndrome or T2D with elevated TG and decreased HDL-C. Studies have evaluated the addition of metformin to insulin to improve insulin resistance in people with T1D with mixed effects on A1c but some improvement in lipids [117]. The role of insulin sensitizers in T1D and the potential benefits on the lipid profile and CVD to follow require further evaluation [118]. As noted above, both the thyroid function and the presence of kidney disease should be evaluated as part of routine screening in T1D and specifically as part of the evaluation of dyslipidemia in T1D.

Complications

Dyslipidemia in T1D is a risk factor for mortality and CVD as in patients without diabetes. However, given the increased risk of CVD in T1D, goals for lipids and thresholds for treatment are more aggressive than in the nondiabetic population both for adults and for adolescents. In addition to CVD, dyslipidemia is a risk factor for microvascular disease in T1D.

The FinnDiane study reported that in 4197 patients with T1D increased levels of TC, TG, and high LDL-C and reduced levels of HDL2-C were risk factors for all-cause mortality, in addition to kidney disease, insulin resistance, and abdominal obesity [119]. In a further analysis in 4084 patients, apo B, apo B/apo A-1 ratio, and the intermediate-density lipoprotein cholesterol (IDL-C) were the best lipoprotein predictors of mortality [120]. The EDC study using NMR reported that in addition to TG and overt nephropathy, all three VLDL subclasses, small LDL, medium LDL, and medium HDL were increased in CHD cases compared to controls without CHD [121]. Paradoxically, Costacou and coworkers reported that women with HDL-C > 80 mg/dl had increased CHD risk, highlighting the need for further study on how levels of HDL-C in patients with T1D relate to risk [122].

Data also link dyslipidemia to surrogate markers of CVD in people with T1D. Increased non-HDL-C in the EDC study [123] and reduced HDL-C in the CACTI study [124] were associated with progression of coronary artery calcification (CAC). Oxidized LDL immune complexes were associated with the development of CAC in a subgroup of the DCCT [125] and oxidized LDL and advanced glycation end product-modified LDL in circulating immune complexes were associated with carotid intima-media thickness (cIMT) and its progression [126]. A cross-sectional study has also linked apoE polymorphisms with cIMT [127], and in adolescents and young adults with T1D HDL-C, and the LDL-C/HDL-C ratios were associated with cIMT in males [128].

Data also link dyslipidemia with microvascular disease in T1D. In the DCCT-EDIC study, progression of microalbuminuria after 13 years of persistent microalbuminuria was reduced in patients with lower LDL-C and TG, in addition to other factors [129]. Incident microalbuminuria was predicted by high TG, apo B, apo A-II, and HDL3-C in the 2304 adults with T1D in the FinnDiane study [130]. The EDC reported that non-HDL-C, small LDL-C, and LDL particle size were risk factors for overt nephropathy in T1D [131] and high levels of HDL-C as well as better glycemic control were associated with regression from microalbuminuria or more macroalbuminuria in children and adolescents with T1D [132]. Severity of retinopathy was positively associated with TG and negatively with HDL-C in the DCCT-EDIC study [130]. Moreover, using NMR, small LDL, LDL particle concentration, apo B, and small HDL were positively associated with retinopathy and large LDL and large HDL were negatively associated with retinopathy [133]. There is also evidence that elevated serum lipids were associated with clinically significant macular edema (CSME) and retinal hard exudates, suggesting lipid-lowering may lower risk for CSME [134]; similar associations of serum lipids with CSME were also seen in an Australian cohort [135]. Less data are available on lipids and neuropathy, but the EURODIAB prospective study reported TC and LDL-C were risk factors for vibration perception threshold, a marker of large nerve fiber

dysfunction [136]. The same group reported elevated TG as well as BMI, smoking, and hypertension (HTN) are risk factors for neuropathy [137].

Consideration must also be given to the possibility of severe hypertriglyceridemia and pancreatitis in uncontrolled T1D, especially in DKA. While a lipid profile is not a standard evaluation in DKA, any complaints of abdominal pain should prompt an urgent evaluation for pancreatitis and a lipid panel to determine whether or not hypertriglyceridemia maybe contributory. In general, this becomes an issue when TG are >1000 mg/dl.

Prognosis and Clinical Course

As reviewed above, outcomes in T1D continue to improve as care for T1D improves. Although data suggest that the mean HbA1c has improved post DCCT, these improvements are not as rapid as clinicians or patients wish. For example, the DPV study which includes 30,708 children and adolescents with T1D in Germany and Austria reported a decrease in A1c of 0.038%/year from 8.7% in 1995 to 8.1% in 2009 [138]. While encouraging, at this pace of improvement, mean A1c will not reach the adolescent goal of 7.5% for many years to come. With improved glycemic control, there is a concern that this will lead to increases in weight and altered lipoprotein profile as seen in a subset of patients in the DCCT [56]. There are data to suggest that properly focused intensification of glycemic control can be achieved without increases in weight [139] and such efforts are important to avoid the untoward effects of weight gain on insulin resistance and lipids.

An additional factor in treatment of dyslipidemia in T1D will be the continued advance in pharmacologic therapy. The introduction of statins and the accumulation of safety and efficacy data as well as a decrease in price have led to their increased use. Data on the use of lipid-lowering medications in T1D are more limited than in T2D. As noted above, the Cholesterol Treatment Trialists' (CTT) Collaborators reported a 21% proportional reduction in major vascular events per millimoles per liter reduction in LDL-C in people with T1D ($n=1466$ in a meta-analysis from 14 randomized statin trials) [55]; however, there is some concern that adequate documentation of T1D was lacking. The Heart Protection Study was the largest study with T1D patients in the CTT report and the effect of LDL-C reduction on major vascular events was similar to that on the T2D population [140].

In the pediatric population, one trial of statins in adolescents with T1D was safe and had an expected decrease in LDL-C, although the improvement in vascular stiffness measures was not quite statistically significant, perhaps due to the short duration of the study, small sample size, or relatively low LDL-C levels at start of the study [141]. Currently, the Adolescent type 1 Diabetes Cardio-renal Interventions Trial (ADdit) study is testing the effects of a statin and angiotensin-converting enzyme (ACE)-inhibitor on vascular disease in adolescents with T1D [142]. Results of such studies are certain to provide clinical guidance on treatment of dyslipidemia in adolescents with T1D. There are currently insufficient data to state whether the response to lipid-lowering medications differs in people with T1D than in those with T2D or without diabetes. In addition to healthy lifestyle changes (diet and exercise) and pharmacologic treatment, glycemic control is an important component of lipid health for people with T1D.

Treatment

Intensification of glycemic control will always be a first treatment for dyslipidemia in patients with T1D, if A1c is above goal. In addition, as with dyslipidemia in general, achieving healthy weight and increasing physical activity and improving diet are cornerstones of care. For example, the ADA recommends optimization of glycemic control and MNT, basically a diet that includes multiple servings of fruits and vegetables, whole grains, and lean poultry and fish and contains limited amounts of saturated fat in the diet. Statin therapy is recommended for LDL-C>160 mg/dl or >130 mg/dl with one or more CVD risk factor after MNT and lifestyle changes in patients with T1D>10 years of age

[115], and the AHA has published recommendations for medication initiation, titration, and monitoring [87]. Potential adverse effects of statins include myopathy, exercise intolerance, elevated liver enzymes, and rarely, central nervous system (CNS) effects. Presently, the ADA considers an adult T1D patient statin eligible unless they have an LDL-C < 100 mg/dl and/or are pregnant, nursing, or attempting to conceive [115]. At what age the statin should be started or given to T1D patients who have an LDL-C < 100 mg/dl remains an uncertainty [91].

In patients who are statin intolerant and/or cannot achieve an LDL-C < 100 mg/dl with a statin, additional lipid-altering therapy may be needed. For LDL-C-lowering ezetimibe, a bile acid sequestrant, a fibrate, or niacin may be considered. However, additional considerations are needed. For instance, ezetimibe-induced lowering of LDL-C has not been proven to reduce CVD events when added to statin therapy or when used alone. Bile acid sequestrants increase TG when TG are already elevated, e.g., > 250 mg/dl, and although fenofibrate may lower LDL-C to some extent, gemfibrozil is not very effective. Bile acid sequestrants also commonly cause gastrointestinal (GI) upset. Niacin is perhaps a better choice, but in some patients, glucose tolerance may worsen and are not indicated in the setting of renal disease. If TG are still > 500 but < 1000 mg/dl on a high dose potent statin, then a fibrate or high omega-3 fatty acids should be used. If levels are > 1000 mg/dl, the patient should be referred to a lipid specialist and dietician or certified diabetes educator for further workup and treatment. Presently, low levels of HDL-C are not a target for pharmacologic therapy.

Conclusion

The pathophysiology of dyslipidemia in T1D is frequently a function of underinsulinization and related poor glycemic control or peripheral hyperinsulinization in intensive control (with obesity as a potential contributor). As such, intensive diabetes control to achieve near euglycemia with a healthy diet and physical activity for a healthy weight are the basis of lipid health in patients with T1D. In addition, lipid-lowering medications are frequently required to achieve lipid goals and the thresholds for initiation of treatment and the goals of treatment are evolving as data accumulate on safety, efficacy, and health outcomes. With potential advances in therapy for glycemic control in T1D such as the artificial pancreas on the horizon, the dyslipidemia characteristic of T1D may evolve to be more consistent with that of peripheral normo-insulinemia and relative euglycemia. Lipid health with its effects on microvascular and macrovascular disease will continue to be an important aspect of health care in patients with T1D. At present, most adult patients with T1D should be statin treated and guidelines on what age to start and what lipid goals should be attained are evolving.

Addendum

Recently, the American Heart Association (AHA) and American Diabetes Association published a joint scientific statement on cardiovascular disease (CVD) in T1D which includes a summary of dyslipidemia in T1D [143]. In addition to this, an AHA scientific statement specific to CVD risk factors in youth with diabetes provides a more specific review, including the role of lipids, in the pediatric population [144]. The SEARCH for Diabetes in Youth study found that decreases in BMI after 2-year follow up were associated with modest reduction in serum triglycerides and increases in HDL cholesterol [145]. The Swedish Diabetes Register reported a 2-3 fold increase in all cause and CVD mortality in 33,915 adults with T1D who had an HbA1c <6.9% compared to 169,249 non T1D controls over 8 years [146]. Mortality risk in people with T1D further increased in a step-wise manner with elevated HbA1c. Furthermore, LDL-C increased in stepwise manner with increasing HbA1c and mortality, although it did not enter the final models. Statin use was reported as 43.1 at any time after 2005 (compared to 9% of controls).

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Dyslipidemia in Chronic Kidney Disease and Nephrotic Syndrome

8

Nosratola D. Vaziri

Introduction

The association of kidney disease with lipid abnormalities has been known for many decades [1–3]. More recent studies employing experimental animals and cultured cells have helped to expand the understanding of the molecular mechanisms by which kidney disease alters lipid metabolism and plasma lipid profile. In addition, much has been learned about the role of the associated lipid disorders in progression of kidney disease and its cardiovascular, metabolic, and other complications. The nature of lipid abnormalities in patients with kidney disease varies depending on the presence and severity of proteinuria, reduction in glomerular filtration rate (GFR), renal replacement modalities (hemodialysis, peritoneal dialysis, and renal transplantation), dietary and drug regimens, and coexistent genetic disorders of lipid metabolism. An overview of the features, mechanisms, consequences, and treatment of kidney disease-associated lipid disorders is provided in this chapter.

Historical Perspective The association of kidney disease with changes in serum lipids has been known for many decades. For instance, hyperlipidemia has long been considered as one of the cardinal manifestations of nephrotic syndrome [1, 4]. In addition, numerous studies conducted in the 1960s and 1970s demonstrated the association of chronic renal failure with elevated serum triglyceride and triglyceride-rich lipoproteins [5–9]. Building upon these pioneering investigations, during the past two decades, considerable progress has been made in elucidation of the nature, mechanisms, consequences, and potential treatment of lipid disorders caused by renal disease.

Part I: Lipid Disorders in Nephrotic Syndrome

A variety of primary and secondary kidney diseases impair the glomerular filtration barrier which leads to proteinuria (Table 8.1). Glomerular proteinuria exceeding 3.5 g/day in adults or urine protein/creatinine ratio of 2–3 or greater in children results in nephrotic syndrome which is characterized by the tetrad of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. The magnitude of hyperlipidemia and the associated alteration in lipoprotein metabolism in nephrotic syndrome parallels the severity of proteinuria. Plasma concentrations of cholesterol, triglycerides, apoB-containing lipoproteins (very-low-density lipoprotein, VLDL; intermediate-density lipoprotein, IDL; and low-density lipoprotein, LDL), and lipoprotein(a)(Lp(a)) are elevated in nephrotic syndrome [10, 11]. However, high-density lipoprotein (HDL) cholesterol concentration is usually unchanged or

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Table 8.1 Common causes of nephrotic syndrome in children and adults are listed in this table. The most common cause of nephrotic syndrome in children is minimal change disease (77%) followed by membranoproliferative glomerulonephritis (8%) and focal segmental glomerulosclerosis (7%) with the remainder of listed conditions representing approximately 2% of the cases each. Causes of nephrotic syndrome in adults are listed according to their prevalence

Children
Minimal change disease
Membranoproliferative glomerulonephritis
Focal segmental glomerulosclerosis
Proliferative glomerulonephritis
Mesangial proliferation
Membranous glomerulonephropathy
Focal and global glomerulosclerosis
Adults
Focal segmental glomerulosclerosis
Membranous nephropathy (including lupus)
Minimal change disease
Diabetic nephropathy
IgA nephropathy
Preeclampsia
Post-infectious glomerulonephritis
Primary amyloidosis or the related disorder light-chain deposition disease
Benign nephrosclerosis

IgA immunoglobulin A

reduced and occasionally elevated and the total cholesterol/HDL cholesterol ratio and triglyceride content of HDL are generally increased in the patients with nephrotic syndrome [10, 11]. In addition to these quantitative changes, nephrotic syndrome markedly alters the composition of lipoproteins. In this context, the cholesterol to triglyceride, free cholesterol to cholesterol ester, and phospholipid to protein ratios in the lipoproteins are altered in nephrotic syndrome [11]. This is accompanied by significant increase in apolipoproteins—A-I, A-IV, B, C, and E levels—and the apoC-III/apoC-II ratio.

Pathogenesis of Nephrotic Dyslipidemia

The abnormalities of serum lipids and lipoproteins in nephrotic syndrome are largely due to their impaired clearance and to a lesser extent their altered biosynthesis. The underlying mechanisms by which nephrotic syndrome alters lipid and lipoprotein metabolism are summarized below.

Impaired Triglyceride-Rich Lipoprotein Metabolism in Nephrotic Syndrome

Fasting serum triglyceride, VLDL, and IDL levels are elevated, triglyceride contents of apoB-containing lipoproteins is increased, and postprandial lipemia is prolonged in nephrotic syndrome [12–16]. These abnormalities are primarily due to impaired clearance of VLDL and chylomicrons [14–18]. Nephrotic syndrome results in lipoprotein lipase (LPL), hepatic lipase, and VLDL receptor deficiencies. In addition, by changing the structure and composition of the lipoproteins, nephrotic syndrome impairs effective binding of the triglyceride-rich lipoproteins to the key receptors, their ability to activate lipolytic enzymes, and engage in a proper lipid and apoprotein exchange with HDL. The effects of nephrotic syndrome on the key steps in metabolism of triglyceride-rich lipoproteins are briefly described here.

Several studies have shown marked reduction of post-heparin lipolytic activity in the nephrotic humans [12, 13] and animals [11, 14, 16, 19] pointing to depletion of endothelium-bound LPL pool. In addition, a series of studies conducted in the author's laboratory have shown marked reductions of heparin-releasable and intracellular LPL, along with a significant reduction of LPL protein abundance despite normal LPL messenger RNA (mRNA) abundance in the adipose tissue, skeletal muscle, and myocardium of Imai rats with spontaneous focal glomerulosclerosis [20] and Sprague–Dawley rats with puromycin-induced nephrotic syndrome [21]. These findings point to the posttranscriptional/posttranslational nature of LPL deficiency and dysfunction in nephrotic syndrome. Marked downregulation of hepatic lipase expression and activity, and of VLDL receptor mRNA and protein in the skeletal muscle and myocardium, is also seen in these animals [22–24].

Diminished apoE and apoC-II contents and increased apoC-III/apoC-II ratio in VLDL and chylomicrons further contribute to impaired LPL-mediated lipolysis of triglyceride-rich lipoproteins [25, 26]. This is, in part, due to the HDL dysfunction in nephrotic syndrome [27, 28]. In fact, in vivo studies have shown impaired endothelial binding and LPL-mediated lipolysis of VLDL in nephrotic rats and their correction by infusion of HDL from normal animals. Likewise, in vitro studies using cultured rat aortic endothelial cells have shown impaired binding and LPL-mediated lipolysis of VLDL and chylomicron particles from nephrotic rats and their restoration by addition of HDL from normal rats [18, 28]. In vitro studies have shown a 50% lower heparin-releasable lipase activity in the livers of nephrotic rats compared with the normal rats [14]. Nephrotic syndrome results in increased expression of the key enzymes involved in fatty acid, phospholipid, and triglyceride biosynthesis and downregulation of genes-encoding proteins involved in fatty acid catabolism in the liver [29–31]. These abnormalities suggest that increased production of fatty acids, triglycerides, and phospholipids may contribute to the pathogenesis of hyperlipidemia in nephrotic syndrome.

LDL and Cholesterol Metabolism in Nephrotic Syndrome

Serum total cholesterol and LDL cholesterol are markedly elevated in nephrotic syndrome. This is due to increased production of cholesterol and LDL and impaired catabolism/clearance of apoB and LDL [15, 32]. Data from animal models of nephrotic syndrome reveal that several factors contribute to the defective LDL clearance and increased cholesterol biosynthesis. These include posttranscriptional or posttranslational reduction in LDL receptor (LDLR) protein expression in the liver [33–35] upregulation of 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase [34, 36] and Acetyl-CoA acetyltransferase (ACAT-2) expressions [37] and activities as well as increased apoB-100 biosynthesis [10]. Recent studies conducted in the author's laboratories have revealed upregulation of hepatic proprotein convertase subtilisin/kexin type 9 (PCSK-9) inducible degrader of LDLR (IDOL) as the main cause of the posttranslational deficiency of the LDL receptor in nephrotic syndrome [38]

HDL Metabolism in Nephrotic Syndrome

Nephrotic syndrome results in Lecithin cholesterol acyltransferase (LCAT) deficiency which is caused by its heavy losses in the urine [39] and contributes to impaired cholesterol enrichment of HDL. This is not surprising since molecular weight of LCAT (63 kD) is close to that of albumin whose heavy urinary loss is the defining feature of nephrotic syndrome. HDL receives a significant amount of its cholesterol content from albumin, which serves as a carrier of free cholesterol from the peripheral tissues to the freely floating HDL-3 [40]. Consequently, hypoalbuminemia, which is a cardinal feature of nephrotic syndrome can potentially contribute to the prevalence of cholesterol ester-poor HDL in nephrotic syndrome.

Several studies have shown significant elevation of serum cholesterol ester transfer protein (CETP) in humans with nephrotic syndrome [41–43] which can deplete cholesterol esters and raise triglyceride content of HDL in nephrotic patients. Cholesterol-rich HDL particles serve as the apoE and C donors to the nascent VLDL

and chylomicrons, and thus the impaired maturation of HDL can contribute to the dysregulation of triglyceride-rich lipoproteins in the nephrotic individuals [28]. In addition, a marked reduction in scavenger receptor class B type 1 (SR-B1) protein abundance and significant downregulation of PDZ-containing kidney protein 1 (PDZK1) mRNA and protein expressions are seen in animals with nephrotic syndrome [44, 45]. By compromising the reverse cholesterol transport, the observed SR-B1 deficiency may contribute to the associated atherogenic diathesis.

Increased Plasma Lipoprotein(a) in Nephrotic Syndrome

Nephrotic syndrome results in marked elevations of serum Lp(a) [46–48] which is due to its increased production by the liver [49]. Plasma Lp(a) falls in response to spontaneous remission or anti-proteinuric therapies. Elevation of Lp(a) in nephrotic syndrome contributes to the prothrombotic and atherogenic diathesis in this population.

Part II: Lipid and Lipoprotein Metabolism in Chronic Renal Failure

The serum lipid profile evolves during the course of progression of chronic kidney disease (CKD). Patients with mild-to-moderate CKD and nephrotic proteinuria commonly exhibit hypercholesterolemia and elevated LDL levels [50]. In contrast, serum total cholesterol and LDL cholesterol concentrations are commonly within the normal limits or reduced in most patients with CKD and minimal proteinuria and in patients with end-stage renal disease (ESRD) maintained on hemodialysis. Serum triglycerides and VLDL levels are commonly increased and clearance of VLDL, chylomicrons, IDL, and chylomicron remnants is impaired in advanced CKD or ESRD patients. This is associated with presence of small dense LDL, accumulation of IDL, chylomicron remnants, and oxidized LDL [50–53], reduced serum apoA-1 and HDL cholesterol, impaired HDL maturation, and defective HDL antioxidant, and anti-inflammatory and reverse cholesterol transport properties [50, 51, 54–56].

In patients with ESRD, the renal replacement modality and kidney transplantation significantly affect lipid profile. For instance, serum total cholesterol and LDL cholesterol levels are commonly elevated in patients treated with chronic peritoneal dialysis [50, 52], contrasting normal or reduced values in most hemodialysis-treated patients. In addition, serum lipid profile in this population is affected by severity of inflammation, malnutrition, and lipid-altering drugs (e.g., statins, fibrates, steroids, rapamycin, calcineurin inhibitors, and sevelamer, etc.) as well as coexisting genetic disorders of lipid metabolism. Moreover, oxidative stress and inflammation, which are common features of CKD [57, 58], lower serum cholesterol and simultaneously may cause atherosclerosis by promoting LDL oxidation and monocyte adhesion, infiltration, and foam-cell formation in the artery wall [59]. The mechanisms by which CKD/ESRD alters lipid metabolism are briefly described here:

Abnormalities of Triglyceride-Rich Lipoprotein Metabolism in CKD

Accumulation of triglyceride-rich lipoproteins in CKD is exclusively due to impaired clearance of VLDL and chylomicrons and their remnants which is caused by downregulation of lipoprotein lipase and VLDL receptor in the skeletal muscle, adipose tissue, and myocardium and hepatic lipase and LDL receptor-related protein (LRP) in the liver as well as increased ratio of apoC-III to apoC-II. Several studies have demonstrated marked reduction of plasma post-heparin lipolytic activity in patients with chronic renal failure [60–62]. In addition, studies conducted in experimental animals have demonstrated marked reduction of LPL mRNA and protein expression [63] accompanied by downregulation of glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) in the adipose tissue, myocardium, and skeletal muscles of rats with CKD [64] and secondary hyperparathyroidism [65]. Indeed, Akmal et al. [60] showed improvement in post-heparin lipolytic activity with parathyroidectomy in patients

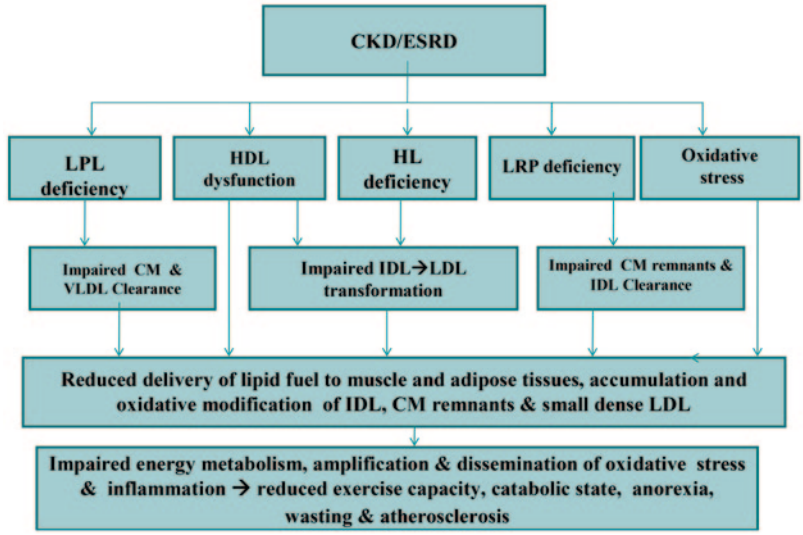

Fig. 8.1 CKD-induced downregulation of LPL, hepatic lipase (HL), and LRP and HDL deficiency and dysfunction lead to impaired delivery of lipid fuel to skeletal muscles and adipose tissue, accumulation and oxidative modification of LDL, IDL, and chylomicrons. Together, these abnormalities contribute to reduced exercise capacity, amplification of oxidative stress, inflammation, catabolic state, and atherosclerosis. CKD chronic kidney disease, LPL lipoprotein lipase, LRP LDL receptor-related protein, HDL high-density lipoprotein, IDL intermediate-density lipoprotein

with ESRD. In addition, a number of other factors, such as reductions of apoC-II and apoE may contribute to LPL deficiency and dysfunction in patients with advanced CKD. Other contributing factors include insulin resistance [66], reduced physical activity, and diminished thyroxin (T_4) to-triiodothyronin (T_3) conversion which are common consequences of advanced CKD and are known to downregulate expression and activity of LPL. Finally, by promoting the release and degradation of the endothelium-bound LPL, recurrent heparinization used with each hemodialysis procedure may contribute to LPL depletion in ESRD patients [67]. LPL deficiency and dysfunction plays a major part in the pathogenesis of the CKD-associated hypertriglyceridemia and impaired energy metabolism [68].

Patients and animals with CKD also have hepatic lipase deficiency, in part, caused by secondary hyperparathyroidism [69] which can result in accumulation of IDL particles. Reduced hepatic *LRP* gene expression and protein abundance is seen in rats with CKD [70] which can further contribute to accumulation of the highly atherogenic IDL and chylomicron remnants. In addition, CKD results in marked reduction of VLDL receptor abundance in skeletal muscle, adipose tissue, and myocardium [71], a phenomenon which can contribute to elevation of VLDL in this population.

Consequences of Triglyceride-Rich Lipoprotein Abnormalities in CKD (Fig. 8.1) The CKD-associated impairment of clearance of triglyceride-rich lipoproteins and accumulation of their remnants have numerous adverse consequences [68]. Accumulation of oxidation-prone IDL, chylomicron remnants, and triglyceride-containing small dense LDL may lead to accelerated atherosclerosis. In addition, binding of oxidized LDL and phospholipids to their receptors on the leukocytes, macrophages, and resident cells stimulates production and release of cytokines and chemokines which participate in the development and intensification of CKD-associated inflammation [72]. Finally, by initiating lipid peroxidation chain reaction, the circulating oxidized lipoproteins and their remnants facilitate

the dissemination and maintenance of oxidative stress throughout the body. Therefore, the presence of oxidized lipids and lipoproteins is both a cause and a consequence of oxidative stress.

Effect of CKD on Cholesterol and LDL Metabolism

Despite having normal or subnormal serum total cholesterol and LDL cholesterol levels, the risk of atherosclerosis and cardiovascular disease is greatly increased in CKD patients. It is likely that oxidative stress and inflammation as opposed to elevated plasma cholesterol or increased cholesterol biosynthesis play a more important role as the cause of atherosclerosis in CKD. It is, therefore, not surprising that clinical trials of statins have proven ineffective in lowering the incidence of cardiovascular disease in patients with ESRD maintained on hemodialysis [73]. As noted earlier, due to the constellation of LPL and hepatic lipase deficiencies and scarcity of cholesterol-rich HDL particles, conversion of IDL to triglyceride-depleted cholesterol-rich LDL is impaired in advanced CKD. Accumulation of these abnormal oxidation-prone LDL particles contributes to the atherogenic diathesis in this population.

When present, nephrotic proteinuria compounds the effect of renal insufficiency causing hypercholesterolemia [33, 36]. It is tempting to speculate that a similar phenomenon may be involved in the pathogenesis of hypercholesterolemia in peritoneal dialysis patients due to losses of proteins in the peritoneal dialysate effluent.

HDL Abnormalities in Chronic Renal Failure

Serum HDL cholesterol concentration is commonly reduced, triglyceride content of HDL is increased, maturation of pre-beta-migrating cholesterol ester-poor to alpha-migrating cholesterol ester-rich HDL is impaired, and proportion of cholesterol ester-poor HDL to cholesterol ester-rich HDL is increased in patients with advanced chronic renal failure [50, 51, 56, 74]. The chronic renal failure-induced structural and quantitative abnormalities of HDL are accompanied by its impaired antioxidant and anti-inflammatory activities and reverse cholesterol transport capacity [56]. Interestingly, HDL cholesterol is markedly elevated and is paradoxically associated with increased cardiovascular and overall mortality/morbidity in a sizeable minority of this population [75]. The HDL particles in these patients are highly oxidized and pro-inflammatory in nature and contain high levels of the acute phase protein, serum amyloid A1, albumin, lipoprotein-associated phospholipase A2, and apoC-III. In addition, the HDL particles from hemodialysis patients have reduced phospholipid and increased triglyceride content and impaired ability to promote cholesterol efflux from macrophages [76]. The following factors contribute to the abnormalities of HDL function and metabolism in CKD:

Serum apoA1 and apoA2 levels are significantly reduced in ESRD patients [56]. Studies in ESRD patients maintained on hemodialysis have shown increased catabolism of apoA1 as a major cause of its reduced serum concentration [77]. In addition, in vivo and in vitro studies have shown marked reduction in hepatic biosynthesis of apoA1 in uremic animals due to mRNA instability in cultured hepatocytes exposed to uremic milieu [78–80]. Interestingly, there is a high prevalence of anti-apoA1 autoantibodies in maintenance hemodialysis patients which is associated with dialysis vintage [81].

Deficiency and diminished hepatic production of LCAT [82–85] may impair maturation of cholesterol ester-poor pre-beta-HDL to mature cholesterol ester-rich HDL in chronic renal failure. In vitro studies have shown that the ability of HDL in removing cholesterol from lipid-laden macrophages is significantly lower in hemodialysis-dependent patients than healthy control individuals [86]. Chronic renal failure also results in marked upregulation of ACAT1 in the artery wall and the diseased kidney [87–90]. The combination of LCAT deficiency and upregulation of ACAT works in concert to impair HDL maturation and reverse cholesterol transport in chronic renal failure. In fact, oxidative modification of

HDL has been demonstrated in ESRD patients [54, 91, 92], which can also contribute to the defective HDL maturation, impaired reverse cholesterol transport, and accelerated atherosclerosis in chronic renal failure.

Serum CETP levels and activity are normal in hemodialysis patients [93, 94]. Elevated HDL triglyceride content in advanced CKD is primarily due to deficiency of hepatic triglyceride lipase [22, 69].

In a recent study, we found marked reductions of HDL antioxidant capacity in ESRD patients maintained on hemodialysis [54], in part, due to significant reduction of paraoxonase1, and glutathione peroxidase [54, 95]. In addition, we have found marked reduction of anti-inflammatory activity of HDL in ESRD patients [54, 55]. These observations were subsequently confirmed by Yamamoto et al. [86] who found that in contrast to the HDL from healthy individuals, the HDL from hemodialysis patients stimulated production of inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)6, and IL1 beta) in isolated macrophages in vitro. This was associated with significant reduction of the HDL antichemotactic activity. Likewise, Weichhart et al. [96] have shown that HDL from the majority of ESRD patients lacked anti-inflammatory property and in many cases promoted production of inflammatory cytokines by macrophages in vitro. They attributed the pro-inflammatory activity of HDL to the presence of serum amyloid A in this population.

Oxidative modification and reduced antioxidant and anti-inflammatory functions of HDL in this population are largely due to the prevailing oxidative stress and inflammation as seen in other conditions [97, 98] and can, in turn, intensify the inciting oxidative stress and inflammation and, thereby, participate in a vicious cycle. In fact, oxidative modification of HDL is associated with a high risk of cardiovascular and overall mortality [75].

The abnormalities cited above have serious consequences: The reduction in the antioxidant capacity of HDL and the oxidative milieu of CKD lead to accumulation of oxidized LDL and phospholipids, their avid uptake by macrophages, and resident cells leading to foam-cell formation and atherosclerosis. This is compounded by oxidative modification of HDL [54, 75] which limits its binding affinity for ATP-binding cassette transporter A1 (ABCA-1) transporter, increased ACAT1 activity which favors intracellular retention of cholesterol, and LCAT deficiency which limits uptake of cholesterol by HDL. Together, these abnormalities severely limit cholesterol efflux and can contribute to accelerated atherosclerosis and cardiovascular disease in CKD. In addition, HDL deficiency and dysfunction contribute to the prevailing oxidative stress and inflammation [72] which are the major cause of morbidity and mortality in this population. Finally, given the antithrombotic effect of normal HDL, its deficiency and dysfunction may contribute to blood-access thrombosis in the dialysis population.

Lp(a) in CKD

Plasma Lp(a) concentration is generally elevated in patients with chronic renal failure. Comparison of patients receiving continuous ambulatory peritoneal dialysis with patients receiving hemodialysis has shown significantly higher Lp(a) levels in former population in whom significant losses of proteins in the peritoneal dialysis fluid effluent simulates nephrotic syndrome in functionally anephric subjects [99]. Both free and LDL-bound Lp(a) fractions are elevated in the plasma of patients with ESRD. This is, in part, due to the lack of renal catabolism of this lipoprotein in this population [100].

Part III: Superimposing Factors that Modify Lipid Metabolism in CKD/ESRD

Effect of Chronic Peritoneal Dialysis on Lipid Metabolism in ESRD Patients Peritoneal dialysis results in significant losses of proteins in the dialysate effluent averaging 10 g/day. In addition, high glucose concentrations in peritoneal fluids used as an osmotic agent to facilitate

fluid removal leads to the unintended absorption of large amounts of glucose through peritoneal membrane. Loss of substantial amounts of protein in the peritoneal fluid can raise serum LDL and total cholesterol levels by simulating nephrotic syndrome. Moreover, influx of large amounts of glucose from the peritoneal fluid can further raise plasma triglyceride levels by activating Carbohydrate-responsive element-binding protein (chREBP) and thereby de novo fatty acid synthesis and lipogenesis in these patients. In fact, compared to the hemodialysis patients, LDL cholesterol, triglyceride and Lp(a) concentrations and LDL/HDL ratio are significantly higher in the majority of patients maintained on peritoneal dialysis [101–107]. The role of excess glucose load in the pathogenesis of peritoneal dialysis-associated dyslipidemia was demonstrated by Babazono et al. [108] who found significant reduction of serum LDL cholesterol, triglycerides, and small dense LDL particles using an icodextrin-containing instead of glucose-containing peritoneal dialysis solution. However, despite more atherogenic lipid profile in peritoneal dialysis patients, the risk of cardiovascular mortality in them is comparable to hemodialysis patients [109]. This is primarily due to lower incidence of dialysis-induced hypotension and cardiac arrhythmias caused by the rapid rise and fall in electrolytes as well as better control of fluid balance with peritoneal dialysis.

Effect of Kidney Transplantation on Lipid Profile The kidney transplant recipients frequently exhibit dyslipidemia which is typically marked by increased total cholesterol, LDL cholesterol, and triglyceride levels and normal or reduced HDL concentration [110–112]. The associated dyslipidemia plays an important part in the pathogenesis of cardiovascular disease in this population [113]. Dyslipidemia in transplant recipients is largely due to the use of immunosuppressive agents, particularly prednisone, cyclosporine, and sirolimus, which are commonly used in this population to prevent graft rejection. In this context, glucocorticosteroids promote insulin resistance and raise hepatic production and blood concentration of glucose which stimulates production of fatty acids and triglycerides and lowers HDL cholesterol [114]. In addition, cyclosporine-A increases serum triglyceride, cholesterol, and Lp(a) levels. Animal studies conducted in our laboratories to discern the mechanism by which cyclosporine-A raises serum cholesterol and triglycerides have identified downregulation of hepatic cholesterol 7-alpha-hydroxylase (which limits conversion of cholesterol to bile acids) and of LPL in the skeletal muscle and adipose tissue [115]. In fact, Artz et al. [116] have shown that the replacement of cyclosporine by tacrolimus, azathioprine, or mycophenolate mofetil results in a significant decrease in total cholesterol and LDL cholesterol concentrations and triglyceride levels in transplant patients. Likewise, rapamycin can cause hypertriglyceridemia and hypercholesterolemia by as-yet unknown mechanism(s). Among the commonly used immunosuppressants, azathioprine and tacrolimus have little or no impact on lipid metabolism.

Part IV: Treatment of Dyslipidemia in Patients with Kidney Disease

It is critical to tailor and modify the treatment on an individualized basis and make the necessary changes as the kidney disease evolves over time. This viewpoint is supported by the results of several clinical trials which have shown the futility of the application of uniform therapeutic strategies in this population. The available data concerning the efficacy or lack thereof of various classes of lipid-modulating therapies are briefly described below.

Statins

Statins are generally effective in attenuating hypercholesterolemia and reducing the risk of adverse outcomes in patients with nephrotic syndrome. However, as described below, the efficacy of these products in reducing the risk of cardiovascular and overall morbidity and mortality in patients with CKD varies depending on the nature and severity of renal disease, the renal

Table 8.2 Results of statin trials in patients with chronic renal failure undergoing dialysis. The primary end points of these trials was fatal and nonfatal cardiovascular events

	n	Duration (year)	Baseline LDL-C	LDL-C ↓ (%)	Statins (dose)	Primary end point (95% CI)
4D	1255	4	125 mg/dl	42	Atorvastatin (20 mg/d)	0.77–1.10
AURORA	2276	2.4	100 mg/dL	43	Rosuvastatin (10 mg/d)	Not significant 0.84–1.11
SHARP	2527	4.9	100 mg/dL	30–43	Simvastatin (20 mg/d) ± Ezetimibe (10 mg/d)	Not significant 0.78–1.15 (hemodialysis patients)

LDL-C low-density lipoprotein cholesterol, *SHARP* study of heart and renal protection, *4D* Die Deutsche Diabetes Dialyse, *AROURA* an assessment of survival and cardiovascular events

replacement modalities, and presence or absence of hypercholesterolemia. A brief description of the available data is provided below.

Statins for Prevention of Cardiovascular Disease in Patients with Mild-to-Moderate CKD Post hoc analyses of several, large, randomized, placebo-controlled statin trials evaluating the effect of statins on cardiovascular (CV) outcomes have shown that statin therapy results in a similar risk reduction and medication-related toxicity in patients with mild CKD and those with normal kidney function [117–119]. (Table 8.2) However, since the event rates in people with CKD are higher, the absolute risk reduction conferred by statin therapy was greater in the presence of impaired kidney function. It should be noted, however, that less than 1% of the patients enrolled in these studies had stage 4 CKD, and none had end-stage renal disease requiring dialysis. Therefore, these conclusions cannot be extended to patients with advanced CKD. The subgroup analysis of the secondary prevention trials suggest that statins may reduce CV morbidity and mortality and all cause mortality in patients with stage I-IV CKD [119–121]. These findings were supported by the results of the Study of Heart and Renal Protection (SHARP) Trial which showed significant reductions in major atherosclerotic events, nonhemorrhagic stroke, and coronary revascularization, and a trend toward reduction of the nonfatal myocardial infarction with a combination of simvastatin and ezetimibe in patients with mild-to-moderate CKD as compared to their placebo-treated counterpart [122]. In contrast, the Prevention of Renal and Vascular End-Stage Disease Intervention Trial (PREVENT IT) study, a prospective randomized trial designed to examine the efficacy of pravastatin in patients with very mild CKD yielded inconclusive results [123]. This trial, which had randomized patients with microalbuminuria to receive fosinopril, pravastatin, or matching placebos for 4 years, showed only an insignificant reduction in the cardiovascular mortality and hospitalization for cardiovascular events in the pravastatin-treated group. The reason for discrepancy between the latter study and the abovementioned studies is not clear. However, it may be due to the difference in severity of proteinuria among the patients enrolled in these trials. As noted earlier, heavy proteinuria results in downregulation of hepatic LDL receptor and HDL docking receptor which leads to hypercholesterolemia by increasing hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and cholesterol synthesis [11, 33, 36, 44]. Consequently, HMG-CoA reductase inhibitor can have salutary effect in such individuals. The PREVENT IT trial had enrolled patients with microalbuminuria, whereas patients enrolled in the former trials had stage I-IV CKD, a significant subset of whom exhibited significant proteinuria.

Effect of Statins on Progression of CKD The available data on this topic are derived from secondary or post hoc analyses of secondary prevention studies and a few randomized clinical trials. For instance, compared to placebo-treated patients, the simvastatin-treated patients

experienced a significantly lower rate of decline in GFR in the Heart Protection Trial [124]. Likewise, the post hoc analysis of data from the Greek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study showed an approximately 5% fall in the estimated GFR in the untreated but not in the statin-treated patients with dyslipidemia, coronary disease, and normal baseline renal function during the 3-year study period [125]. These findings were confirmed by subanalysis of data from the "Treatment to New Targets" (TNT) study [126] which revealed a significant improvement in estimated GFR in patients with coronary heart disease over the 5-year study period. The subgroup analysis of the Cholesterol and Recurrent Events (CARE) trial revealed that pravastatin slowed the annual rate of decline in GFR (2.5 mL/min/year) in individuals with GFR below 40 mL/min/1.73 m^2, but not in the entire study population [127]. The meta-analysis of 12 small randomized controlled trials (RCTs) which directly explored the impact of statins on renal function including 362 participants revealed that statins may retard progression of renal disease and reduce proteinuria [128]. These findings were confirmed by meta-analysis of data from 27 published or unpublished randomized, controlled trials or crossover trials of statins (encompassing nearly 40,000 participants) which showed a significant reduction in the annual rate of decline in estimated GFR (1.22 mL/min per year) in the statin-treated individuals [129]. Interestingly, the subgroup analysis of the data revealed a statistically significant favorable effect of statin therapy in the participants with cardiovascular disease but not in patients with preexisting kidney disease, i.e., glomerulonephritis, diabetic nephropathy, or hypertensive nephrosclerosis.

There is evidence that statin therapy can modestly reduce proteinuria in patients with kidney disease. This assumption is supported by the meta-analyses of data derived from 50 trials including large numbers of CKD and transplant patients [130]. It should be noted, however, that in contrast to most statins, rosuvastatin does not have an anti-proteinuric effect and instead it can cause or intensify proteinuria. This phenomenon was confirmed in the Prospective Evaluation of Proteinuria and Renal Function in diabetic and non-Diabetic Patients with Progressive Renal Disease Trials (PLANET I and II studies, respectively) [131]. The PLANET I and II studies were undertaken to determine the efficacy of rosuvastatin (10 or 40 mg/day) and atorvastatin (80 mg/day) on progression of renal disease in CKD patients with and without diabetes. The studies showed significant reductions in proteinuria and the rate of decline in estimated GFR in atorvastatin-treated patients but significant increase in proteinuria and faster decline in GFR in rosuvastatin-treated group. The likely mechanism for this phenomenon is accumulation of rosuvastatin and its metabolites in the renal tissue where it can cause injury, especially at high doses.

Thus, except for rosuvastatin which can worsen proteinuria, most statins can be beneficial in slowing progression of CKD, particularly in patients who have significant proteinuria and hypercholesterolemia. Nonetheless, statins should be prescribed with caution since high doses of these agents can lead to renal and extrarenal complications. For instance, proteinuria can occur in some patients when treated with a high dose of simvastatin (40 mg/day), regress following discontinuation, and recur with the resumption of the drug [132]. The mechanism by which rosuvastatin and occasionally other statins cause proteinuria is not entirely clear. However, it appears to be, in part, due to impaired reabsorption of filtered proteins in proximal tubules. This supposition is supported by in vitro experiments which showed concentration-dependent inhibition of protein uptake by simvastatin, pravastatin, and rosuvastatin in cultured human renal proximal tubular epithelial cells [133].

Statins in ESRD Patients Maintained on Hemodialysis Randomized prospective clinical trials of different statins have consistently shown no reduction in the risk of cardiovascular events and cardiovascular or overall mortality in ESRD patients maintained on hemodialysis. The first among these studies was the Die Deutsche Diabetes Dialyse (4D) trial [134] which had enrolled 1255 hemodialysis patients with type 2 diabetes randomized to receive atorvastatin, 20 mg/day or

placebo for 4 years. The trial showed no significant reduction in mortality from cardiac causes or nonfatal myocardial infarction and stroke (95% CI 0.77–1.10) despite about 42% reduction in LDL cholesterol in patients receiving atorvastatin. While atorvastatin reduced the rate of all cardiac events combined (95% CI.68–99), the deaths from stroke were increased (95% CI 1.05–3.93). The second trial, an assessment of survival and cardiovascular events (AURORA), was a double-blind, randomized, placebo-controlled trial designed to compare the effect of rosuvastatin 10 mg/day versus placebo on cardiovascular morbidity and mortality in hemodialysis patients [135]. The study had enrolled 2776 patients with identical mean baseline LDL cholesterol values of 100 mg/dL in the rosuvastatin-treated group and 99 mg/dL in the placebo-treated group. The mean length of the treatment was 2.4 years and the mean length of follow-up was 3.2 years. The rosuvastatin-treated group showed an approximately 43% reduction in LDL cholesterol concentration within the first year of trial. Despite the dramatic reduction in serum cholesterol level in the rosuvastatin-treated group, no significant difference was found in the incidence of nonfatal myocardial infarction, cardiovascular, or overall mortality between the rosuvastatin- and placebo-treated groups. In fact, the rosuvastatin-treated patients with diabetes experienced a significant increase in the incidence of fatal hemorrhagic stroke as was seen in the 4D study.

The latest and the largest primary prevention trial in this series was the Study of Heart and Renal Protection (SHARP) Trial [122] which was different from the former trials as in addition to dialysis patients it included a large cohort of CKD patients who did not require dialysis. The estimated glomerular filtration rate (eGFR) in the CKD groups averaged 27 mL/min/1.73 m^2. The study was designed to determine the effectiveness of LDL cholesterol reduction on major vascular events and the rate of progression of CKD in as-yet dialysis-independent patients. Patients were randomized to receive either simvastatin 20 mg/day with or without ezetimibe 10 mg/day or placebo. The median duration of follow-up was 4.9 years. Mean baseline LDL cholesterol levels (108 mg/dL in the entire group and 100 mg/dL in the dialysis subgroup) were reduced by 30 mg/dL with simvastatin alone and by 43 mg/dL with simvastatin plus ezetimibe at 1 year. Patients on simvastatin alone were re-randomized to simvastatin and ezetimibe after 1 year. Compared to the placebo-treated arm, the simvastatin and ezetimibe-treated arm showed a 17% reduction in major atherosclerotic events, a 25% reduction in nonhemorrhagic stroke, a 21% reduction in coronary revascularization, and trend toward a reduction in nonfatal myocardial infarction. It is of note, however, that cholesterol-lowering therapy failed to reduce either mortality rates or cardiovascular events in the dialysis-dependent ESRD patients in this trial thus recapitulated the results of the earlier studies. The reason for the favorable results of this trial was inclusion of a large cohort of patients with less advanced CKD in whom the underlying mechanisms of cardiovascular disease resembles that in the general population.

The failure of statins to confer protection against cardiovascular disease in hemodialysis patients observed in the above clinical trials contrasts their favorable effects in the general population [136] and the majority of CKD patients who do not require dialysis. Several factors account for the ineffectiveness of statins in the majority of hemodialysis population. As mentioned above, accelerated atherosclerosis and cardiovascular disease in the majority of hemodialysis patients are associated with normal or subnormal serum cholesterol level. This observation excludes elevated cholesterol production or concentration as the central player in the pathogenesis of the associated cardiovascular disease in the majority of these patients. Instead, cardiovascular disease in this population may be primarily driven by systemic inflammation, oxidative stress, accumulation of atherogenic VLDL and chylomicron remnants, formation of small dense LDL, and HDL deficiency and dysfunction, hypertension, vascular calcification, and arrhythmogenic electrolyte disorders which are not amenable to statin therapy. It should be noted, however, that due to preexisting genetic or other unrelated mechanisms a minority of hemodialysis patients exhibit

hypercholesterolemia which can contribute to the cardiovascular disease. Statin therapy can have salutary effects in such patients as demonstrated by the post hoc analysis of the 4D study [137]. In this study, the authors demonstrated that atorvastatin significantly reduced the rates of adverse cardiovascular and overall outcomes in hemodialysis patients with the highest quartile of baseline LDL cholesterol (≥145 mg/dL, 3.76 mmol/L) but not in patients with the other quartiles of LDL cholesterol at baseline.

Statin in Peritoneal Dialysis Population Statins may lower the risk of cardiovascular complications in peritoneal dialysis patients primarily by lowering serum cholesterol, but may be by improving endothelial function, reducing neointima formation, and inhibiting vascular smooth muscle cell proliferation, platelet activation, and aggregation [138]. In addition, statins may help to protect peritoneal membrane by limiting deposition of fibrin and development of adhesion in these patients [139].

Statins in Kidney Transplant Population In a long-term randomized clinical trial comparing fluvastatin XL 80 mg/day with placebo including over 1600 kidney transplant recipients, the fluvastatin-treated group showed a significant reduction in mean LDL cholesterol (from 159 mg/dL at baseline to 98 mg/dL at last follow-up) [140]. This was associated with a significant reduction in the risk of major adverse cardiac events ($p=0.036$), and a 29% reduction in cardiac death or nonfatal myocardial infarction ($p=0.014$). However, the treatment did not significantly impact total mortality or graft loss in the study population.

Importance of Individualized Care Approach in Prescribing Statins in CKD Population Patients with advanced CKD generally suffer from uremic myopathy, mitochondrial dysfunction, and type 2 diabetes and insulin resistance; events that can make them more vulnerable to the unintended actions of statins. Thus, the author believes that the use of statins in CKD and ESRD patients should be restricted to those with hypercholesterolemia and should be avoided in those with normal serum cholesterol levels. In addition, given the vulnerability of this population, the lowest effective dose should be prescribed. Finally, statins which are metabolized/excreted by the kidney such as rosuvastatin should be avoided in patients with kidney disease.

Fibrates

In view of the prevalence of hypertriglyceridemia and HDL deficiency in CKD patients, PPAR-α agonists (fibrates) which can lower triglyceride and raise HDL cholesterol levels can be useful in the management of CKD-induced dyslipidemia. The trial of PPAR-α agonist, gemfibrozil, has shown significant reduction in serum triglyceride, increase in serum HDL cholesterol, and reduced incidence of coronary death and nonfatal myocardial infarction in patients with mild-to-moderate CKD who had coronary disease and low HDL cholesterol level [141–143]. However, the treatment did not attenuate progression of renal disease in the study population. On the contrary, the drug tended to increase the risk of persistent elevations of serum creatinine in participants with or without CKD. These concerns have greatly curtailed the use of fibrates in the management of dyslipidemia in the CKD population. However, according to a recently published meta-analysis [144], the initial spike in serum creatinine following the onset of therapy with fibrates reverses over time. The authors further found reduction in proteinuria in the fibrate-treated groups and suggested that the drug might have renal protective effect. It should be noted that the safety and efficacy of fibrates in patients with advanced CKD have not been definitively established. The National Kidney Foundation Clinical Practice Guidelines (K/DOQI; 2003) recommend gemfibrozil as the fibrate of choice for use in patients with CKD. This was based on its dual biliary- and urinary-excretion pathways which require less intense dose adjustment in patients with mild CKD. The National Lipid Association recommended a 50% reduction in the dose of gemfibrozil for patients with GFR below 60 mL/min/1.73 m^2 and avoidance of all

fibrates in patients with GFR less than 30 mL/min/1.73 m^2. Caution should be exercised to avoid or minimize interaction of gemfibrozil and other fibrates with other drugs, specially statins and Coumadin, that can lead to serious consequences. Among statins, fluvastatin is the only product whose plasma level does not increase when co-administered with gemfibrozil; therefore, this combination may be preferred in CKD patients with mixed dyslipidemia [145]. With the exception of fenofibrate, all other fibrates can elevate serum level of statins and predispose the patients to rhabdomyolysis and liver injury [146]. However, the dose of fenofibrate should be markedly reduced in patients with diminished GFR and the drug should be avoided when the GFR is below 15 mL/min/1.73 m^2 [146].

Niacin

Low doses of niacin can increase serum HDL cholesterol and high doses of niacin can raise HDL cholesterol and lower LDL, triglyceride, and Lp(a) concentrations. Moreover, the antioxidant and anti-inflammatory properties of niacin may further help to slow progression of atherosclerosis and kidney disease. However, due to its poor tolerability, use of niacin in CKD population has been limited. The dose-dependent hyperglycemic effect of niacin on serum glucose is of particular concern in patients with diabetes which is the most common cause of CKD.

Experimental Lipid-Modulating Agents (ACAT Inhibitors)

In a series of studies, we found significant improvements in proteinuria, renal function, and plasma lipid profile with administration of the ACAT inhibitor, avasimibe, in animal models of nephrotic syndrome and chronic renal failure [147, 148]. These findings in experimental animals suggest that ACAT inhibitors may be effective in ameliorating kidney disease and preventing atherosclerosis in selected patients with CKD or chronic nephrotic proteinuria. However, clinical trial of avasimibe was prematurely halted due to acute cardiovascular events [149].

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Dyslipidemia in HIV-Infected Patients

9

Frederick J. Lee and Andrew Carr

Introduction

Since the advent of effective combination antiretroviral therapy (ART), patterns of mortality in HIV infection have changed and the rates have fallen, with the acquired immunodeficiency syndrome (AIDS) now overtaken by non-AIDS events [1]. Cardiovascular disease is a common cause of death in this population, and will become increasingly so as these patients age [2, 3].

This is partly because some traditional risk factors, notably smoking, are more common amongst HIV-infected individuals, placing them at risk of events at ages younger than their HIV-negative peers [4, 5]. Additionally, both the infection and especially ART are significantly associated with the development of an abnormal metabolic milieu that can promote cardiovascular risk. The resulting derangements in plasma lipids are believed to be a key pathway by which accelerated atherosclerosis may occur in HIV-infected individuals [6].

In this chapter, we review the phenotypic changes in lipid metabolism observed in both untreated ('treatment-naïve') and treatment-experienced HIV infection, the current state of knowledge about their respective pathogenic mechanisms and the approach to management. All the information presented relates to HIV-1, by far the dominant cause of HIV cases, and not the less virulent HIV-2, for which very little metabolic data are available.

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Dyslipidemia Associated with HIV Viremia

Observations in HIV-Infected, Treatment-Naïve Populations

Derangements in lipid metabolism have long been a recognized feature of untreated HIV infection, predating the advent of ART [7]. The precise time course in the untreated setting is unknown, but the key early change is a fall in cholesterol fractions [8]. A fall in high-density lipoprotein (HDL) cholesterol levels appears to be the initial change, followed by a fall in low-density lipoprotein (LDL) cholesterol levels somewhat later.

The first prospective description of evolving dyslipidemia was made via a subgroup analysis of the Multicenter AIDS Cohort Study. In 50 HIV-infected but treatment-naïve men, mean plasma HDL and LDL cholesterol (and triglyceride levels) fell between seroconversion and initiation of ART [9]. As this was documented by comparing paired samples (preseroconversion vs. pre-ART, median separation 99 months), an accurate illustration of the time course does not exist. The net effect may be pro-atherogenic as HDL cholesterol remains a strong inverse predictor of cardiovascular events even with

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concurrently low levels of LDL cholesterol [10]. This is likely compounded because HDL cholesterol loses its efficacy as an antioxidative agent in the setting of active infection and inflammation [11]. These changes to HDL and LDL cholesterol were corroborated in a later prospective study of HIV-infected adults prior to treatment [12].

Data from the Strategies for Management of Antiretroviral Therapy (SMART) study provide a striking illustration of the relationship between lipid metabolism and HIV infection [13]. In this study, patients received CD4-guided intermittent ART with the aim of limiting dyslipidemia and other metabolic toxicities. While total and LDL cholesterol levels fell during periods of treatment interruption, there was a proportionally greater decline in HDL cholesterol, such that the total-to-HDL cholesterol ratio actually increased, consistent with a higher cardiovascular risk. This was borne out in the clinical outcomes, where the group receiving intermittent therapy had more frequent cardiovascular events and deaths.

With advanced disease, there is a significant elevation in triglycerides and very low-density lipoprotein (VLDL) cholesterol levels, with the latter further increasing cardiovascular risk [8]. The rise in triglycerides appears related to the HIV viral load; in an early study of the antiretroviral drug zidovudine, used as monotherapy in patients with AIDS, mean triglyceride levels fell only in those patients receiving zidovudine [14]. Correlations also exist between falling HDL cholesterol levels, high plasma viral load and low CD4 counts of late disease. However, this lipid profile is now uncommon in developed countries, where there is access to combination ART.

A limitation of the aforementioned studies is that the participants were almost exclusively male and sourced from resource-rich populations. Subsequent studies confirm that dyslipidemia is also a prominent metabolic feature in untreated women and non-Caucasians, although no data directly comparing different ethnicities are available. One large cross-sectional study of 12,513 adults pre-ART in Tanzania (65% female) showed a high prevalence of dyslipidemia as defined by the US National Cholesterol Education Program (NCEP) guidelines, with decreased HDL cholesterol (<1 mmol/L) in 67%, and elevated triglycerides (>1.7 mmol/L) in 28% [15]. Interestingly, males had significantly lower total, HDL, and LDL cholesterol levels; the reasons for this are unclear. As observed in other studies, triglyceride levels were positively associated with more advanced HIV disease. In a smaller Nigerian study, active tuberculosis infection (a marker for advanced disease and inflammation) was associated with a significantly higher mean LDL cholesterol level [16].

A very small minority (<0.5%) of HIV-infected individuals remain clinically well for decades without developing AIDS despite not receiving ART. There are no data on circulating lipid levels in these long-term nonprogressors and so-called 'elite controllers', although some suggestion of increased cardiovascular risk exists [17].

Mechanisms of Dyslipidemia Associated with HIV Viremia

A number of possible mechanisms have been identified to explain the dyslipidemia of untreated HIV infection, although none are definitive. Broadly, they may be categorized as:

- Direct viral effects
- Consequences of the inflammatory response triggered by the infection

These mechanisms act to disrupt existing metabolic pathways, and in some cases the effects feed back to exacerbate the initial insult.

HIV infection appears to directly interfere with steps in the reverse cholesterol transport pathway, whereby HDL cholesterol facilitates clearance by the liver of cholesterol from the extrahepatic tissues. The HIV protein *nef* is a virulence factor expressed to enhance viral replication that has been shown to block the efflux of cholesterol from macrophages mediated by the adenosine triphosphate (ATP)-binding cassette protein A1 (ABCA1). *Nef* induces a post-transcriptional downregulation of the normal expression of ABCA1 as well as redistributing it to the plasma membrane [18]. In doing so, lipid accumulates in macrophages, promoting their conversion into the foam cells involved in the genesis of atherosclerotic plaques.

HIV infection also upregulates expression of the cholesterylester transfer protein (CETP), a later step in reverse cholesterol transport. CETP exchanges triglycerides from LDL and VLDL particles for cholesterol esters from HDL particles [19]. This leads to the HDL particles becoming saturated with triglycerides, and their accelerated clearance by hepatic lipases.

Although not directly related to lipid metabolism, another HIV protein, *tat,* in concert with tumour necrosis factor (TNF)-α can induce endothelial proliferation and activation, permitting adhesion and translocation of leukocytes into the vasculature, creating a pro-inflammatory, pro-atherogenic state [20]. The HIV envelope protein gp120 may also increase endothelial activation and monocyte adhesion [21]. Such dysfunction promotes local thrombosis as well as inflammation.

But it must be remembered that most of these findings are from in vitro studies; it remains unclear which of these, if any, play the predominant role in causing dyslipidemia in untreated HIV infection. For instance, there is no clear in vivo evidence to demonstrate that the HIV virus directly reduces circulating levels of HDL cholesterol, although that is what is observed clinically. Persons with noninfective, chronic inflammatory diseases such as systemic lupus erythematosus and rheumatoid arthritis display lipid derangements similar to those found in untreated HIV; furthermore, these lipid changes correlate well with disease activity [22, 23]. This suggests that the state of active inflammation in untreated HIV may play a greater role in generating dyslipidemia (and subsequently, increased cardiovascular risk) than direct effects of the virus. Inflammation stimulates endothelial phospholipase A2, which reduces HDL cholesterol, and in turn attenuates cholesterol efflux from macrophages in the arterial wall [24]. Hypertriglyceridemia results from increased hepatic fatty acid synthesis, activation of adipose tissue lipolysis, and suppression of ketogenesis. This is mediated by multiple cytokines—TNF-α, interleukin (IL)-1, IL-2, and IL-6 [25].

The strong association between serum triglyceride levels and the degree of inflammation is seen in AIDS, which is marked by increased levels of interferon (IFN)-α and TNFα [26]. As seen with noninfective chronic inflammatory states, IFN-α decreases lipoprotein lipase activity, impairing the clearance and/or storage uptake of lipoproteins, raising the circulating levels of triglycerides and VLDL cholesterol [27]. Levels of TNF-α further rise during opportunistic infections, and permits lipolysis by attenuating the anabolic effect of insulin and interfering with free fatty acid metabolism [28]. In the resulting lipoprotein-rich environment, HDL cholesterol becomes enriched with triglycerides, marking it for clearance by hepatic lipase. This complements the aforementioned enhanced activity of CETP.

These mechanisms (and their interplay with metabolic disturbances generated by ART, discussed below) are summarized in Fig. 9.1.

Whether the reduction of HDL cholesterol levels and modest increase in serum triglyceride concentrations, when associated with a fall in LDL cholesterol levels, will increase risk of atherosclerosis or coronary heart disease in treatment-naïve HIV-infected patients remains unknown. In the pre-ART era, most HIV-infected patients died of AIDS and its complications, and not from coronary heart disease.

Dyslipidemia Associated with ART

The metabolic derangements associated with ART are ultimately of greater clinical relevance than those attributed to HIV viremia alone. This is because in the current absence of a cure, ART requires a high degree (>90 %) of lifelong adherence to maintain its efficacy. Furthermore, guidelines are trending towards a 'test-and-treat' approach, whereby ART is initiated progressively earlier [29].

Although the first clinical trial of zidovudine was in 1986, the current era of effective combination ART began in 1996 [30]. Currently, there are almost 30 drugs in six classes with widespread regulatory approval for use as ART:

- Nucleoside reverse transcriptase inhibitors (NRTI)
- Nonnucleoside reverse transcriptase inhibitors (NNRTI)

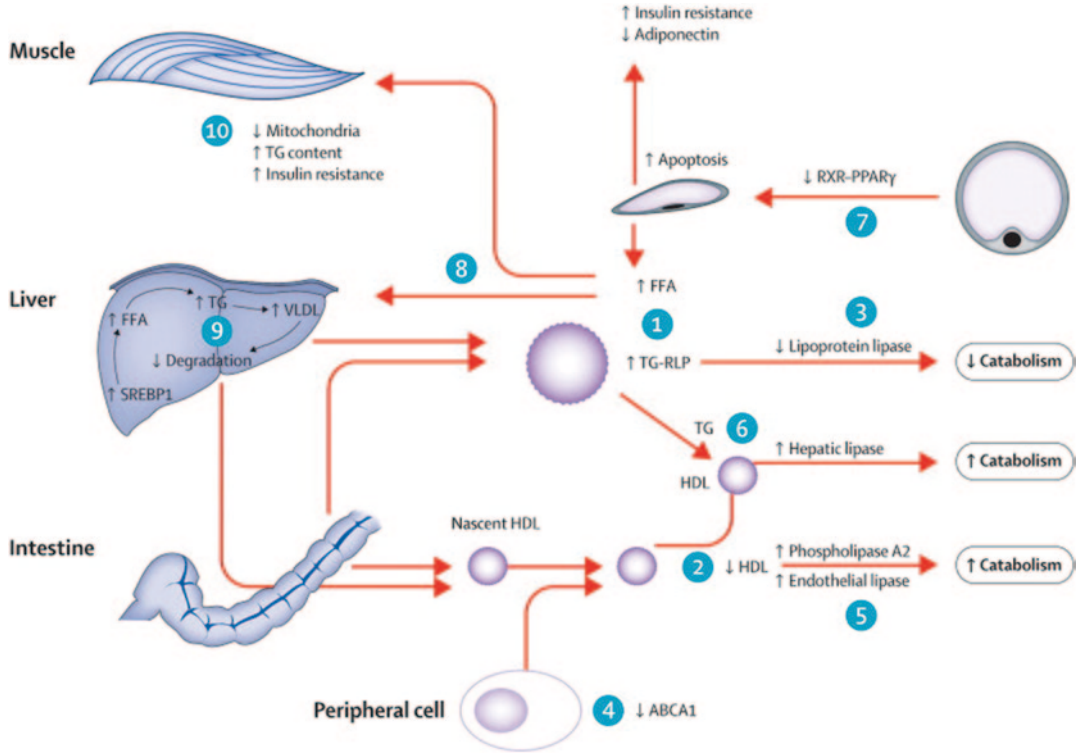

Fig. 9.1 Schematic representation of possible mechanisms underlying HIV-associated dyslipidemia. Derangements of lipid metabolism are a result of direct effects of the HIV itself, the inflammatory response to infection. The addition of antiretroviral therapy (ART) further adds to the complexity. *1* Increased triglyceride (TG)-rich lipoproteins (RLP), particularly very low-density lipoprotein (VLDL cholesterol; and *2* decreased high-density lipoprotein (HDL) cholesterol. The inflammatory cytokine response to HIV infection: *3* decreases lipoprotein lipase activity, which results in accumulation of triglycerides; *4* decreases cholesterol efflux from peripheral cells via the ATP-binding cassette protein A1 (ABCA1), which results in decreased formation of HDL cholesterol; and *5* increases activity of phospholipase A2 and endothelial lipase, which results in increased catabolism of HDL cholesterol. Increased plasma TG results in *6* abnormal TG-enrichment of HDL cholesterol, which increases catabolism via hepatic lipase. ART causes redistribution of adipose tissue as a result of *7* decreased retinoid X receptor-peroxisome proliferator-activated receptor γ (RXR-PPAR-γ) activity. *8* Free fatty acid (FFA) spills over from apoptotic peripheral adipocytes, increasing FFA flux to the liver and skeletal muscle. In the liver *9*, increased FFA supply and upregulation of the TG synthetic pathway, through the sterol-regulatory element-binding protein (SREBP) 1c and downstream targets, increase hepatic TGs and ultimately secretion of TG-rich VLDL, while protease inhibitors interfere with intracellular degradation of VLDL and related particles. In the muscle *10*, ART is associated with mitochondrial depletion, which in turn compromises FFA oxidation; as a result, intra- and intermyocellular TG content increases. Insulin resistance in liver and skeletal muscle compounds the metabolic disturbances, including dyslipidemia. *HIV* human immunodeficiency virus, *ATP* adenosine triphosphate. (Reproduced with permission from: Oh J and Hegele RA. HIV-associated dyslipidemia: pathogenesis and treatment. Lancet Infect Dis. 2007;7:787–796. Copyright ©2007 ScienceDirect)

- Protease inhibitors (PI)
- Integrase strand transfer inhibitors (INSTI)
- CC chemokine receptor 5 (CCR5) entry antagonists
- Viral fusion blockers

The therapeutic targets of the ART classes within the HIV-1 lifecycle are represented in Fig. 9.2. Initial therapy is usually a combination of three medications: Typically, to a 'backbone' of two NRTIs is added a third agent, drawn from the NNRTI, PI or INSTI classes. The role of CCR5 entry antagonists is still being defined, while viral fusion blockade is reserved for 'salvage' therapy in cases of multiresistant virus. Considering the

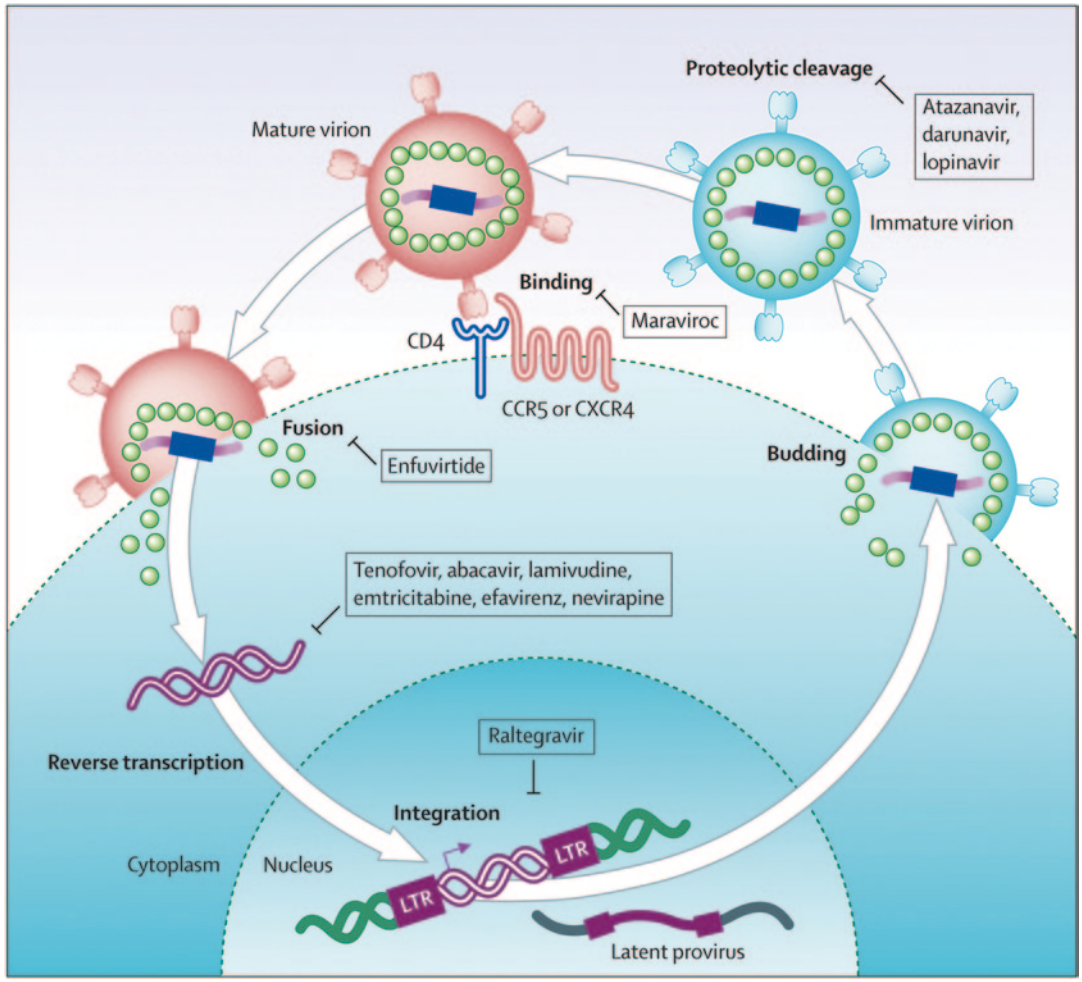

Fig. 9.2 HIV life cycle and antiretroviral targets. Present antiretroviral drugs span six classes that target five unique steps in the HIV life cycle (binding, fusion, reverse transcription, integration, and proteolytic cleavage). The most common drugs currently used in resource-rich regions to target each step are shown. Extracellular virions enter their target cell through a complex three-step process, which is (1) attachment to the CD4 receptor, (2) binding to the CCR5 or CXCR4 co-receptors, or both, and (3) membrane fusion. Maraviroc blocks CCR5 binding and enfuvirtide blocks fusion. The HIV reverse transcriptase enzyme catalyses transcription of HIV RNA into double-stranded HIV DNA, a step inhibited by nucleoside analogues and nonnucleoside reverse transcriptase inhibitors (NNRTIs). The HIV integrase enzyme facilitates incorporation of HIV DNA into host chromosomes, and this step is inhibited by raltegravir and other integrase inhibitors. After transcription and translation of the HIV genome, immature virions are produced and bud from the cell surface. The HIV protease enzyme cleaves polypeptide chains, allowing the virus to mature. This last step is inhibited by HIV protease inhibitor. *HIV* human immunodeficiency virus, *CD4* cluster of differentiation 4, *CCR5 C-C* chemokine receptor type 5, *CXCR4 C-X-C* chemokine receptor type 4. (Reproduced with permission from: Volberding PA and Deeks SG. Antiretroviral therapy and management of HIV infection.Lancet. 2010;376:49–62. Copyright ©2010 ScienceDirect) Dietary factors can also play an important role in advanced disease. Patients with AIDS are frequently malnourished, with protein depletion being a particular problem, and this may contribute to lipid metabolism derangements [7, 26].

number of possible permutations, it is not helpful to attempt to define any single lipid profile as being representative of ART-associated dyslipidemia. Rather, individual drugs, and where possible, different classes need to be considered separately. The possible mechanisms of ART-associated dyslipidemia are outlined in Table 9.1. It is also useful to examine the evolution of observed phenotypes concurrent with developments in ART, starting with HIV lipodystrophy.

Table 9.1 Proposed possible mechanisms of ART-associated dyslipidemia

Mechanism	Effect
Protease inhibitors	
Fall in activated retinoic acid levels	Blocks activity of activity of peroxisome proliferator-activated receptor (PPAR)-γ, inhibiting adipocyte differentiation and promoting apoptosis (and therefore lipodystrophy) [34]
	FFA from apoptotic adipocytes flux to the liver and skeletal muscle, leading to insulin resistance
Protease inhibitor binding to CRABP1 (60% homology between HIV-1 protease and CRABP1)	Stops activation of retinoic acid, blocking PPAR-γ activity [34]
Cytochrome P450 (CYP) 3A4 inhibition (particularly by ritonavir)	Reduces amount of activated retinoic acid synthesized [118]
Proteasome blockage (demonstrated with lopinavir and ritonavir)	Retards degradation of the sterol-regulatory element-binding protein (SREBP) 1c, leading to increased hepatic production of lipoproteins [119, 120]
Accumulation of intramyocellular fat	Insulin resistance, and elevated levels of triglycerides and apolipoprotein B [121]
PIs binding to the LDL-receptor-related protein (LRP)-1 (LRP 1 shares homology with HIV-1 protease)	Blocks LRP 1 from binding to endothelial lipoprotein lipase. Stops hydrolysis of free fatty acids from circulating triglycerides [122]
Inhibition of the expression of lipoprotein lipase (indinavir)	Reduced clearance of lipids, particularly by the liver [123]
Possible decreased expression of LDL receptors	Reduced uptake of LDL cholesterol particles with resulting higher plasma levels [124]
Decreased lipid intake by adipocytes, increased lipolysis in adipocytes (demonstrated at supratherapeutic levels with nelfinavir, saquinavir, and ritonavir, but not amprenavir or indinavir)	Increased plasma triglyceride levels [125]
Elevated expression of lipogenic genes in cultured hepatocyte models (may not occur at therapeutic plasma concentrations)	Increased production of VLDL cholesterol [119, 125]
Reduced CD36 expression in human monocytes	Lipid accumulation in macrophages, promoting apoptosis and conversion into atherogenic foam cells [126]
Inhibition of the zinc metalloproteinase ZMPSTE24	Dysregulation of nuclear lamin A processing, leading to prelamin A accumulation within fibroblasts and adipocytes [38]
Nucleoside reverse transcriptase inhibitors	
Inhibition of mitochondrial DNA polymerase γ (particularly by thymidine analogues, stavudine, and zidovudine)	Depletion of mitochondrial DNA and suppression of the respiratory chain [127]
	Leads to lipoatrophy in adipocytes, and insulin resistance in skeletal muscle
CCR5 entry antagonists and integrase strand transfer inhibitors	
No described mechanisms	

ART antiretroviral therapy, *FFA* free fatty acid, *CRAPB1* cytoplasmic retinoic acid binding protein I, *CYP* cytochrome P-450A, *LDL* low-density lipoprotein, *HIV* human immunodeficiency virus, *VLDL* very-low-density lipoprotein, *CD4* cluster of differentiation 4

HIV Lipodystrophy and Dyslipidemia

HIV lipodystrophy was first described in 1998 [31]. The hallmarks are a loss of subcutaneous fat (lipoatrophy) in the face and limbs, and the accumulation of fat (lipohypertrophy) centrally, especially intra-abdominally. Both lipoatrophy and lipohypertrophy may occur independently, to varying degrees and in an anatomically localized fashion (Fig. 9.3). As it remains a clinical diagnosis without a validated case definition, the reported prevalence of HIV lipodystrophy can

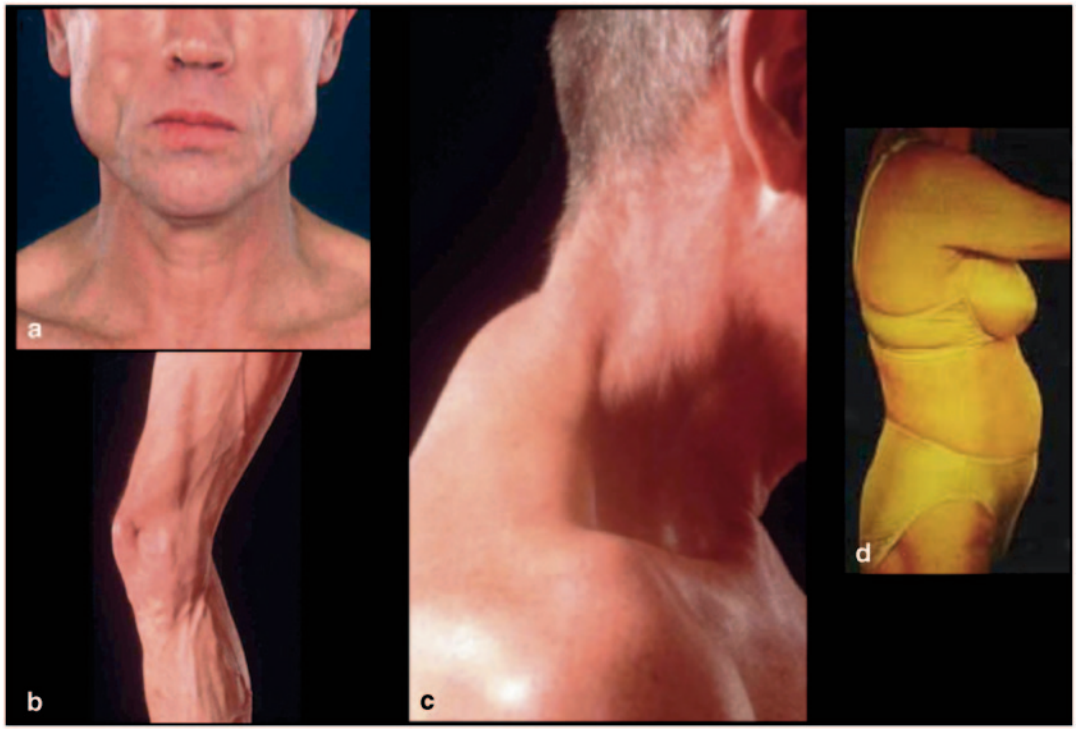

Fig. 9.3 Morphological changes of HIV lipodystrophy. Examples of lipodystrophy, most strongly associated with the use of thymidine analogues. Typical features include a prominent, hollowed-out facial appearance due to lipoatrophy of buccal fat (**a**) and appendicular fat (**b**), causing leg and arm veins to become prominent. Enlargement of the cervico-dorsal fat, commonly referred to as a 'buffalo hump' (**c**) is another typical feature. Concurrent deposition of fat intra-abdominally leads to central obesity. Computed tomography and dual-energy X-ray absorptiometry can be used to quantify the extent of lipodystrophy. These imaging modalities show that both superficial and deep fat is affected. HIV human immunodeficiency virus. (Photos courtesy of A. Carr)

vary significantly—studies from the late 1990s describe rates of 20–35% at 12–18 months post-ART initiation [32, 33].

Insights into the pathogenesis of this complex syndrome remain few and unclear, particularly as an appropriate biomarker is lacking. The first case descriptions and early clinical studies identified PI use as a strong predictor of lipodystrophy [34–36]. There is certainly some in vitro study evidence supporting a role for PIs. Indinavir may inhibit adipocyte differentiation, probably acting via the sterol-regulatory element-binding protein (SREBP) [37]. Lopinavir and tipranavir inhibit the zinc metalloproteinase ZMPSTE24, disrupting the processing of nuclear lamin A; this is significant because genetic defects in lamin A metabolism result in syndromes that feature lipodystrophy [38]. However, accumulated clinical trial data appears to implicate use of thymidine analogue NRTIs (stavudine, zidovudine) as the major causative factor, rather than PIs [33, 39, 40]. Lipodystrophy may occur without exposure to thymidine analogues, suggesting that other patient or disease factors, presently unidentified, are involved. Although assessing the contribution of PIs to lipodystrophy is confounded because almost all participants of early ART studies also received concurrent zidovudine or stavudine, they may act synergistically with thymidine analogues to potentiate its development [41].

Dyslipidemia is a very common feature of lipodystrophy; up to 70% of affected individuals will have a lipid profile conferring an increased risk of cardiovascular disease [31, 42]. Greater proportions of lipodystrophic patients have elevated triglycerides (up to 57%), total cholesterol (up

to 57%) and LDL cholesterol (up to 22%), and low levels of HDL cholesterol (up to 46%) when compared to those without lipodystrophy [43]. Nascent insulin resistance (up to 25%) and type 2 diabetes mellitus (8–10%) are also common findings. These metabolic changes are unlikely to be solely due to the changes in body fat distribution, as direct effects of ART drugs also play a role, not to mention diet and premorbid body habitus. A number of nonmedication risk factors for developing HIV lipodystrophy have been identified, mainly from large cross-sectional studies [32, 33, 44]. These are older age, lower body weight pre-ART, previous diagnosis of AIDS, and a lower nadir CD4 cell count. Women may be more susceptible to central adiposity than men [45].

With currently preferred ART regimens, which use an NRTI backbone of tenofovir and emtricitabine, and anchored with later-generation drugs such as efavirenz (NNRTI) and raltegravir (INSTI), new-onset cases of lipodystrophy are very uncommon. Where possible, patients affected with lipoatrophy should be switched from thymidine analogues. This slows progression, and any subsequent gains in fat volume are modest and not usually clinically evident, making lipoatrophy effectively irreversible [46]. Nor does the lipid profile significantly improve—depending upon the replacement ART drug, the dyslipidemia may actually be exacerbated [47]. Injectable temporary facial fillers are the mainstay of treating lipoatrophy but are solely cosmetic and have no effect on lipid metabolism; poly-L-lactic acid and calcium hydroxylapatite are approved by the US Food and Drug Administration (FDA) for this purpose [48, 49]. The thiazolidinedione pioglitazone was demonstrated to significantly increase HDL cholesterol (0.04 mmol/L) and limb fat (0.38 kg), although the improvement in lipoatrophy was insufficient to be clinically perceived [50]. Its use in lipodystrophy remains investigational.

Accumulation of intra-abdominal fat in HIV lipodystrophy is largely visceral and not amenable to liposuction. The growth hormone-releasing factor analogue tesamorelin is an effective medical treatment, producing significant reductions in visceral adipose tissue (15%), triglycerides (0.57 mmol/L) and total cholesterol (0.2 mmol/L) without affecting glycemic control [51]. It does not significantly alter HDL cholesterol or the total-to-HDL cholesterol ratio, however [52]. Although FDA-approved for the treatment of excess abdominal fat in HIV lipodystrophy, fat rapidly re-accumulates after discontinuation [53]. Long-term safety data are lacking, and cost can be a limiting factor, so it is best reserved for patients who have not responded to regular exercise and dietary modifications. Metformin has been shown to reduce insulin resistance and both subcutaneous and visceral fat, but has no significant effects upon lipids [54]. It should be regarded as investigational in nondiabetic HIV-infected patients, and be used in caution in those with significant lipoatrophy.

Data from Clinical Trials: 1996 to the Present

The majority of lipid ART data come from prospective, randomized trials that directly compare two or more regimens. These trials fall into one of two categories:

- Initial treatment
- Changing ART in treatment-experienced patients (so-called switch studies)

Important ancillary data are derived from large observational cohorts, such as the Danish Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D Study), and systematic analyses of pooled prospective trials that allow comparisons between individual drugs and classes for a variety of risk and safety outcomes.

Of all the ART classes, PIs are the most strongly associated with dyslipidemia—typically proatherogenic increases in total, LDL and VLDL cholesterol, as well as hypertriglyceridemia, with minimal effect upon HDL cholesterol levels [55–57]. This association was founded largely upon studies of early-generation PIs; patients receiving indinavir, nelfinavir or saquinavir were reported to have a significantly higher prevalence of dyslipidemia (38–70%) compared to PI-naïve individuals (5 to 25%) [58]. Treatment with indinavir is associated with significant median increases from baseline of total, LDL cholesterol and triglycerides (17, 21, and 27%, respectively), while saquinavir may result in smaller, but still-

significant rises (8, 6, and 12%, respectively) [59]. These increases are evident even after only 4 weeks of treatment and maintained at 48 weeks. Similar results have been described for nelfinavir and full-dose ritonavir when compared to non-PI controls, with mean increases in total cholesterol of 0.8–2.0 mmol/L, depending upon the PI [56]. Apart from the particular PI used, factors associated with greater dyslipidemia are duration of PI exposure and use of dual-PI therapy [60].

Switching away from full-dose ritonavir improves the lipid profile, suggesting at least a partial reversibility [56]. Recent switch studies have borne out this principle in clinical practice, positioning it as a feasible treatment option for dyslipidemia in those patients receiving a ritonavir-boosted PI [61, 62].

Early-generation PIs are no longer favoured for first-line therapy (although low-dose ritonavir remains as a pharmacokinetic booster of other PIs); nor is lopinavir, which causes similar lipid derangements. In large prospective studies, the present first-line PIs, atazanavir and darunavir, both display a significantly favourable lipid effect compared to lopinavir, with relatively modest increases in all lipid fractions, including HDL cholesterol, of between 11 and 19% from baseline after 96 weeks [63, 64]. As the mean lipids for both PIs remained within NCEP target ranges for optimal lipids, these increases are of doubtful clinical relevance. The only trial directly comparing these two PIs showed greater increases of all lipid fractions with darunavir, although the differences were all nonsignificant [65]. Studies conducted in vitro with atazanavir demonstrate no effect upon adipocyte metabolism, suggesting that it may be altogether lipid neutral [66].

Results from the D:A:D Study analysis provide a useful illustration of the differences between the individual PIs by examining the impact upon cardiovascular risk. When compared to patients not exposed to any PIs, early-generation PI-based ART regimens were associated with a 1.16 times per year increased risk of myocardial infarction, of which approximately 50% could be attributed to PI-associated dyslipidemia [67]. Further analysis implicated cumulative exposure to indinavir and lopinavir (and the NRTIs, didanosine and abacavir), but not nelfinavir or saquinavir, as being independently associated with a statistically significant increased risk of myocardial infarction [68]. Atazanavir-based therapies are. however, not associated with increased risk of stroke or myocardial infarction [69]. Similar data for darunavir are yet to be reported. While the risk reduction seen with atazanavir may be driven by greater awareness and thence more aggressive treatment of cardiovascular risk, including lipid-lowering therapies, it may also suggest a progressive diminution of lipid derangement with newer agents.

While NNRTIs are not as well known for their dyslipidemic properties, they do exert lipid effects. The key difference compared to the early-generation PIs appears to be that pro-atherogenic shifts are offset by increases in anti-atherogenic HDL cholesterol. For instance, nevirapine is linked with increases in LDL cholesterol (to levels similar to the PI indinavir). Yet it is also associated with significant increases in HDL cholesterol of up to 50% from baseline, conferring a favourable decrease in the total-to-HDL cholesterol ratio, as well as a decline in triglycerides [60]. The first-line NNRTI, efavirenz, is associated with significant increases in triglycerides and lesser increases in HDL cholesterol, similar to the lipid effects of lopinavir [70]. Interestingly, there is no association between the NNRTIs and increased risk of myocardial infarction [67, 68], emphasising that the expectation of neither particular lipid profiles nor cardiovascular risk can be deduced simply based upon the drug class.

Amongst the NRTIs, stavudine and zidovudine are associated with dyslipidemia [71, 72]. This is in part due to their strong link with lipodystrophy. The currently preferred NRTI for first-line therapy, tenofovir, and the alternate agent, abacavir, both have significantly less lipid effects than their predecessors [73]. In studies of initial ART, tenofovir is associated with modest elevations of total (0.7 mmol/L), HDL (0.3 mmol/L), LDL cholesterol (0.2–0.4 mmol/L), and triglycerides (0.1–0.5 mmol/L), as well as a fall in the total-to-HDL cholesterol ratio (0.4–0.9). In comparison, abacavir shows a similar pattern of changes when used in initial treatment, although the elevations in total, LDL cholesterol and triglycerides tend to be significantly higher than with tenofovir, while

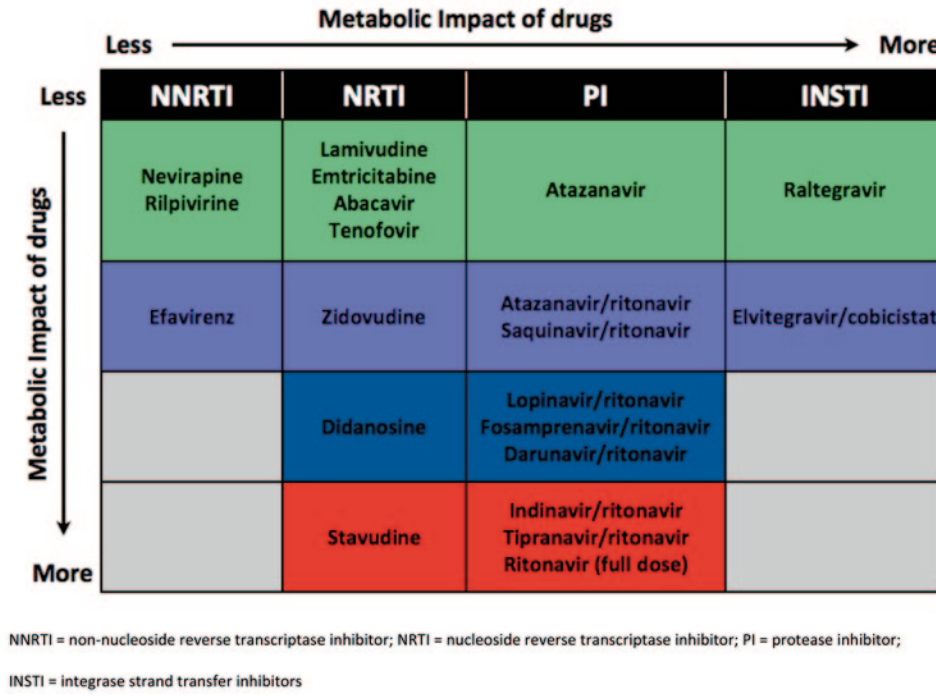

Fig. 9.4 Estimated lipid and metabolic changes associated with ART drugs and classes. Limited data from use of fusion blockers (enfuvirtide) and CCR5 entry antagonists (maraviroc) suggest these drugs to have little metabolic impact, but length of experience with these agents is limited. *ART* antiretroviral therapy, *NNRTI* nonnucleoside reverse transcriptase inhibitor, *NRTI* nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *INSTI* integrase strand transfer inhibitors. (Adapted with permission from: Lundgren JD, Battegay M, Behrens G et al. European AIDS Clinical Society (EACS) on the prevention and management of metabolic diseases in HIV. HIV Med. 2008;9:72–81. Copyright ©2010 Wiley-Blackwell)

the fall in total-to-HDL cholesterol ratio is smaller but nonsignificant [74–76]. In treatment-experienced patients, switching to a tenofovir-based regimen, either from abacavir or a thymidine analogue, results in significantly greater falls in total and LDL cholesterol [77, 78].

A 2008 D:A:D Study analysis reported that abacavir was associated with a significantly increased risk of myocardial infarction that was not associated with dyslipidemia [79]. Rather, IL-6 and high-sensitivity C-reactive protein levels were found to be higher in patients receiving abacavir, fuelling speculation that it caused vascular inflammation, triggering coronary artery disease. Cessation of abacavir may revert risk to the pre-abacavir baseline [80]. More recent systematic analyses have failed to replicate the D:A:D Study results [81, 82], leaving this an area of uncertainty; abacavir remains an accepted alternate agent in current treatment guidelines.

There are insufficient data on emerging classes of ART agents (INSTIs, CCR5 entry antagonists) and the viral fusion blocker, enfuvirtide, to make definitive assessments of their long-term metabolic effects. Thus far, phase 2 –4 clinical trials indicate that these drugs have minimal effects upon lipid levels [83–87]. Results of in vitro studies indicate that like atazanavir, the INSTI raltegravir may be lipid neutral [66]. Cobicistat (GS-9350) is a recently approved, potent inhibitor of human cytochrome P450 (CYP) 3A isoforms intended as an alternate pharmacokinetic booster to low-dose ritonavir. It is not a PI, and devoid of anti-HIV activity. However, compared to low-dose ritonavir in prospective studies, there appears to be no significant difference in lipid effects [88].

The relative effects of individual ART agents on lipid metabolism are summarized in Fig. 9.4.

ART-Associated Dyslipidemia: The Risk and Clinical Relevance

Determining the metabolic effects of ART drugs remains of particular clinical relevance when deciding which agents to recommend as components of a preferred initial regimen. However, a number of considerations make assessing the clinical impact potentially confusing. Firstly, there are the number of available ART agents and the mandatory requirement for combination treatment to consider. As we have seen, there can also be considerable heterogeneity in the lipid effects of individual ART drugs, both between and within classes. Even amongst different drugs with similar lipid effects (for instance, lopinavir and efavirenz), it is particularly curious that the downstream impact on cardiovascular risk diverges significantly. The reasons for this are unknown, but it does serve to demonstrate that the dyslipidemia is ultimately but a component of and not the sole arbiter of the clinical endpoint.

Secondly, it is important to remember that many patients initiating combination ART already have varying degrees of dyslipidemia due to viremia and inflammation, while ART trials report metabolic changes relative to the point of ART initiation. In contrast, prospective data for lipids relative to preinfection are limited to one study; triglycerides and LDL cholesterol rose following ART, to levels, respectively, greater and similar to preseroconversion levels, with no significant change in HDL cholesterol [9]. But it is worth noting that these data were collected from patients initiating therapy before 1997, with now nonpreferred PIs. Part of the seemingly proatherogenic lipid changes seen post ART may in fact reflect a 'return to health', or at least the premorbid baseline, rather than being wholly due to metabolic derangement. Making an assessment of the net effect upon cardiovascular risk solely attributable to ART is therefore difficult. Pilot studies in HIV-negative healthy volunteers have demonstrated that individual drugs—mainly PIs—have effects upon fasting and postprandial lipid profiles even after very short periods [89, 90]. Typically, these are limited by the small number of participants and that the findings are based solely on monotherapy, not a full therapeutic regimen. While it could reasonably be expected that the impact of untreated infection on lipids will be negated because of progressively earlier ART initiation, this is a relatively recent shift in the management paradigm.

Finally, the dyslipidemia of ART is a mixed, heterogeneous entity; not all patients will develop disturbances to the same extent on the same regimen; many have only minimal disturbances. This suggests that genetic factors may play a role in the metabolic response to ART. Examples include polymorphisms of the *APOA5* and *APOC3* genes being associated with greater hypertriglyceridemia in PI-treated patients [91, 92]. Polymorphisms of the CYP enzymes can affect ART pharmacokinetics, leading to variable lipid effects with efavirenz and nevirapine [93, 94]. Study design is also an influence. In trials of darunavir, treatment-experienced participants developed greater lipid derangements compared to studies in treatment-naïve participants [95].

Management of ART-associated Dyslipidemia

Diagnosis and Evaluation

Dyslipidemia in HIV-infected patients is essentially a variable, mixed hyperlipidemia—isolated elevations of lipid fractions are uncommon and warrant other diagnostic consideration. Hence, there are no formal criteria for its diagnosis. It is therefore important that clinicians be familiar with the components of a patient's ART and their possible actions (and interactions). HIV-infected individuals are subject to the same vascular risk factors as the noninfected population, and these comorbidities should be assessed for, and treated, or excluded before attributing the dyslipidemia to the HIV or the ART (Table 9.2). D:A:D Study data indicate that the incidence of diabetes mellitus has increased with cumulative exposure to ART, and the European AIDS Clinical Society guidelines recommend that all HIV-infected persons be screened for diabetes mellitus at diagnosis, prior to initiating ART and annually thereafter [96].

Table 9.2 Secondary causes of dyslipidemia to consider in HIV-infected individuals

Condition	Relevant assessments
Lipodystrophy	Use of thymidine analogues (stavudine, zidovudine)
	Use of protease inhibitors
Smoking	Medical history
Type 2 diabetes mellitus	Fasting glucose regularly in all patients
	Consider oral glucose tolerance testing
	Glycoslyated hemoglobin (HbA1c)
Obesity	Body-mass index
	Waist-to-hip circumference ratio
Hypothyroidism	Thyroid function testing
Medications (nonantiretroviral)	Medications history:
	Thiazide diuretics
	β-blockers
	Corticosteroids
	Oral contraceptive pill
	Atypical antipsychotics (clozapine, olanzapine)
Hepatic or biliary disease	History of excessive alcohol intake
	Viral hepatitis serology
	Serum transaminases, bilirubin levels
Chronic renal disease	Proteinuria
	Elevated serum creatinine
Familial hyperlipiademias (rare)	Known family history
	Presence of corneal arcus, xanthelasmata, and xanthomata

HIV human immunodeficiency virus

Guidelines issued by the Infectious Diseases Society of America and the AIDS Clinical Trials Group recommend that the NCEP targets be used as the basis for evaluating and treating HIV-infected patients [97, 98]. This means that the goals of treatment are currently no different than for an HIV-negative individual.

These guidelines recommend that HIV-positive individuals undergo screening of their fasting lipid profile (total, HDL, LDL cholesterol and triglycerides) prior to initiating ART and within 3–6 months of starting or switching an established regimen. While the utility of postprandial lipid measurements have been assessed in pilot studies, there are insufficient data to recommend their use.

The NCEP targets aim to tailor the intensity of any lipid-lowering intervention(s) to a patient's overall risk. Factors assessed for risk stratification include cigarette smoking, systolic hypertension (≥140 mmHg), depressed HDL cholesterol (<1 mmol/L), family history of premature coronary heart disease, age (men >45 years, women >55 years), and the calculated risk score. Several algorithms are available to quantify risk, but the Framingham Risk Score (FRS), which provides a percentage risk at 10 years, is the most widely used. However, clinicians should note that although the FRS is a useful tool for conventional risk factors, it likely underestimates the risk in the HIV-infected population [99].

The only risk-scoring algorithm to cater specifically for HIV-infected persons is the D:A:D risk equation, which produces a 5-year risk score (rather than 10-year score, pending longer follow up of the cohort) [100]. The D:A:D algorithm factors in traditional risk factors (age, family history, gender, diabetes mellitus, blood pressure, total and HDL cholesterol, smoking) as well as current and prior exposure to particular ART drugs associated with increased risk of myocardial infarction (indinavir, lopinavir, abacavir), thereby countering a key shortcoming of the FRS. The D:A:D algorithm has the additional advantage of being derived from a much larger population than the FRS.

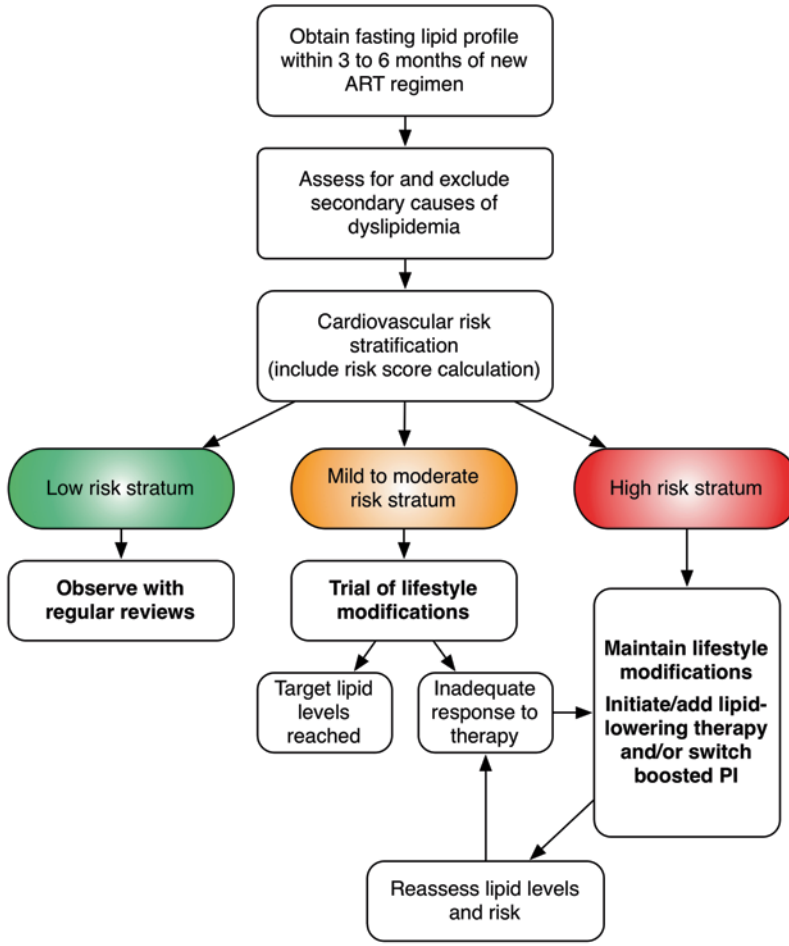

Fig. 9.5 Management approach for ART-associated dyslipidemia. *ART* antiretroviral therapy, *PI* protease inhibitor

Those with the highest risk, such as those with established heart disease, diabetes mellitus, or a calculated risk score >20% are targeted for the most aggressive therapy. Some HIV physicians may choose to intervene at a lower-risk score of 10–15%, taking the view that it may be an underestimate of the actual risk.

Management Approach

The overall management approach (including screening) is outlined in Fig. 9.5.

As with assessment, the management and goals of treatment are derived from the studies of HIV-negative cohorts. This is tacit acknowledgement that there are no randomized trial data on the benefits of lipid lowering in HIV-infected populations, although the D:A:D study reports that the cardiovascular event rate stabilized between 1999 and 2006 despite the greater risk of an aging population, perhaps due to more aggressive management of risk factors, including prescription of lipid-lowering drugs, and the use of newer ART drugs [101].

For all patients, treatment should begin with the same nonpharmacological strategies as those employed in HIV-negative patients—namely dietary modification and regular exercise prescription as lifestyle changes. For the former, review by a dietician may be of value. Attempts at smoking cessation are mandatory, as it is the modifiable factor with the single greatest impact on calculated risk scores.

The success of nonpharmacological therapies is dependent upon the patient's long-term adherence. For those patients in which this is achieved, these measures alone may prove sufficient; and they may be half as likely to require pharmacological intervention [102].

In those patients with established coronary heart disease or a risk equivalent, such as diabetes mellitus, concomitant use of pharmacotherapy is usually required. But for mild-to-moderate risk individuals, it is not clear how long lifestyle alterations should be trialled before proceeding to pharmacological therapies. The NCEP guidelines simply state that they should be 'given a thorough trial'—and how this should be interpreted for HIV-infected patients is not known in the absence of prospective data.

For HIV-infected patients on ART, the pharmacological options are:

- Switching to an alternate ART combination with a more favourable lipid profile, or
- Lipid-lowering drugs

Both methods have their respective advantages and disadvantages (Table 9.3). To date, there has only been one prospective study directly comparing these two strategies [103]. While statin and fibrate therapy appeared to have a modest advantage over switching to an NNRTI for lipids, the study was limited by using efavirenz as a switch option, and examining now nonpreferred PIs (indinavir, nelfinavir, saquinavir). As yet, there are no clinical data comparing these two approaches for the endpoint of risk reduction.

Switching ART

Switching assumes that the ART is the source of dyslipidemia. It should be considered firstly for those patients receiving a ritonavir-boosted PI. Switching to another PI has the advantage of maintaining the same drug class, thus preserving future non-PI treatment options—atazanavir can be used unboosted, and with its favourable lipid profile, may be a feasible option.

Switching must be done only after careful consideration, taking into account the pill burden, patient lifestyle, and possible effects on adherence, and the availability of switch options due to previous treatment failures and resistance mutations. Too broad a selection of patients as switch candidates may prove suboptimal, resulting in higher rates of virological failure, as was seen in the SWITCHMRK study [104]. In contrast, patients switched in the course of the SPIRAL study maintained suppression of HIV viral loads, while improving lipid levels [62]. Both studies used the INSTI raltegravir as the switch drug. Switching

Table 9.3 Comparison of switching and statin strategies for ART-associated dyslipidemia

Advantages	Disadvantages
Statin therapy	
Proven efficacy in reducing cardiovascular risk in the general population both as primary and secondary prevention	Potential side effects (hepatitis and myopathy/rhabdomyolysis
Well tolerated	Using a drug to treat another drug toxicity
Several statins (pravastatin, atorvastatin or rosuvastatin) can be used safely with PI therapy, although dose adjustment may be required	Increases pill burden and treatment costs indefinitely; both undesirable in a disease in which pill burden associates with ART efficacy
Possible anti-inflammatory effects independent of their lipid-lowering effects that may incur additional cardiovascular benefit	
Minimal risk of virological failure	
Switching ART	
Removes cause of the hypercholesterolemia	Boosted PI therapy may not be the cause of dyslipidemia in many patients
Less likely to increase pill burden or costs	Virological failure
	Drug toxicity from the new ART drug
	Reduces available ART choices

ART antiretroviral therapy, *PI* protease inhibitor

Table 9.4 Potential switch ART options for patients receiving a ritonavir-boosted protease inhibitor

Baseline regimen	Switch options (as per DHHS guidelines)	Notes
2 NRTIs + rPI	rPI → NNRTI	Efavirenz may lead to lipid changes similar to that of some boosted PIs
	rPI → INSTI	
	rPI → unboostedatazanavir	Unboostedatazanavir should not be used if NRTI backbone includes tenofovir[a]
NNRTI + rPI	rPI → 2 NRTIs	
INSTI + rPI	rPI → NNRTI	

ART antiretroviral therapy, *DHHS* Department of Health and Human Services (USA), *NRTI* nucleoside reverse transcriptase inhibitor, *NNRTI* nonnucleoside reverse transcriptase inhibitor, *INSTI* integrase strand transfer inhibitor, *rPI* ritonavir-boosted protease inhibitor

[a]Tenofovir decreases the bioavailability of unboosted atazanavir

of boosted PIs to NNRTIs has also demonstrated favourable changes in both total cholesterol and triglycerides with both nevirapine and rilpivirine [105, 106]. Even the switching of efavirenz to nevirapine has been associated with significant decreases in LDL cholesterol levels [107].

For suitable patients not receiving novel or salvage ART regimens, potential switch options are outlined in Table 9.4.

Statin Therapy

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, are the best known and most effective pharmacotherapy for treating hypercholesterolemia. They are the first choice for reducing elevated LDL cholesterol in HIV-negative individuals, thereby improving cardiovascular risk. Statins show similar efficacy in HIV-infected individuals, with a mean reduction in total and LDL cholesterol levels of up to 50% from baseline. The actual risk reduction, however, is unknown [108].

The mechanisms and additional benefits of statin therapy are described in greater detail elsewhere in this book. For HIV-infected patients receiving ART, however, the main issue is not a lack of efficacy, but the potential for drug-to-drug interactions with PIs (Table 9.5) [109]. Some of the statins are metabolized by the same CYP 3A4 isoform inhibited by PIs, leading to increased exposure to statins. This can predispose to a greater risk of statin-induced myopathy. In the case of simvastatin, the exposure can increase by as much as 3000% [110]. For this reason, simvastatin (and lovastatin) are contraindicated with PI use. Other commonly prescribed PIs are less affected by this interaction and may be used in conjunction with PIs, but still require dose reduction (atorvastatin, rosuvastatin).

In prospective studies of HIV-infected patients, rosuvastatin has proven to be the most effective statin, reducing LDL cholesterol significantly higher than the least-potent agent, pravastatin (37 vs. 20%) [111]. Both rosuvastatin and atorvastatin are significantly more likely to achieve NCEP target goals for total and LDL cholesterol than pravastatin, with similar toxicity rates of between 5% and 7% [112].

Other Hypolipidemic Agents

Other lipid-lowering options include fibrates, ezetimibe, omega-3 fatty acids (found in fish oil supplements), niacin, and bile–acid sequestering resins.

Fibrates (gemfibrozil, fenofibrate, bezafibrate) are agonists of peroxisome proliferator-activated receptor α, and are well tolerated in HIV-infected patients and have few interactions with ART. Their principal action is reduction of triglycerides. Their utility in HIV is limited, however, by the presence of mixed dyslipidemia; in hypertriglyceridemic states, relatively common in HIV, fibrates can lead to increases in LDL cholesterol of up to 0.5 mmol/L, potentially blunting their

Table 9.5 Statin–protease inhibitor interactions[a]

Statin	Affected PIs	Pharmacokinetic interaction	Prescribing recommendations
Atorvastatin	Tipranavir/r	Moderate inhibition of statin metabolism	Avoid
	Lopinavir/r		
	Darunavir/r	Mild inhibition of statin metabolism	Use with caution, use lowest dose necessary
	Fosamprenavir/r		
	Saquinavir/r		
Fluvastatin	Nelfinavir	Limited data available (possible induction of statin metabolism via non-CYP mechanism)	Alternative to rosuvastatin, pravastatin and atorvastatin
	Ritonavir (full-dose)		
Lovastatin	All PIs	Marked inhibition of statin metabolism	Avoid
Pravastatin	Darunavir/r	Induction of statin metabolism, with possible reduced statin effect	No special limitations to dose
	Lopinavir/r		
Rosuvastatin	Atazanavir	Inhibition of statin metabolism (via non-CYP mechanisms)	Limit to 10 mg daily (5 mg daily in Asians[b])
	Atazanavir/r		
	Lopinavir/r		
Simvastatin	All PIs	Marked inhibition of statin metabolism	Avoid

PI protease inhibitor, *r* with ritonavir-boosting, *CYP* cytochrome P450
[a]Drug interactions are regularly updated at the University of Liverpool's online database (http://www.hiv-druginteractions.org/)
[b]Includes individuals of SouthEast and South Asian ethnicity

reduction of cardiovascular risk [113]. Combined statin–fibrate therapy can be considered where the triglyceride level is >5.6 mmol/L (500 mg/dL), but should be generally cautioned against because of the potential for myopathy [98].

Eicosapentaenoic acid and docosahexaeneoic acid—the omega-3 fatty acids—significantly lower triglycerides in HIV-infected patients with dyslipidemia by up to 20% [114]. However, fish oil is also associated with a >20% rise in LDL cholesterol. It is therefore unclear if omega-3 fatty acids will have an overall benefit for cardiovascular risk.

Ezetimibe blocks cholesterol absorption in the gastrointestinal tract and is free of interactions via the CYP pathway. As monotherapy in HIV-uninfected persons, it can reduce circulating LDL cholesterol by >20% and by up to 50% in combination with statins [115]. As a relatively recent addition to lipid-lowering therapies, data in HIV-infected persons are limited, but it is associated with reductions in LDL cholesterol [116]. Risk reduction is yet to be demonstrated, but using a statin–ezetimibe combination may prove to be useful and preferable to increasing statin doses.

Niacin (nicotinic acid) is well-known for its side effects of flushing and headaches, and can be effective for reducing triglycerides and LDL cholesterol in HIV-infected patients [117]. Other common self-limiting side effects include cutaneous rash and pruritus. But importantly for cardiovascular risk, one study reported the nascent onset of insulin resistance and glucose intolerance. It should therefore be avoided as a first-line option. Hepatotoxicity is uncommon, but can be severe. Bile–acid sequestering resins such as cholestyramine are known to decrease the serum concentration of many orally administered drugs by limiting gastrointestinal absorption. Although no such interactions with any of the ART drug classes have been reported, no studies to assess this question have been performed, so clinicians should be mindful of the potential for interaction.

Conclusions

Dyslipidemia in HIV-infected patients is a product of viremia and ART; both contribute independently to an increase in cardiovascular risk. The

precise pathogenesis is unclear, being a highly complex portmanteau of direct and inflammatory effects, and no predominant mechanism has hitherto been identified.

As ART is initiated ever earlier, patients spend less time in a state of active viral replication following diagnosis, diminishing the relative contribution of viremia to dyslipidemia. ART is therefore ultimately of greater long-term clinical relevance on lipid levels. As the full metabolic profile of the newer ART classes remains to be determined, dyslipidemia will continue to be a commonly encountered problem by HIV physicians.

It is important to remember that HIV-infected patients have a greater likelihood of cardiovascular disease even after correcting for traditional risk factors. The management of HIV-associated dyslipidemia may therefore require a lower threshold for intervention, and should be aimed at the reduction of overall cardiovascular risk, not just restitution of a normal lipid profile. Statins remain the first choice for pharmacotherapy, but may require dose reduction. Switching of ART is a treatment option in carefully selected patients.

Further studies are required to assess the most efficacious intervention to reduce cardiovascular risk attributable to HIV-related dyslipidemia. Elucidating pathogenic mechanisms, particularly those of each class of ART drugs, may also identify future therapeutic targets.

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Monogenic Hypercholesterolemias

10

Fernando Civeira and Miguel Pocovi

Definition and Classification

Monogenic hypercholesterolemias (MHs) are a heterogeneous group of single-gene defects with Mendelian transmission in the family characterized by elevated plasma low-density lipoprotein (LDL) cholesterol levels and very high risk for premature atherosclerotic disease, especially coronary heart disease (CHD) [1] (Table 10.1).

Approximately, one in 200–500 people is affected by MH in most populations explored so far, so this group of diseases is among the most frequent genetic metabolic defects [2]. The study of MH has provided decisive evidence of the linkage between high LDL cholesterol concentration and atherosclerosis development in humans. Furthermore, the metabolic and genetic characterization of MH in the past decades has supplied crucial information about the cholesterol homeostasis, metabolism, and regulatory pathways. The scientific information generated around the MH has contributed decisively to the development of many drugs in common use today, such as hydroxy-methyl-glutaryl coenzyme A reductase (HMG-CoAR) inhibitors or statins, which have contributed to change the evolution of arteriosclerotic disease. The information generated around the MH remains very active today, and the discovery of new genes responsible for high LDL cholesterol is promoting the development of very promising new drugs, such as inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), and others.

MH traditionally included two common diseases of autosomal dominant inheritance: familial hypercholesterolemia (FH), due to mutations in the LDL receptor gene (*LDLR,* Online Mendelian Inheritance in Man (OMIM) 143890) causing isolated high LDL cholesterol (type IIa hyperlipoproteinemia) and familial combined hyperlipidemia (FCHL) of unknown etiology (OMIM 144250) usually associated with mixed hyperlipidemia secondary to high concentrations of very LDLs (VLDL) and LDL particles (type IIb hyperlipoproteinemia); as well as several rare recessive diseases such as sitosterolemia (OMIM 210250) and autosomal recessive hypercholesterolemia (ARH;OMIM 603813) [3]. However, FH is heterogeneous from the genetic standpoint, and mutations in the *LDLR* are found only in 60–80 % of patients with a clinical diagnosis of FH [4]. Functional mutations in other genes produce indistinguishable clinical phenotypes of FH, including a missense mutation p.(Arg3527Gln) located in the LDLR-binding domain of

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Table 10.1 Monogenic hypercholesterolemias (MHs) causing high LDL cholesterol

Inheritance	Disease name	Defective gene	Prevalence[a]	Plasma LDL cholesterol (mg/dL)
Dominant				
	Familial hypercholesterolemia (FH)	*LDLR*	1 in 500	200–500 (heterozygous) 500–800 (homozygous)
	Familial defective apo B-100	*APOB*	1 in 2000	200–400 (heterozygous) 500–800 (homozygous)
	FH3	*PCSK9*	<1 in 10,000	200–500 (heterozygous)
	FH4	*APOE*	?	200–500 (heterozygous)
	Hyperlipoproteinemia(a)	*LPA*	1 in 2500	200–300
	Autosomal dominant familial combined hyperlipidemia	*LDLR, C5L2, APOE, PCSK9,?*	1/500	200–350
Recessive				
	Autosomal recessive hypercholesterolemia	*LDLRAP1*	<1 in 10^6	400–600
	Sitosterolemia	*ABCG5/ABCG8*	<1 in 10^6	Variable
	Lysosomal acid lipase deficiency	*LIPA*	<1 in 50,000	200–300
	Cholesterol 7-alpha-hydroxylase deficiency	*CYP7A1*	Very rare	150–210

[a] Some prevalences are highly heterogeneous among populations. *LDL* low-density lipoprotein

apolipoprotein (apo) B-100 that produces familial defective apo B-100 (FDB, OMIM 144010) [5]; gain-of-function mutations in PCSK9, a protein that binds to the LDL receptor inducing its degradation along with the LDL particle, and are termed FH3 (OMIM 603776) [6, 7]; and, a deletion in a codon of APOE (p.Leu167del) has been recently associated with autosomal dominant hypercholesterolemia in two different studies [8, 9]. Very high levels of lipoprotein(a) ((Lp(a)), named as hyperLp(a), is a single-gene condition also causing MH [10]. Except for the presence of apo(a) in the LDL particle surface, Lp(a) is essentially indistinguishable from LDL, so Lp(a) carries in some cases substantial amounts of cholesterol. Lp(a) varies from 0.1 to 300 mg/dL among individuals due to the *LPA* gene locus which codes for the apo(a) [11]. *LPA* kringle IV-2 sequence is present in a variable number of identical repeated copies (from 3 to >60) and the number of kringle IV-2 repeats is inversely correlated with the Lp(a) concentration [12]. Lp(a) is discussed in extension in another chapter of this book.

The genetic heterogeneity of isolated high LDL cholesterol in MH, the clinical similarities among them, which make their clinical diagnosis in most cases indistinguishable, their common high cardiovascular risk, and their uniform response to the different lipid lowering treatments, mean that all of them are referred to collectively as FH, regardless of the presence of mutations in the *LDLR* [13, 14]. Hence, FH should be defined as a group of monogenic genetic defects resulting in severely elevated serum LDL cholesterol concentrations with autosomal codominant transmission pattern of inheritance.

In contrast, most cases of FCHL, the most common genetic form of hyperlipidemia identified in survivors of myocardial infarction [15, 16], do not correspond to a monogenic disease, rather they are complex genetic diseases resulting from the interaction of multiple genetic and environmental factors mainly overweight, obesity, saturated fat- and sugar-enriched diets, and physical inactivity [17]. Many families with FCHL combine adipose tissue dysfunction [18, 19], insulin resistance [20], hepatic overproduction of VLDL particles [21], and peripheral slow clearance of triglycerides-rich lipoproteins [22]. Different association and linkage studies have shown more than 40 different genes associated with FCHL that have been recently reviewed by Brouwers et al. [23] although with great differ-

ences among studies. Therefore, FCHL is currently considered to be a complex phenotype consequence of multiple genetic defects, each one mostly with minor effects, which differ among families, and among populations. In over 50% of the families with the clinical diagnosis of FCHL, all effected members are overweight or obese, and with high frequency they develop diabetes mellitus with time [24]. This predisposition within certain families, to develop mixed hyperlipidemia only in the presence of increased body fat deposits, we have proposed to be named as "adiposity-related familial hyperlipidemia" [25]. However, the actual definition of FCHL, familial transmission of high apo B levels with high plasma total cholesterol and/or triglycerides, also includes some forms in which the lipid phenotype is largely determined by a single gene [23]. These less common forms of autosomal dominant FCHL demonstrate that, in some cases, the FCHL phenotype is largely determined by a single genetic defect, and behaves as an MH [8, 26] (Table 10.1).

Familial Hypercholesterolemia

As defined above, FHs are a group of monogenic genetic defects resulting in severely elevated serum LDL cholesterol concentrations with autosomal codominant transmission pattern of inheritance. Hence, patients with two defective alleles (FH homozygotes or compound heterozygotes) have much higher LDL cholesterol than those with one mutant allele (FH heterozygotes). Lifelong elevated plasma concentrations of LDL cholesterol are responsible for the major clinical manifestation of FH: premature CHD and extravascular cholesterol deposits as tendon xanthomas or corneal arcus [27]. The frequency of FH heterozygotes (1 in 500 individuals) is much higher than FH homozygotes (<1 in 1 million). However, some populations such as French Canadians [28], Afrikaners in South Africa [29], Lebanese, and Finns [30] have a much higher prevalence due to a founder effect.

Etiology and Pathogenesis

FH caused by mutations in *LDLR* was the first disease of lipid metabolism to be genetically defined, the best known at present time, and the most frequent MH in most countries around the world. Different mutations in the LDLR affect the LDL receptor protein functionality [27]. LDL receptor in cell membranes binds LDL and the complexes enter the cell by endocytosis [31]. The LDL receptor is synthesized as a 120-kDa precursor protein, which is converted to a mature form of apparent molecular mass of 160 kDa. The increase in molecular mass is correlated with extensive *N*- and *O*-glycosylation in the Golgi apparatus during transfer to the cell surface [32]. In addition to the glycosylation, in the endoplasmic reticulum (ER), the 21-amino-acid signal peptide of the LDL receptor is cleaved to give rise to a mature receptor. The transmembrane LDL receptor (glycoprotein of 839 amino acids) is present at the surface of most cell types and mediates the transport of lipoproteins containing apo B or apo E into cells, through receptor-mediated endocytosis. The mature LDL receptor reaches the cell surface and is directed towards clathrin-coated pits where it binds to apo B- and apo E-enriched lipoproteins via its extracellular domain [33]. The lipoprotein–LDL receptor complex is endocytosed and migrates to endosomes. At the acidic pH of the lysosomes, LDL is released, allowing LDL receptor to return to the membrane and entering into a new cycle [34] (Fig. 10.1). Although the LDL receptor was initially thought to play the single role of helping to achieve cholesterol homeostasis, its expression in neurons suggests it may also play other functional roles [35, 36].

Cholesterol homeostasis is among the most regulated processes in biology. Cellular cholesterol balance is achieved by both synthesis and uptake through LDL receptor. When cellular cholesterol levels rise, *LDLR* transcription is reduced and de novo synthesis is inhibited. When cellular cholesterol storage is depleted, *LDLR* transcription is activated and de novo synthesis activated. Two major transcriptional *LDLR* regulation pathways have evolved in mammals to

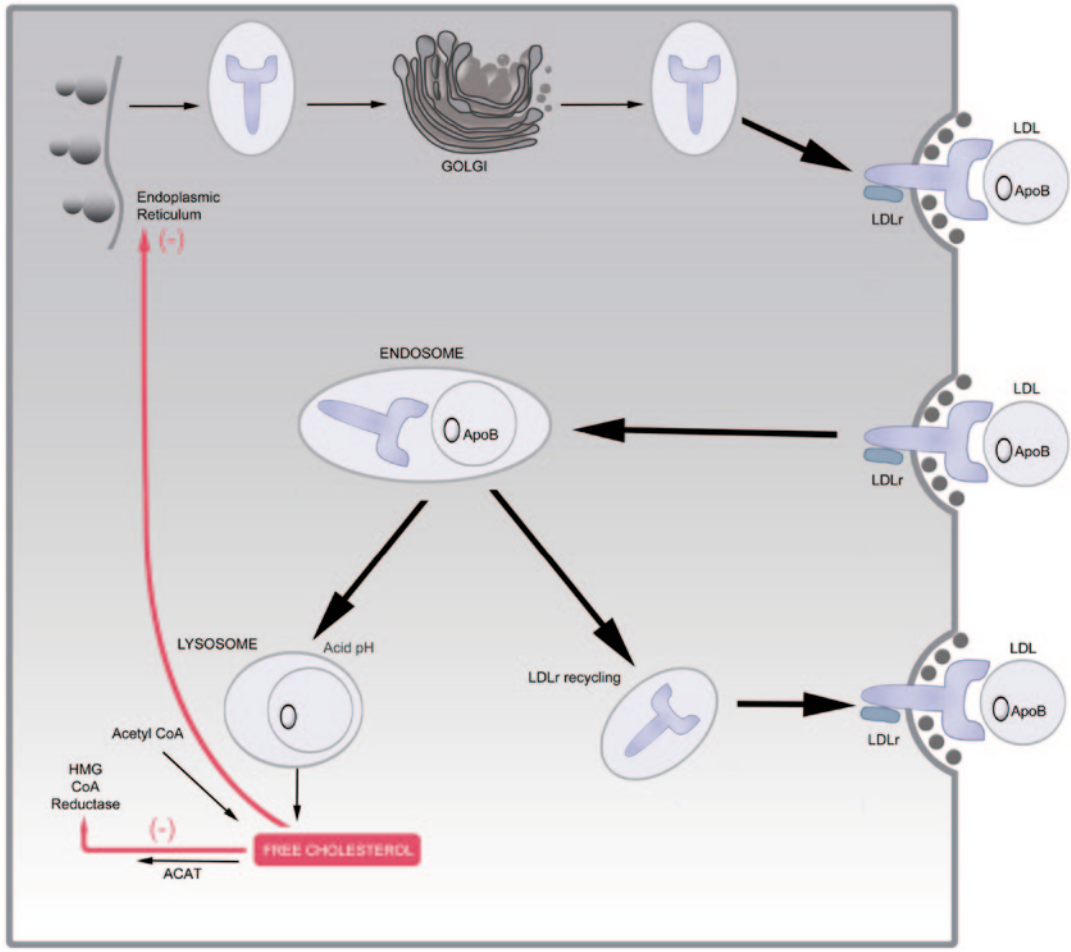

Fig. 10.1 Schematic representation of the itinerary of the low-density lipoprotein (LDL) receptor in human cells. The LDL receptor is synthesized in the endoplasmic reticulum (ER) as a precursor of apparent molecular weight of 120 kd and transported at the Golgi complex where the N-linked carbohydrates are processed. Once transferred to the surface of the cell, the receptor recognizes the apolipoprotein B-100 component of the LDL. Binding leads to cellular uptake and lysosomal degradation of the LDL by receptor-mediated endocytosis. This uptake process satisfies the cholesterol needs of the cells, and hence keeps cholesterol synthesis suppressed

coordinate responses to both elevated and reduced cellular cholesterol content: the sterol regulatory element-binding proteins (SREBPs) and the liver X receptors (LXRs). SREBP-2 promotes the expression of the *LDLR,* thereby increasing LDL uptake and cholesterol delivery to cells [37]. The SREBP-2 precursor protein resides in the ER and is transported to the Golgi apparatus under low intracellular cholesterol content, where it undergoes proteolytic processing. The mature SREBP protein translocates to the nucleus and switches on the transcription of *LDLR,* as well as other genes involved in cholesterol biosynthesis, including HMGCoAR and HMGCoA synthase (HMGCoAS) [38]. An additional modulator of LDL receptor-dependent cholesterol uptake independent of the SREBP pathway is the LXR [39]. LXR induces expression of E3 ubiquitin ligase inducible degrader of the LDL receptor (Idol), which in turn catalyzes the ubiquitination of the LDL receptor and targets it for degradation [40].

Characterization of Idol-deficient cells has also provided insights into to the functional relationship between PCSK9 and Idol pathways. PCSK9 and Idol share the same protein substrates, but PCSK9 is still able to induce LDL receptor degradation in Idol$^{-/-}$ cells, suggesting that Idol and PCSK9 may be complementary but independent pathways [41]. PCSK9 is secreted into plasma and binds to the first domain (EGF-A) of epidermal growth factor (EGF) homology repeats of LDL receptor [42–44]. Although the C-terminal domain of PCSK9 is not required for LDL receptor binding, it is required for LDL receptor degradation [33]. The complete mechanism by which PCSK9 binding to the LDL receptor targets the receptor for degradation is not understood. Although PCSK9 is a protease, it does not cleave LDL receptor, nor is the proteolysis of LDL receptor required to downregulate *LDLR*. The LDL receptor–PCSK9 complex is internalized via clathrin-mediated endocytosis and then routed to lysosomes via a mechanism that does not require ubiquitination and is distinct from the autophagy and proteosomal degradation pathways [45].

Posttranscriptional regulation of *LDLR* expression is also a major determinant of lipoprotein metabolism. LDLR adaptor protein 1 (LDLRAP1) is a protein required for the efficient activity of LDL receptor. It has been demonstrated that LDLRAP1 is essential for the efficient internalization of the LDL–LDL receptor complex and cells from patients with ARH fail to internalize the LDL receptor because they carry two defective alleles of LDLRAP1, a gene that encodes a specific clathrin adaptor protein [46]. LDLRAP1 is an endocytic sorting adaptor that actively participates in the internalization of the LDL–LDL receptor complex, possibly enhancing the efficiency of its packaging into the endocytic vesicles [47]. LDLRAP1 is required not only for internalization of the LDL-LDL receptor complex but also for efficient binding of LDL to the receptor. LDLRAP1 stabilizes the associations of the receptor with LDL and with the invagination portion of the budding pit, thereby increasing the efficiency of LDL internalization [48].

The *LDLR* Gene

The *LDLR* is mapped to chromosome 19p13.1-13.3 and spans 45 kb and contains 18 exons and 17 introns encoding the six functional domains of the mature protein: signal peptide, ligand-binding domain, EGF-like, O-linked sugar, transmembrane, and cytoplasmic domain [49, 50] (Fig. 10.2). The human LDLR complementary DNA (cDNA) and gene were cloned and characterized in 1984 and 1985, respectively [51, 52]. The gene sequencing of the *LDLR* suggested that the LDL receptor is a mosaic protein built up of exons shared with different proteins, and it therefore belongs to several supergene families [52].

Exon 1 encodes a hydrophobic sequence of 21 amino acids that correspond to the signal peptide, which is cleaved from the protein into the ER during the translocation process. Around 4.5% of the total *LDLR* mutations described including frameshift, missense, and nonsense sequence variants have been located in this exon (http://www.ucl.ac.uk/fh).

Exons 2–6 encode the ligand-binding domain, a cysteine-rich sequence of seven tandem structurally homologous repeats of 40 amino acids each, which is responsible for binding lipoproteins. The structure of the ligand-binding domain has been partially elucidated. Each repeat contains a cluster of negatively charged amino acids, Asp-X-Ser-Asp-Glu and six cysteine residues that form three disulfide bonds [53–55]. Binding of lipoproteins to the LDL receptor appears to be mediated by an interaction between acidic residues in the LDL receptor binding domain and basic residues of apo E and apo B-100 [53, 56]. Repeats R3–R7 are necessary for LDL binding (apo B-100-mediated), whereas remnant lipoproteins binding (apo E-mediated) is impaired only when R5 is deleted. Repeats R4 and R5 are sufficient to bind to apo E-phospholipids vesicles [54]. We have proposed a new mechanism for the release of LDL particles in the endosome; it is based on the instability of repeat 5 at endosome low pH and low Ca^{2+} [57]. Under these conditions, repeat 5 is unable to bind Ca^{2+} and appears in an unfolded conformation not expected to bind

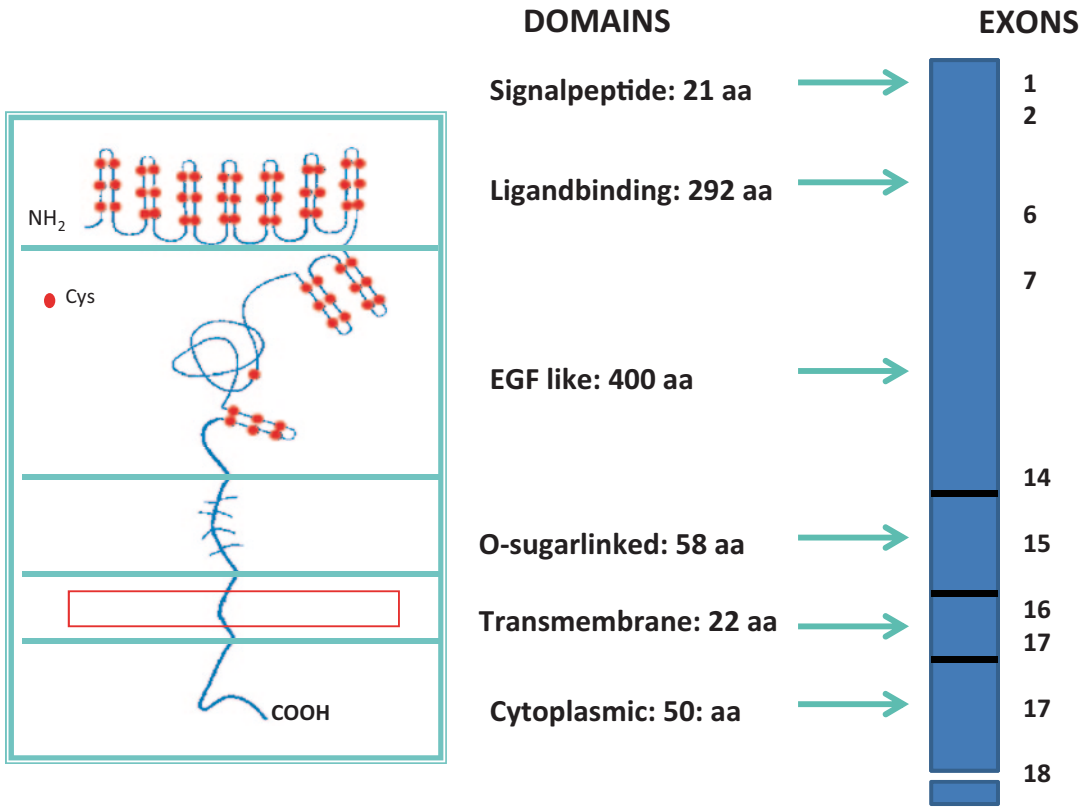

Fig. 10.2 Schematic representation of the five domains in the structure of the human low-density lipoprotein (LDL)-receptor protein and their corresponding exons in the LDLR gene. *Red dots* represent the six cysteine residues that form three disulfide bonds in each tandem structurally homologous repeats or class A repeats in the ligand-binding domain of the LDL receptor protein

LDL particles. In the ligand-binding domain, 40% of the total allelic variants associated with FH have been found to date (http://www.ucl.ac.uk/fh).

Exons 7–14 encode a region that shares 33% sequence identity to the human EGF gene. This domain consists of a 411-amino-acid sequence, encoded by exons 7–14. Like the ligand-binding domain, this region also contains three repeats of 40–50 amino acids with cysteine-rich sequences. The first two repeats, designated A and B and encoded by exons 7 and 8, are contiguous and separated from the third repeat, C encoded by exon 14, by a 280-amino-acid sequence that contains five copies of the conserved motif Tyr-Trp-Thr-Asp, encoded by exons 9–13. PCSK9 binds to EGF-A repeat, decreasing receptor recycling and increasing degradation [43]. The EGF-like domain is required for the acid-dependent dissociation of the LDL particles from the LDL receptor and clathrin-coated pits during receptor recycling. When the EGF-like domain is deleted from the LDL receptor, the receptor can no longer bind LDL particles but it still binds lipoproteins that contain apo E [58]. The majority of FH mutations described (55% of total) have been associated with the EGF homology region.

Exon 15 encodes an LDL receptor domain of 58 amino acids rich in Thr and Ser residues. The function of this domain is unknown, but it has been observed that this region serves as an attachment site for O-linked carbohydrate chains and it is thought that it plays a role in the stabilization of the receptor. This domain shows

minimal sequence conservation among six species analyzed, and Davis et al. reported that deletion of clustered O-linked carbohydrates does not impair function and turnover of human LDL receptor in Chinese hamster ovary (CHO) cells transfected with the human *LDLR* gene [59]. However, from the analysis of LDL receptors in CHO mutant cells with defective uridine diphosphate (UDP)-galactose and UDP-*N*-acetylgalactosamine 4-epimerase, Kingsley et al. proposed that O-linked carbohydrate chains may be crucial for receptor stability [60]. A total of 41 allelic variants within exon 15 are registered in *LDLR* databases.

Exon 16 and the 5′-end of exon 17 encode a domain of 22 hydrophobic amino acids that is essential for anchoring the LDL receptor to the cell membrane.

The cytoplasmic domain of the LDL receptor, that compromises 50-amino-acid residues, is encoded by the remainder 3′ region of the exon 17 and the 5′ end of the exon 18 [27]. This domain contains two sequence signals for targeting the LDL receptor to the surface and for localizing the receptor in coated pits [61]. This domain is the most conserved region of the LDL receptor, which is more than 86% identical among six species [27]. Only a few allelic variants, 6% of the total, have been identified within these domains.

The DNA motifs essential for the transcriptional regulation of the LDLR are located within 177 bp of the proximal promoter. The LDL receptor production is tightly regulated by a sophisticated feedback mechanism that controls the transcription of the *LDLR* in response to variations in the intracellular sterol concentration and the cellular demand for cholesterol [62]. The promoter region contains all the *cis*-acting elements for basal expression and sterol regulation and includes three imperfect direct repeats of 16 bp each, repeats 1–3. Repeats 1 and 3 contain binding sites for Sp1 transcription factor, and contribute to the basal expression of the gene, requiring the contribution of the repeat 2 for a strong expression. Repeat 2 contains a regulatory element, sterol regulatory element (SRE)-1, that enhances transcription when the intracellular sterol concentration is low through interaction with SREBP [63]. Several naturally occurring mutations have been mapped to the transcriptional regulatory elements of the *LDLR*.

Nowadays, over 1500 naturally occurring *LDLR* mutations have been described in FH patients (http://www.ucl.ac.uk/fh). The *LDLR* mutations can produce defects in transcription, posttranscription processes, translation, and posttranslation processes. FH mutations have been classified into five classes depending on phenotypic behavior of mutant protein [64]. Class 1 mutations are known as “null alleles,” which fail to produce immune-precipitable LDL receptor protein. Most of them are due to *LDLR* promoter deletion, rearrangements, frameshift, nonsense, or splicing mutations in a way that messenger RNA (mRNA) is not produced [64]. Class 2 mutations are transport-defective alleles which encode for proteins that cannot adopt an adequate tridimensional structure after being synthesized and keep them blocked, completely or partially (2A and 2B, respectively) in transport process between ER and Golgi apparatus. This defect is caused, usually, by missense mutations or small deletions in *LDLR* avoiding partially or completely the folding of the protein. These mutations are the most common at the *LDLR* locus [64]. Class 3 mutations are binding-defective alleles which encode for LDL receptor that are synthesized and transported to cell surface but fail to bind LDL particles. This is a heterogeneous group, because LDL binding activity goes from 2 to 30% of normal. This defect is due to rearrangements in repeat cysteine residues in binding ligand domain or repeat deletions in EGF-like domain [65]. Class 4 mutations are known as internalization-defective alleles. These alleles produce proteins that are unable to cluster into clathrin-coated pits, therefore LDL receptor is not internalized [66]. Finally, class 5 mutations result in receptors that are able to bind and internalize LDL, but they fail to release LDL in the sorting endosomes and fail to recycle. Instead, they are rerouted to the lysosomes for degradation [67, 68].

Several studies have shown that different mutations are associated with differences in lipid levels, and it is likely that these will be associated with clinically different effects [29, 69]. In

addition, the phenotypic effect of the mutation is modulated by other genetic or environmental factors [29, 70]. Even the LDL lowering effect of statins in FH patients may depend on the nature of the *LDLR* mutation [71, 72]. Our group observed that FH patients with a molecular diagnosis show different advanced carotid and femoral atherosclerosis in relation to *LDLR* mutational class, thus FH patients with null allele mutations of *LDLR* show a more severe clinical phenotype and worse advanced carotid atherosclerosis than those with receptor-defective mutations, independently of age, gender, lipid, and nonlipid risk factors [73, 74].

APOB Gene

APOB was the second locus identified to be responsible for MH. A group of individuals with a clinical phonotype similar to FH and also reduced LDL catabolism were found to have normal LDL receptor activity. The disease was secondary to a defective apo B that displays low affinity for the LDLR, and was named FDB. FDB is as common as FH in some European populations [75, 76] but much higher in Old Order Amish living in the USA [77]. The interaction between LDL and the LDL receptor is essential for the regulation of plasma cholesterol in humans. Apo B-100, the major protein component of LDL, is also a ligand for the LDL receptor; therefore, apo B-100 mediates the binding of LDL particle to the LDL receptor [78]. Studies using immunoelectron microscopy have shown that the N-terminal 89 % of apo B-100 enwraps the LDL particle like a belt and that the –COOH terminal 11 % constitutes a bow that crosses over the belt, bringing residues 4154–4189 and 4507–4513 close to amino acid 3527 [79].

Vega and Grundy observed that a group of patients with hypercholesterolemia have reduced clearance of LDL because of a defect in the structure or composition of LDL that reduces its affinity for receptors [80]. Innerarity et al. found that this type of hypercholesterolemia could be attributed to a defective receptor binding of a genetically altered apo B-100 to the LDL receptor [81].

The first mutation found in *APOB* as FDB cause was demonstrated by Soria et al. They observed a mutation in the codon for amino acid 3527 that results in the substitution of Gln for Arg (p.R3527Q) [5]. So far, ten true mutations at the *APOB* locus have been identified that alter the binding properties of apo B-100 indicating that FDB is more heterogeneous than previously assumed. Two mutations causing FDB were described in 1995: a change of Gln for a Trp in the amino acid 3527 (p.R3527W) and a substitution of Arg for Cys in 3558 codon (p.R3558C) [82, 83].

The binding affinities of p.R3527Q and p.R3558C to the LDL receptor are reduced to 30 and 70 %, respectively. Nevertheless, the deleterious effect of the p.R3558C variation was reconsidered not sufficient to cause hypercholesterolemia suggesting that it is more a susceptibility variation than a causative mutation [84].

The p.E3432Q mutation binds to LDL receptor at the same rate as normal LDL, but LDL particles containing this mutant protein are taken up and degraded at significantly reduced rates [85]. The *APOB* mutation p.R3507W has been associated with FDB because of its position near Trp 4396 that was shown to interact with Arg 3527 and facilitate the protein conformation required for normal receptor binding of LDL [86].

Another *APOB* mutation was found in codon 3543, located between known FDB mutations at codons 3527 and 3558, this mutation, p.N3543K, introduces a positively charged amino acid Lys, while other FDB mutations remove a positively charged residue Arg. The p.N3543K mutation influences conformation of LDL apo B and its interaction with the LDL receptor [82].

Other four *APOB* mutations, p.H3570Y, p.R3527L, p.R4385H, and p.V4394L were detected in hypercholesterolemic patients [87, 88].

However, the causative effect of these four mutations has not been yet demonstrated. Recent data reveal that compared with FH patients with *LDLR* mutations, FDB patients have lower LDL cholesterol levels by 20–25 %, respond better to statins, and have lower risk of CHD [88, 89]. This difference could be due to normal clearance of VLDL remnants through apo E-mediated uptake in FDB [90].

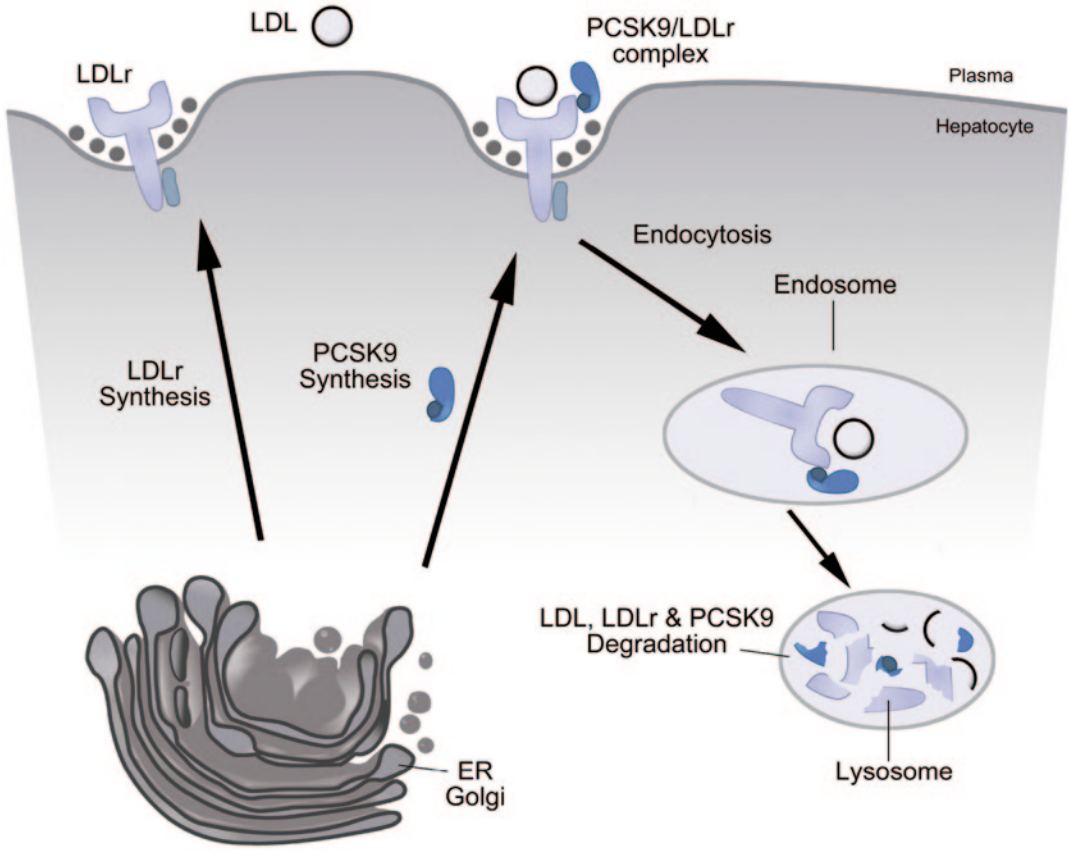

Fig. 10.3 Proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in the metabolism of low-density lipoprotein (LDL) receptor. PCSK9 is synthesized in hepatocytes and released into blood. On the hepatocyte surface PCSK9 binds to the LDL receptor. The binding of LDL particles with the complex PCSK9/LDL receptor produces its internalization by endocytosis and lysosomal degradation of the LDL receptor. In the absence of PCSK9, LDL receptor degradation does not occur and the LDL receptor is recycled back to the cell surface

Proprotein Convertase Subtilisin/ Kexin Type 9 Gene

In 1999, Varret et al. identified by linkage analysis a third major autosomal dominant locus (HCHOLA3) at 1q34.1-p32 chromosome and showed that HCHOLA3 was in fact *PCSK9* [6, 91]. The *PSCK9* gene comprises 12 exons transcribed into a cDNA that spans 3617 bp. PCSK9 was first identified as a member of proprotein convertase family with hepatic, intestine, and kidney expression. PCSK9 is a 692-amino-acid glycoprotein that contains a 22-residue signal sequence followed by a pro-domain and a catalytic domain that shares structural homology with the proteinase K family of subtilisin-like serine proteases [7]. PCSK9 is a secreted protein that promotes degradation of the LDL receptor, and variants in *PCSK9* gene that cause hypercholesterolemia in humans are gain-of-function mutations [3, 92].

Initially, it was thought that PCSK9 has a role in LDL receptor degradation at the cell surface [92]. As we have described above, there is enough evidence to think that PCSK9 participates in LDL receptor lysosomal degradation via a mechanism that does not require ubiquitination and is distinct from the autophagy and proteosomal degradation pathways [45] (Fig. 10.3).

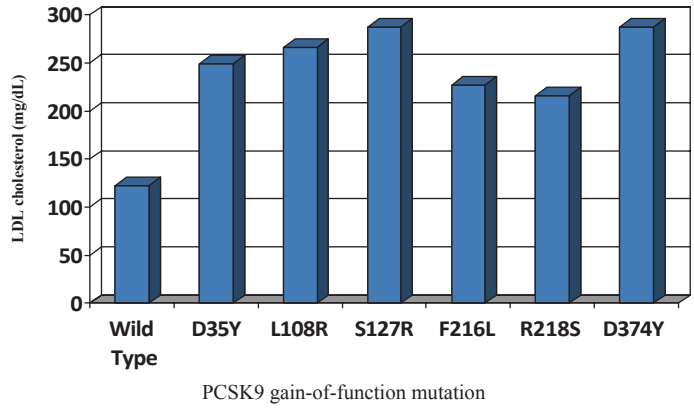

Fig. 10.4 Effect of proprotein convertase subtilisin/kexin type 9 (PCSK9) mutations on plasma low-density lipoprotein (LDL) cholesterol concentration (Modified from reference [96])

PCSK9 mutations have been also classified into five classes, including "null alleles," mutations that affect autocatalytic scission, avoiding the protein transport through ER or from the ER to cell surface, alleles that affect PCSK9 stability and, finally, mutations that produce gain of function because of gene overexpression [93–96] (Fig. 10.4). By contrast, mutations in *PCSK9* that produce loss of function (Y142X, C679X, and R46 L) are associated with low LDL cholesterol [97, 98].

Other FH *Loci*

The proportion of individuals with autosomal dominant hypercholesterolemia without a mutation in LDLR, APOB, or PCSK9 ranges from 12 to 60% (ADH-) [88, 99–102]. This variability is due mainly to the clinical–biological criteria used to select FH subjects as well as due to ethnicity [103]. Our group has demonstrated that hyperLp(a) is responsible for FH phenotype in approximately 6% of nonLDLR/nonAPOB subjects [10].

More recently, two groups independently have demonstrated that a rare mutation in *APOE,* c.500_502delTCC/p.Leu167del causes a lipid phenotype indistinguishable from classical FH in Spain and France [8, 9]. In the Spanish study, the mutation was found in probands with the clinical diagnosis of FCHL, but the family studies demonstrated that the most common phenotype in mutation carriers' family members was isolated high LDL cholesterol rather than combined hyperlipidemia [8]. The mechanism of high LDL cholesterol associated with this mutation is unknown, but is predicted to interrupt an alpha-helix in the binding domain of apo E and reduce the catabolism of particles containing apo E, including LDL [9].

Several genome-wide linkage scan have suggested susceptibility FH *loci* on chromosomes 3q25–26, 8q24.22, 16q22.1, and 21q22. However, no gene nor disease-causing mutation was identified in these loci so far [104–106].

Clinical Findings

Atherosclerotic Vascular Disease in FH Approximately 80% of FH heterozygotes and almost 100% of homozygotes will suffer and die of atherosclerosis vascular disease if they are not treated to lower their LDL cholesterol during long periods of time [107]. Symptomatic atherosclerosis disease presents as CHD before age 55 and 60, in over 50% of FH heterozygotes, men and women, respectively, while homozygotes with much higher LDL cholesterol typically suffer CHD very early in life and usually die before age 20 without treatment. In homozygotes, atherosclerosis begins in the aortic root, causing CHD and supravalvular aortic stenosis [27]. The mean age of onset of a cardiovascular event in men with heterozygous FH is in the early 40s and in women with FH in the early 50s. Approximately, 85% of males will suffer a coronary event before 65 years if they are not treated. Atherosclerotic vascular disease in FH is mostly CHD. FH repre-

Table 10.2 Major CVD risk factors in heterozygous FH subjects

Risk factor	Cut points
Age	Men >30 years of age
	Women >40 years of age
LDL cholesterol	>250 mg/dL
Smoking	Current smoker
Family history of premature CHD	First-degree male relative <age 55
	First-degree female relative <age 65
Blood pressure	>140 mm Hg systolic or >90 mm Hg diastolic
Low HDL cholesterol	<40 mg/dL
High lipoprotein(a)	>50 mg/dL
Physical findings	Tendon xanthoma
Diabetes mellitus	Presence
Genetic defect	LDL receptor-negative mutations

LDL low-density lipoprotein, *CHD* coronary heart disease, *HDL* high-density lipoprotein

sents 1–2% of all premature (<55 years of age in men and <65 years of age in women) myocardial infarctions in most countries [13, 14], and up to 9% of total premature CHD in Eastern Finland [108] and Germany [109] are caused by FH. The mechanism of this excess of coronary lesions in FH with respect to other vascular beds such as lower limbs or carotid arteries is not known, but is probably related to the type of LDL particle which accumulates in plasma.

Risk factors associated with CHD in heterozygous FH are the traditional risk factors for the general population, but however, the effect of each risk factor is greater in FH [2, 13]. Besides, FH specific clinical or molecular features as tendon xanthomas or receptor-negative or null mutations in the *LDLR* gene have also been reported to increase risk of CHD in FH heterozygotes. Major risk factors for CVD in heterozygous FH are presented in Table 10.2 [2, 13]. The presence or absence of these factors modifies the LDL cholesterol treatment goals in FH [13].

Extravascular Cholesterol Deposits The presence of tendon xanthomas is common in people over age 40 in FH heterozygotes and almost constant in the first decade of life in FH homozygotes. The most characteristic location of tendon xanthomas is the Achilles tendon, but they are also common in elbows and fingers (Fig. 10.5). The presence of tendon xanthomas is associated with an increased risk of premature CVD, especially in women [110], and increased risk of tendinitis. In recent years, due to the availability of effective treatments for hypercholesterolemia from youth, the prevalence of tendon xanthomas has dropped sharply. Achilles tendon sonography improves the detection of xanthomas, and facilitates the clinical diagnosis [111] (Fig. 10.6).

The corneal arcus in the first decades of life is another surface lipid deposition characteristic of FH (Fig. 10.7), which is sometimes used as a criterion in some diagnostic algorithms.

Coronary Diease-Genotype Correlations in FH Because of the large number of allelic variants (more than 1500) differently affecting LDL receptor clearance function, *LDLR* allele-specific differences may be predicted for the FH phenotype. *LDLR* mutations may be classified into different functional types: (1) receptor-negative or null alleles, which include disruptions of the promoter sequence, large rearrangements, nonsense, frameshift, or mutations resulting in a deletion of the translation initiation signal and early stop codons, which result in no protein synthesis; (2) receptor-defective alleles, that is, transcription and missense defects that do not completely suppress the function of the protein, which has residual receptor activity; and (3) undetermined receptor activity alleles, which are splicing defects with an unknown effect on protein function [27].

Different studies have analyzed [29, 112–120] whether *LDLR* mutational class affects the prevalence of CHD risk in heterozygous FH by

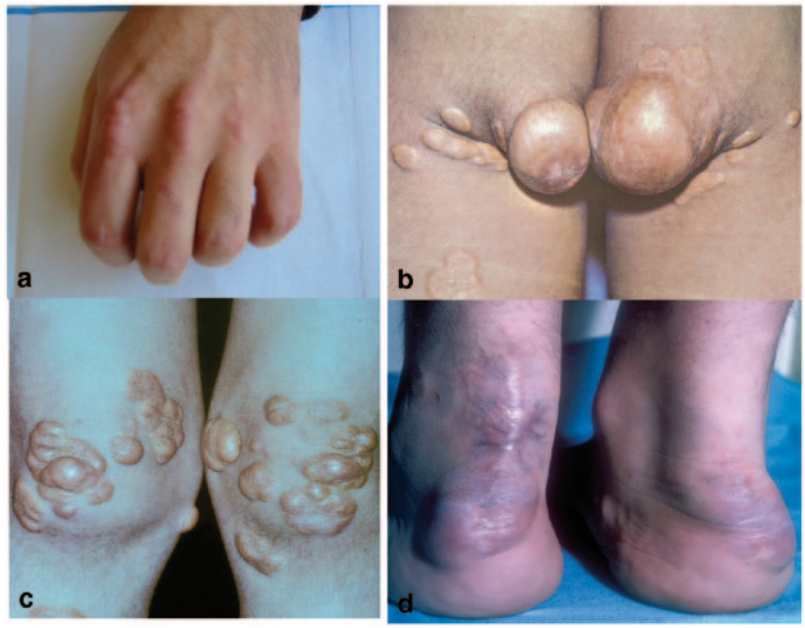

Fig. 10.5 Xanthomas in familial hypercholesteromia (FH). **a** Xanthomas on the extensor tendons of the hand in FH heterozygote. **b** and **c** tendon and tuberous xanthomas on the elbows and knees in FH homozygote. **d** Achilles tendon xanthomas in FH heterozygote. (Courtesy Prof. Francisco Carapeto)

comparing receptor-negative versus receptor-defective alleles (Table 10.3). In population with large genetic heterogeneity, most of the studies have usually found higher prevalence of xanthomas and CHD in patients with receptor-negative alleles than in those with receptor-defective alleles. However, this association was partially due to higher total and LDL cholesterol in receptor-negative subjects [119].

Low-density Lipoprotein Concentration in FH FH is defined by severely elevated serum LDL cholesterol concentrations from birth onwards. LDL cholesterol usually ranges from 200 to 400 mg/dL in heterozygous adults, and over 500 mg/dL in homozygous subjects from childhood. *LDLR* mutations usually present higher LDL cholesterol concentration than subjects with *APOB* or *PCSK9* mutations; and receptor-negative alleles higher LDL cholesterol than defective alleles. Those subjects with clinical diagnosis of FH, but without mutation in *LDLR, APOB,* or *PCSK9* tend to have lower LDL cholesterol than genetically well-defined FH, 237±49 mg/dL versus 302±69 mg/dL, respectively [4].

FH Diagnosis

An early diagnosis of ADH is extremely important since lipid-lowering drugs are highly effective, safe, and cost-effective in FH. The diagnosis of FH has been traditionally performed based on blood lipid values within a family, deposition of cholesterol in extravascular tissues such as tendon xanthomas or corneal arcus, and personal and family history of premature CHD.

Homozygous FH The diagnosis of homozygous is typically based on the presence of very high LDL cholesterol, in absence of secondary causes of hypercholesterolemia, and high LDL cholesterol in both parents. Appearance of cutaneous xanthomas, especially interdigital planar xanthomas, or tendon xanthomas prior to age 10 years is almost with high LDL cholesterol is almost pathognomonic of homozygous FH. The genetic

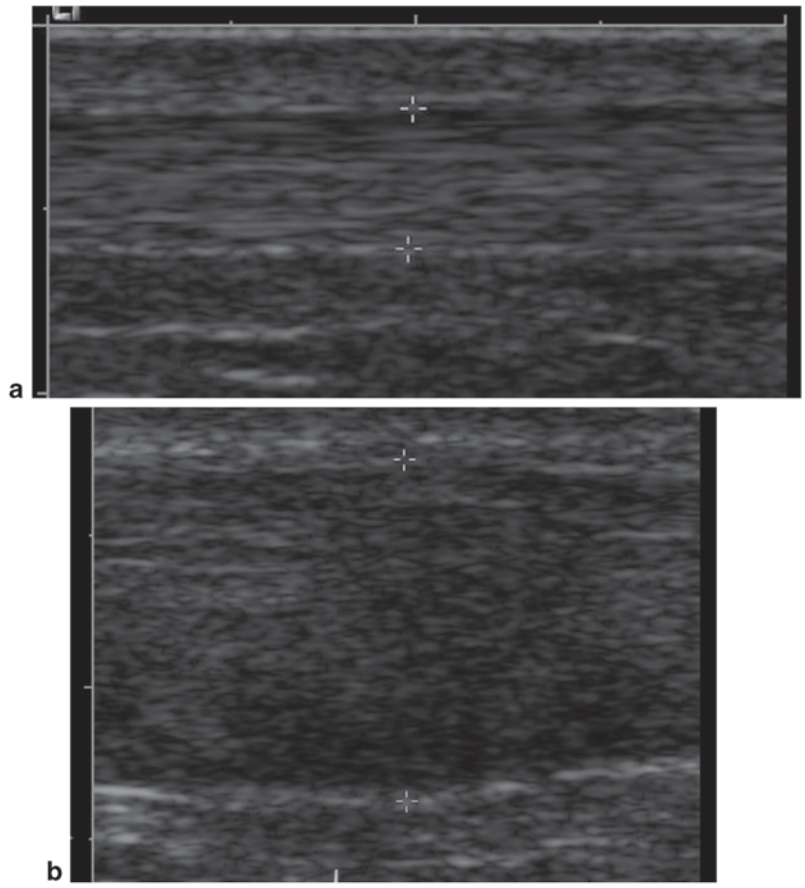

Fig. 10.6 Achilles tendon sonographic longitudinal images. **a** normal. **b** presence of tendon xanthoma. Calipers are located in the proximal and distal borders of the Achilles tendons

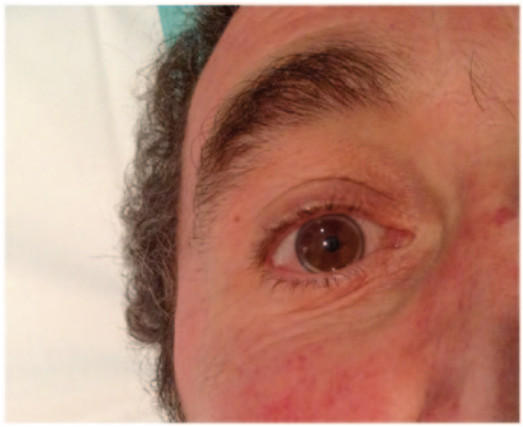

Fig. 10.7 Corneal arcus in familial hypercholesterolemia (FH)

confirmation of two mutated *LDLR, APOB, or PCSK9* alleles and the genetic diagnosis of true homozygosity, compound heterozygosity, or double heterozygosity for FH genes is highly recommended in these subjects. The most common diagnostic criteria are presented in Table 10.4 [121].

Heterozygous FH Three important diagnostic criteria have been extensively used for the clinical diagnosis of FH: The Simon Broome Register Group in the UK [122], the US Make Early Diagnosis to Prevent Early Deaths (MedPed) Program [123], and the Dutch Lipid Clinic Network [124] (Table 10.5). The accuracy of these three diag-

Table 10.3 Odds ratio (OR) for the presence of coronary heart disease (CHD) in heterozygous familial hypercholesterolemia (FH) with receptor-negative (R−) versus receptor-defective (R+) *LDLR* alleles

Country (reference)	Year	Study sample	Total cholesterol (mg/dL)	OR (95 % confidence interval)
South Africa (29)	1993	148	R−: 418 R+: 364	2.6 (0.92–7.2)
Canada (109)	1997	94	R−: 315 R+: 283	2.7 (1.03–7.24)
Italy (110)	2000	185	R−: 408 R+: 353	2.6 (1.37–4.83)
Greece (111)	2004	78	R−: 333 R+: 298	2.6 (0.44–15.4)
Spain (112)	2003	118	R−: 344 R+: 394	Not significant
The Netherlands (113)	2005	645 (children)	R−: 311 R+: 265	1.22 (0.76–1.95) parental CVD
Spain (114)	2005	181	–	3.14 (1.00–9.87)
Spain (115)	2008	811	R−: 420 R+: 411	2.09 (1.04–4.21)
Italy (116)	2013	1795	R−: 371 R+: 327	38.1 %(R−) versus 27 % (R+), $P = 0.0008$

OR odds ratio, *CVD* cardiovascular disease

Table 10.4 Diagnostic criteria for homozygous familial hypercholesterolemia (FH)

Untreated high LDL cholesterol > 500 mg/dL after exclusion of secondary causes
Plus at least one:
Genetic confirmation of two mutated *LDLR, APOB, or PCSK9* alleles
Appearance of cutaneous or tendon xanthomas prior to age 10 years
Elevated LDL cholesterol (> 200 mg/dL) and both parents consistent with heterozygous FH
Presence of functional FH mutations in both parents

LDL low-density lipoprotein

nostic methods has not been evaluated in large, independent cohorts. Tendon xanthomas are pathognomonic of FH; however, their identification is not always easy and they are considered insensitive diagnostic markers. A high variability of xanthoma presence has been reported in FH patients [125]. Besides, tendon xanthomas may appear in patients with cerebrotendinous xanthomatosis, sitosterolemia, or dysbetalipoproteinemia. Variability in the frequency of xanthomas observed in different studies depends in part on the clinical criteria used for FH (some of them included the presence of xanthomas), as well as the methods used for the identification of xanthomas.

There are not absolutely predictive clinical criteria for the diagnosis of FH, and arbitrary criteria must be used. The criteria established by the Simon Broome Register Group from the UK for FH were based on elevated total plasma cholesterol concentration greater than 7.5 mmol/L (300 mg/dL) in the proband, together with either tendon xanthomas in the proband or in a first-degree relative or the presence of premature CHD or hypercholesterolemia in a first-degree relative [122]. The US MedPed program focuses the diagnostic criteria, principally, on high LDL cholesterol levels in the individual, and on the family history of hypercholesterolemia with evidence for a dominant transmission [123]. The presence of children with hypercholesterolemia increases the diagnostic probability. The US National Lipid Association Expert Panel on FH advises that LDL cholesterol levels > 250 mg/dL in a patient aged 30 or more > 220 mg/dL for patients aged 20–29; and > 190 mg/dL in patients under age 20, should prompt the clinician to strongly consider a diagnosis of FH and obtain further family informa-

Table 10.5 Dutch Lipid Clinic Network criteria for diagnosis of heterozygous familial hypercholesterolemia (FH) in adults

Group 1: family history	Points
First-degree relative with known premature (<55 years, men; <60 years, women) coronary heart disease (CHD) OR First-degree relative with known LDL cholesterol >95th Percentile by age and gender for country	1
First-degree relative with tendon xanthoma and/or corneal arcus OR Child(ren) <18 years with LDL cholesterol >95th percentile by age and gender for country	2
Group 2: clinical history	
Subject has premature (<55 years, men; <60 years, women) CHD	2
Subject has premature (<55 years, men; <60 years, women) cerebral or peripheral vascular disease	2
Group 3: physical examination	
Tendon xanthoma	6
Corneal arcus in a person <45 years	4
Group 4: LDL cholesterol	
155–190 mg/dL (4.0–4.9 mmol/L)	1
191–250 mg/dL (5.0–6.4 mmol/L)	3
251–325 mg/dL (6.5–8.4 mmol/L)	5
>325 mg/dL (>8.4 mmol/L)	8
Group 5: molecular genetic testing (DNA analysis)	
Causative mutation shown in the *LDLR*, *APOB*, or *PCSK9* genes	8

A "heterozygous" diagnosis can be made if the subject scores >8 points. A "probable FH" diagnosis can be made if the subject scores 6–8 points. A "possible FH" diagnosis can be made if the subject scores 3–5 points
LDL low-density lipoprotein

tion [13]. With those LDL cholesterol criteria, the sensitivity is 70% while specificity is 82% for genetically defined FH [4]. The Dutch MedPed Group described a clinical scoring system for the diagnosis of heterozygous FH patients. These criteria include personal and familial LDL cholesterol levels, history of CVD (coronary, carotid, and peripheral arteries), the presence of corneal arcus before the age of 45, and tendon xanthomas. By weighing the occurrence of these clinical signs, alone or in combination with others, a diagnostic scoring table has been constructed in the Netherlands (Table 10.5). These criteria seem to be easy to use in clinical practice and include all the clinical and laboratory features for the diagnosis of FH; and they have recently been proposed as the preferred diagnostic tool by the European Atherosclerosis Society (EAS) [14].

Molecular biology techniques have dramatically improved in recent years and more and more have become highly specific tools to improve the diagnosis of many medical conditions, including FH. Furthermore, the genetic diagnosis is the preferable diagnostic method in FH in most situations because it provides an unequivocal diagnosis. The National Institute for Health and Clinical Excellence (NICE) guidelines on the identification and management of FH recommend cascade screening using a combination of genetic testing and LDL cholesterol concentration measurement [126]. This approach has also been recommended by the European Atherosclerosis Society (EAS). [14].

Several methods are currently used to identify sequence changes in *LDLR*, *APOB,* and *PCSK9* genes including direct sequencing and high-throughput FH resequencing arrays [127]. A microarray for the detection of common point mutations and small deletions in the *LDLR* and *APOB* genes has been developed by our group [117]. By providing either a positive (presence of *LDLR, PCSK9,* or *APOB* mutations) or negative (absence of defects in these genes) diagnosis, this platform has allowed the genetic characterization of >8000 Spanish patients [128]. Even though the diagnosis of FH based on the detection of a functional

Table 10.6 Indication of genetic testing in a suspected familial hypercholesterolemia (FH) proband[a]

Subject phenotype	Indication
1. Subjects with isolated high LDL cholesterol	
a. Positive personal or family history of tendon xanthomas	LDL cholesterol >160 mg/dL
b. Absence of personal and family history of tendon xanthomas	
i. Age 18–30 y	LDL cholesterol >220 mg/dL
ii. Age 30–39 y	LDL cholesterol >225 mg/dL,
iii. Age>40 y	LDL cholesterol >235 mg/dL
2. Subjects with mixed hyperlipidemia (high total cholesterol and triglycerides 200–400 mg/dL)	Total cholesterol >335 mg/dL or Apolipoprotein B >185 mg/dL

[a] Within families with clinical suspicion of FH because of vertical transmission of hypercholesterolemia and bimodal LDL cholesterol distributions in the pedigree and absence of secondary causes of hyperlipidemia (4)
LDL low-density lipoprotein

mutation on a causative gene is the recommended procedure in highly suspicious cases, it cannot be recommended for all cases of hypercholesterolemia. The genetic testing is still complex, expensive, and should be used as complementary to the clinical diagnosis. A group of criteria have been proposed to maximize the likelihood of genetic confirmation in subjects with clinical suspicion of FH based on age, tendon xanthomas presence, and LDL cholesterol levels (Table 10.6) [4].

Lipid-Lowering Therapy for Heterozygous FH

Excess CHD in FH is attributable to high LDL cholesterol in this population; consequently, LDL cholesterol reduction to normal levels is a priority in the management of FH. Long-term therapy is the only way, at this time, to substantially reduce or remove the excess lifetime risk of CHD due to their genetic disorder. A healthy lifestyle is also important in the FH treatment. Lifestyle comprises a healthy diet, ideal body weight, no smoking, and adequate physical activity [4]. A healthy lifestyle provides many benefits beyond LDL cholesterol lowering, and can increase the LDL cholesterol lowering effect of drugs. Although LDL cholesterol is the fundamental CHD risk factor in FH, these subjects are very responsive to other risk factors such as smoking, which should be carefully explored and treated.

Different medical societies and expert panels have published guidelines for the management of FH, and without exception, they highly recommend aggressive LDL cholesterol lowering in all adults and less intensive treatment in children > 10 years of age (Table 10.7).

International Panel on Management of Familial Hypercholesterolemia (2004) [2]. Promoted by the Spanish Society of Arteriosclerosis, a panel of international experts proposed the first global recommendations for the diagnosis and treatment of FH: early diagnosis of the disease, a screening strategy based on finding family affected members, CHD risk stratification in heterozygous subjects according to the presence of other risk factors, early detection of atherosclerosis in preclinical phase, the establishment of three LDL cholesterol treatment goals based on baseline risk, and a therapeutic strategy based on lifestyle and pharmacological treatment with potent statins as first choice. LDL apheresis was recommended after drug treatment when LDL cholesterol is above 200 mg/dL in the presence of coronary artery disease, or above 300 mg/dL without CHD.

NICE in the UK (2008) [126] recommends a clinical diagnosis based on the criteria of Simon Broome British Register. Interestingly, a specific target LDL cholesterol target is not recommended, instead an advice to reduce LDL cholesterol by more than 50%. Baseline risk stratification before beginning the treatment was not considered. Affected children should start drug treatment after 10 years of age.

Belgian consensus for the FH treatment in children and young adults (2011) [129] focused

Table 10.7 Summary of international guidelines for the management of familial hypercholesterolemia (FH)

Recommendation	Country	CHD risk stratification	Recommended screening	LDL cholesterol goal	Year	Reference
International Panel on Management of FH	Spain	*Yes* (Age; gender; family history; smoking; hypertension; diabetes; LDLc > 330 mg/dL; HDLc < 40 mg/dL; Lp(a) > 60 mg/dL)	Familial cascade	<160 mg/dL (low risk) <130 mg/dL (medium risk) <100 mg/dL (high risk or CHD)	2004	[2]
NICE	UK	*NO*	Familial cascade	Reduction > 50%	2008	[126]
Consensus of management of familial hypercholesterolemia in children and young adults	Belgium	*Yes*	Familial cascade	10–14 years: reduction 30% >14 years:<130 mg/dL	2011	[129]
National Lipid Association	USA	*Yes* (Age; gender; family history; smoking; hypertension; metabolic syndrome; tendon xanthomas; LDLc > 250 mg/dL; HDLc < 40 mg/dL; Lp(a) > 50 mg/dL)	LDLc>190 mg/dL Familial cascade	Reduction >50% (all) <160 mg/dL (low risk) <100 mg/dL (high risk)	2011	[130, 131]
European Atherosclerosis Society	Europe	*No*	Total chol >310 mg/dL familial cascade	<100 mg/dL <70 mg/dL (CHD, diabetes)	2013	[14]

CHD coronary heart disease, *LDLc* low-density lipoprotein cholesterol, *HDLc* high-density lipoprotein cholesterol, *NICE* National Institute for Health and Clinical Excellence, *Lp(a)* lipoprotein (a)

on the diagnosis and treatment of children. They recommend the diagnosis and diet treatment of children from 2 years of age and consider drug treatment after 10 years of age when the LDL is >190 mg/dL or >160 mg/dL in the presence of premature CHD in the family or cardiovascular risk factors. LDL cholesterol goal of treatment is to obtain >30% LDL cholesterol reduction between 10 and 14 years, and <130 mg/dL onwards.

Lipid National Association Expert Panel on Familial Hypercholesterolemia (2011) [130, 131] from the USA published a special issue of the *Journal of Clinical Lipidology* in 2011 dedicated to FH. The documents recommend preferably clinical diagnosis based on LDL cholesterol concentrations adjusted for age, and a general population screening in subjects with LDL cholesterol levels > 190 mg/dL in adulthood, or > 160 mg/dL in children. Drug treatment is recommended when LDL cholesterol is > 190 mg/dL, with different therapeutic targets depending on individual risk factors.

Consensus Statement of the European Atherosclerosis Society (2013) [14]. This document emphasizes that FH is underdiagnosed and undertreated in the general population, defines all FH heterozygotes as high-risk patients, promotes the FH screening in subjects with plasma total cholesterol ≥310 mg/dL in adults or ≥230 mg/dL in children, premature CHD in the subject or family members, presence of tendon xanthomas in the subject or family member(s), or sudden premature cardiac death in a family member. The LDL cholesterol goals for lipid-lowering treatment are <135 mg/dL for children, <100 mg/dL for adults, and <70 mg/dL for adults with known CHD or diabetes. Therefore, high doses of potent statins, combined drug regimens usually with ezetimibe or bile acid sequestrans, and, in some cases, LDL-apheresis are required to reach such exigent targets.

Despite current maximal treatment, many heterozygous FH subjects remain with undesired high LDL cholesterol concentration. PCSK9 inhibition with two different monoclonal antibodies against PCSK9 (evolucumab and alirocumab) has been studied in two double-blind, randomized, placebo-controlled trials in heterozygous FH insufficiently controlled with standard treatment [132, 133]. In both studies, the inhibition of PCSK yielded rapid and over 50% reductions in LDL cholesterol with good tolerability, so this therapeutic approach seems very promising in the future treatment of heterozygous FH.

Homozygous FH Treatment

Statins, bile acid sequestrants, and ezetimibe have lesser lipid-lowering effect in homozygous FH than in heterozygous subjects, because these drugs need some LDL receptor functionality to be fully effective. However, they are remarkably safe in homozygous FH and should be tested because in some cases LDL cholesterol reductions between 20 and 40 s% can be obtained especially in LDL receptor-defective patients by increasing residual LDL receptor activity, and by inhibition of cholesterol synthesis [134].

The current treatment of choice for homozygous FH is LDL-apheresis at weekly or biweekly intervals, usually in children over the age of 7. Most homozygous FH obtain substantial reductions in LDL cholesterol, usually >50%, with periodical LDL-apheresis and is the only treatment that substantially lowers Lp(a) in these patients [121].

New drugs have been recently tested for the treatment of homozygous FH: mipomersen, lomitapide, and PCSK9 inhibitors. Mipomersen is an antisense apo B-100 mRNA recently approved for the treatment of homozygous FH. It is administered subcutaneously as a weekly injection. In a randomized, double-blind, placebo-controlled study with 34 patients, the mean LDL cholesterol reduction with this inhibitor of the apo B synthesis was 25%, although the lipid-lowering response was highly variable among individuals [135].

Lomitapide is an inhibitor of the microsomal triglyceride transfer protein (MTTP), a key protein in the assembly of apo B-containing lipoproteins in the liver and intestine. It is a highly potent lipid-lowering drug recently approved for the homozygous FH treatment in the USA and

Europe. In a single-arm, open-label study with homozygous FH, aged 18 years or older with a median dose of 40 mg a day, lomitapide reduced LDL cholesterol by 50% after 26 weeks of treatment. Lomitapide's adverse effects included accumulation of hepatic fat. Mean hepatic fat was 1.0% at baseline and increased to 8.6 and 8.3% at week 56 and at week 78, respectively. The long-term consequence of this accumulation is unknown which seems to stabilize, or even decrease, with time [136].

PCSK9 inhibition has also been evaluated in homozygous FH. Alirocumab (AMG 145) a monoclonal antibody to PCSK9 demonstrates reductions of 19.2% in LDL cholesterol in patients with defective LDL receptor activity, but no efficacy in those who were receptor negative [137].

The prognosis of intensive treated FH subjects has drastically improved in recent years reducing the CHD [138]. The key challenge in the coming years is to expand the diagnosis and treatment to this group of patients in whom cardiovascular prevention is paradigmatic and cost-effective [139].

Autosomal Recessive Hypercholesterolemia

In 1973, a Lebanese family with an autosomal recessive form of severe hypercholesterolemia, clinically indistinguishable from FH, was described by Khachadurian et al. [140]. Afterwards, subjects with similar phenotype were identified in Sardinia [141], in subjects of Turkish and Asian-Indian origin [142], and in Japan [143], and this entity was named ARH. The causative gene, *LDLRAP1,* on chromosome 1, which encodes LDLRAP1, was identified by linkage analysis in 2001 by Garcia et al. [46]. In this gene, both homozygous and compound heterozygous mutations can be found. Most of the ARH-causing mutations are due to premature stop codons, producing no mRNA or truncated proteins [46]. The mutations identified in *LDLRAP1* gene causing ARH can be found in http://www.ucl.ac.uk/ldlr/.

LDLRAP1 is a 32-kDa and 308-amino-acid endocytic adaptor protein required for the function of LDLR in hepatocytes. LDLRAP1 protein serves as an adaptor for LDL receptor endocytosis in the liver and a deficiency in this protein results in a decrease in the LDL cholesterol catabolism [144]. The N-terminal domain of LDLRAP1 contains a phosphotyrosine-binding (PTB) domain, which binds to the internalization sequence (FDNPVY) in the cytoplasmic tail of the LDL receptor. This domain can also simultaneously interact with cell membrane phosphoinositides. Specific sequences within the C-terminal region of LDLRAP1 bind clathrin and its adaptor AP2 [145]. All these interactions together enable LDLRAP1 to function as an endocytic adaptor for the clathrin-mediated endocytosis of LDL receptor in the liver. Accordingly, LDLRAP1 has been classified as a clathrin-associated sorting protein (CLASP) [146], a group of proteins that serve as a molecular bridge between receptors and the clathrin machinery for their endocytic internalization.

ARH subjects have severely elevated plasma LDL cholesterol, tuberous and tendon xanthomata, corneal arcus, and premature atherosclerosis, with severe CHD, that make it clinically indistinguishable from FH [147]. Plasma cholesterol levels and clinical symptoms that present subjects with ARH are intermediate between those of FH heterozygotes and FH homozygotes. The age of onset of symptomatic coronary artery disease in these patients is later and tendon xanthomas tend to be large and bulky [148]. In a phenotypic comparison study between 42 ARH subjects and 42 homozygous FH subjects, Pisciotta et al. [149] reported that in ARH subjects, plasma LDL cholesterol (550 ± 88.6 mg/dL) was lower than in receptor-negative homozygous FH (827 ± 138 mg/dL) but similar to that found in receptor-defective homozygous FH (601 ± 92.5 mg/dL). The risk of coronary artery disease was ninefold lower in ARH patients [150].

However, LDL receptor activity and LDL binding ability in cultured fibroblasts are normal. All *LDLRAP1* mutations characterized to date preclude the synthesis of full-length LDLRAP1, and this LDLRAP1 is required for normal LDLR function in lymphocytes and hepatocytes, but not in fibroblasts. Residual LDL receptor function in cells that do not require LDLRAP1 could explain

the reason why ARH subjects have lower plasma LDL cholesterol levels than homozygous FH patients, who have no functional LDL receptor [150].

LDL turnover studies have demonstrated that the rate of clearance of LDL from plasma is similar in subjects with ARH and in subjects with homozygous FH, and markedly reduced compared with normolipidemic controls [143], suggesting that LDLRAP1 is essential for LDL receptor-mediated uptake of LDL. Despite comparable reductions in the fractional catabolic rate of LDL, the metabolic and clinical phenotype of ARH is less severe than that of homozygous FH, as stated above [151]. ARH patients also respond to lipid-lowering drugs, as statins, with a greater reduction in plasma levels of LDL cholesterol than that observed in homozygous FH patients [152]. As the clearance rates of LDL are similarly decreased in ARH and FH, the less severe clinical phenotype in ARH points to LDL production. LDL is produced as a metabolic product of VLDL, and it has been proposed that the molecular basis for this milder phenotype is the increased removal of VLDL remnants from the circulation [153]. The increased clearance of remnant lipoproteins could contribute to the great responsiveness to statins of ARH patients [154]. Also, studies demonstrate that clearance of postprandial remnant lipoproteins is preserved in ARH in contrast to FH. This preservation of postprandial remnant particles catabolism could also contribute to the mild phenotype of ARH compared with FH [155].

Lysosomal Acid Lipase Deficiency

Deficiency of the enzyme lysosomal acid lipase results in two distinct diseases in humans: Wolman disease and cholesteryl ester storage disease (CESD;OMIM 278000). Wolman disease is a severe lipid infiltration of the liver, spleen, and other organs early in life, causing early death in infants. Wolman disease is very rare, with an incidence of less than one in 100,000 live births. CESD is also a rare disease, around one case in 40,000 people, possibly underdiagnosed, and characterized by the accumulation of cholesterol esters in different organs of the body, especially liver. Clinically, CESD presents as a mixed hyperlipidemia in a young patient with no family history of hyperlipidemia, with hepatosplenomegaly and elevated liver enzymes. Ultrasound typically shows steatosis, but the diagnosis is usually suspected by finding a microvesicular steatosis on liver biopsy. A definitive diagnosis is made by detecting a low lysosomal acid lipase activity, or in the presence of functional mutations in the gene encoding this enzyme, LIPA [156].

The accumulation of cholesterol esters and free cholesterol reduction in the liver of these subjects leads to increased endogenous cholesterol production, and this is the believed mechanism. Evolution of these patients is to chronic liver disease, liver cirrhosis, and increased incidence of atheromatous disease [157]. Recombinant lysosomal acid lipase replacement was shown to be effective in animal models, and recently, a phase I/II clinical trial demonstrated its safety and indicated its potential metabolic efficacy [158].

Cholesterol-7-Alpha-Hydroxylase Deficiency (OMIM 118455)

Pullinger et al. described in 2002 that mutations in the *CYP7A1* gene, encoding the enzyme cholesterol-7-alpha-hydroxylase produced hypercholesterolemia associated with heterozygosity and homozygosity, so it is considered an autosomal codominant hypercholesterolemia OMIM 118455 [159]. The enzyme cholesterol-7-alpha-hydroxylase controls the rate of conversion of cholesterol into bile acids. CYP7A1 deficiency would cause a decrease in the production of bile acids and cholesterol accumulation in the liver, causing a decrease in the expression of LDL receptors, and therefore, an increase in plasma concentration of LDL. In these patients, as liver cholesterol content is increased, statins have the desired effect of inducing the expression of LDL receptors [159].

Summary

MHs are the most frequent inherited metabolic diseases and common causes of premature cardiovascular death and disability in most countries. They are genetically heterogeneous in which similar phenotypes may be caused by mutations in different genes, commonly *LDLR, APOB,* and *PCSK9*. However, there are some MH families in whom the responsible gene/s are unknown. Very high LDL cholesterol, familial presentation, and high prevalence of premature coronary disease are the clinical features to suspect MH. Early diagnosis of MH is very important so that therapy can be initiated as soon as possible. Combination of clinical and genetic test is the preferable diagnostic method. Familial cascade screening, once an index patient is diagnosed, is mandatory. MHs are frequently underdiagnosed and undertreated, so there is a need for a much better diagnostic screening worldwide. High doses of potent statins, combination therapy of statins with ezetimibe, or bile acid sequestrants, and, in some cases, LDL-apheresis are required to reach LDL cholesterol goals. PCSK9, apo B, and MTTP inhibitors are novel and very promising drugs that can substantially improve the treatment in the highest risk patients.

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Primary Hypertriglyceridemia

11

Amanda Brahm and Robert A. Hegele

Introduction

Hypertriglyceridemia (HTG) is often defined by plasma triglyceride (TG) concentration >95th percentile for age and sex. Patients with HTG frequently have concomitant comorbidities such as poor diet, alcohol use, obesity, metabolic syndrome, and type 2 diabetes [1–3]. HTG can be further classified as being of either primary type, in which there is an identified or presumed familial or molecular genetic basis for the condition, or secondary type, in which one of several secondary factors contributes to disease expression [1, 3]. Genetic factors can influence the severity of the plasma TG elevation in the presence of a secondary factor [4]. This chapter focuses on primary HTG, both the rare monogenic and common polygenic forms of HTG, in addition to clinical considerations and treatment.

"Familial" Does Not Mean Monogenic

An important concept that has emerged in the past few years is that while most cases of primary HTG are familial in nature, only a minority is truly monogenic (typically autosomal recessive) [3–6]. In the pregenomic era, primary HTG disorders were presumed mostly to be monogenic, by analogy with and extrapolation from other archetypal monogenic lipid disorders, such as familial hypercholesterolemia (FH). But while FH results from single strong-effect mutations in genes that perturb low-density lipoprotein (LDL) receptor function and show cosegregation with high LDL cholesterol concentrations in families, most cases of "familial" HTG are polygenic rather than monogenic disorders [2–6]. While HTG clusters in families, it usually does not follow classical Mendelian patterns of inheritance, and inconsistently shows vertical transmission in family pedigrees. But despite this, the idea that most HTG states are monogenic has persisted in the literature and textbooks over decades, likely because the term "familial" is included in the names of several classical primary HTG disorders. However, it is generally incorrect to conflate a "familial" disorder with a "monogenic" disorder: While many cases of HTG are familial, they are usually not monogenic [4–6].

Clinical Diagnosis of HTG

HTG is usually diagnosed when fasting plasma TG concentration exceeds a threshold value, such as the 95th percentile when adjusted for age and sex. The 95th percentile for TG corresponds to ~3.0–3.4 mmol/L (~250–300 mg/dL) for most North American adults. Severe HTG is

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Table 11.1 Hypertriglyceridemia (HTG): proposed clinical definitions

General definition (ref. 1)		ATP guidelines (ref. 2)		Endocrine society (ref. 3)	
Category	Serum TG (mmol/L)	Category	Serum TG (mmol/L)	Category	Serum TG (mmol/L)
Normal	<3	Normal	<1.7	Normal	<1.7
Hypertriglyceridemia	>3–3.4 (>95th percentile)	Borderline high	1.7–2.3	Mild	1.7–2.3
				Moderate	2.3–11.2
Severe hypertriglyceridemia	>10	High	2.3–5.6	Severe	11.2–22.4
		Very high	>5.6	Very severe	>22.4

TG triglyceride, *ATP* Adult Treatment Panel III of the National Cholesterol Education Program

Table 11.2 Types of hypertriglyceridemia (HTG)

Name	Primary lipoprotein abnormality	Lipid profile	Population prevalence
Familial chylomicronemia (formerly HLP type 1)	Elevated chylomicrons	↑↑↑TG ↑TC	1 in 1 million
Combined hyperlipidemia (formerly HLP type 2B)	Elevated VLDL, elevated LDL	↑↑TG ↑↑TC	1 in 40
Dysbetalipoproteinemia (formerly HLP type 3)	Elevated IDL, elevated chylomicron remnants	↑↑TG ↑↑TC	1 in 10,000
Primary simple hypertriglyceridemia (formerly HLP type 4)	Elevated VLDL	↑↑TG ↑TC	1 in 20
Primary mixed hyperlipidemia (formerly HLP type 5)	Elevated chylomicrons, elevated VLDL	↑↑↑TG ↑↑↑TC	1 in 600

Abbreviations: as in Table 11.1 plus
HLP hyperlipoproteinemia, *TC* total cholesterol, *VLDL* very low-density lipoprotein, *LDL* low-density lipoprotein, *IDL* intermediate density lipoprotein, *TG* triglyceride

often defined when fasting plasma TG concentration >10 mmol/L (>900 mg/dL) [1–3]. Proposed definitions vary however (Table 11.1). For instance, the Adult Treatment Panel III guidelines of the National Cholesterol Education Program has suggested a classification system with four discrete categories: normal fasting TG is <1.7 mmol/L (<150 mg/dL), borderline high TG is 1.7–2.3 mmol/L (150–199 mg/dL), high TG is 2.3–5.6 mmol/L (200–499 mg/dL), and very high TG is >5.6 mmol/L (>500 mg/dL) [2]. The Endocrine Society has proposed another system with five clinical strata: normal TG is <1.7 mmol/L (<150 mg/dL), mild HTG is 1.7–2.3 mmol/L (150–199 mg/dL), moderate HTG is 2.3–11.2 mmol/L (200–999 mg/dL), severe HTG is 11.2–22.4 mmol/L (1000–1999 mg/dL), and very severe HTG is >22.4 mmol/L (>2000 mg/dL) [3]. Other schemes have been proposed, but no scheme predominates in clinical use.

Classification of HTG Phenotypes

Phenotypic heterogeneity among HTG patients is defined by qualitative and quantitative biochemical differences in plasma lipoproteins. In the pregenomic era, a commonly used classification scheme—the Fredrickson or World Health Organization (WHO) International Classification of Diseases (ICD) hyperlipoproteinemia (HLP) phenotypes—was based on patterns of lipoprotein fractions (summarized in Table 11.2). Five of the six WHO ICD phenotypes include HTG in their definitions [7, 8]. The exception is FH (HLP type 2A), which most often results from mutations in *LDLR* encoding the LDL receptor [8]. The HLP phenotypes defined by HTG include one monogenic pediatric phenotype called familial chylomicronemia (HLP type 1), and four polygenic "familial" phenotypes, called combined hyperlipidemia (HLP type 2B), dysbetali-

poproteinemia (HLP type 3), simple HTG (HLP type 4), and mixed hyperlipidemia (HLP type 5).

Sub-phenotypes of HTG are defined by the specific class or classes of TG-rich lipoprotein particles that accumulate in plasma, including chylomicrons, very-low density lipoprotein (VLDL), or intermediate-density lipoprotein (IDL) [8]. Frequently, the excess of TG-rich lipoproteins coexists with other lipoprotein disturbances. For instance, HLP type 4 is characterized by elevated VLDL concentrations in isolation. HLP type 5 is characterized by elevations in both chylomicron and VLDL concentrations. HLP type 3 is characterized by elevated IDL concentrations. Finally, HLP type 2B is characterized by elevated VLDL and LDL concentrations. Decreased high-density lipoprotein (HDL) cholesterol is very commonly seen in patients with all types of HTG. Implicit in this classification system was the idea that the differences between the HTG-associated phenotypes were due to differences at the molecular genetic level [8], however recent data suggest that this is often not the case [4–8]. We believe that continued use of this traditional nomenclature, while familiar to older clinicians, may be retrogressive. We endeavor in this chapter to refer to this terminology as "formerly known as," where possible.

Secondary Factors Contributing to HTG

Secondary factors that are associated with HTG are discussed in depth elsewhere [3], but include: obesity, metabolic syndrome (where TG concentration ≥ 1.7 mmol/L [≥ 150 mg/dL] is part of the diagnosis), diet with high-positive energy-intake balance and high fat or high glycemic index, alcohol consumption, diabetes (particularly type 2), renal disease (particularly uremia or glomerulonephritis), pregnancy (particularly in the third trimester), autoimmune disorders such as paraproteinemia or systemic lupus erythematosus, and several types of medications, including corticosteroids, oral estrogen, tamoxifen, thiazides, non-cardioselective beta-blockers, bile acid sequestrants, cyclophosphamide, antiretroviral regimens, phenothiazines, and second-generation antipsychotic agents.

Monogenic HTG: Familial Chylomicronemia (Formerly Known as HLP Type 1)

As mentioned above, only one type of HTG is truly monogenic, namely familial chylomicronemia, also known at chylomicronemia syndrome or HLP type 1, which is characterized by the pathological presence of chylomicrons in the blood after a fasting period of 12–14 h [1–3].

Epidemiology Familial chylomicronemia is an extremely rare disorder with an estimated overall prevalence in the population of approximately one in 1 million [1–3].

Clinical Features Familial chylomicronemia usually presents during infancy or childhood, and generally by adolescence [1, 9, 10]. Clinical features include failure to thrive, eruptive xanthomas over extensor surfaces and buttocks, lipemia retinalis, hepatosplenomegaly, recurrent abdominal pain with or without nausea and vomiting, and a strong predisposition for recurrent pancreatitis [9, 10]. Other rarer presentations that may be seen especially include intestinal bleeding, pallor, anemia, irritability, diarrhea, seizures, and encephalopathy; the underlying mechanisms for these uncommon associated symptoms are often unclear [9–11].

Xanthomas are characterized by raised crops of small yellowish papules surrounded by erythematous halos that appear most commonly on extensor surfaces of the extremities, the buttocks and the shoulders [12] (Fig. 11.1). Xanthomas tend to erupt concomitant with severe elevations in plasma TG levels, and gradually disappear over weeks to months as TG levels improve [13]. Microscopic examination of scrapings from xanthomas reveal the presence of lipid-containing macrophages or foam cells within the superficial reticular dermis, as well as infiltration with lymphocytes and neutrophils [12] (Fig. 11.1). The pathophysiology of xanthomas is thought

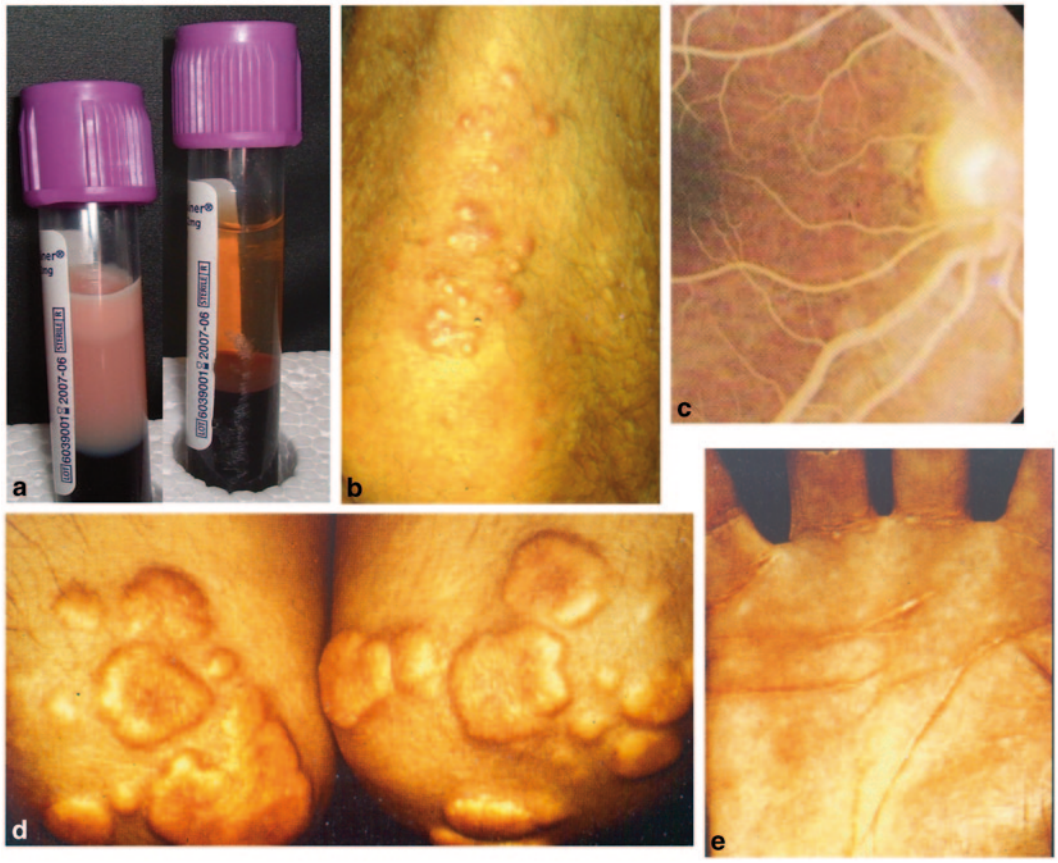

Fig. 11.1 Clinical manifestations of primary hypertriglyceridemia (HTG). **a** Lipemic plasma. Whole blood has been allowed to stand at 4 °C overnight. The sample on the *left* comes from a patient with fasting total cholesterol and triglyceride (*TG*) of 14.2 and 41.8 mmol/L, respectively. The sample on the *right* comes from a normolipidemic subject. **b** Eruptive cutaneous xanthomas. Skin lesions filled with foam cells that appear as *yellow*, morbiliform eruptions between 2 and 5 mm in diameter often with erythematous areolae, which are most often associated with markedly elevated plasma chylomicrons in familial chylomicronemia (HLP type 1) or primary mixed dyslipidemia (HLP type 5) and usually occur in clusters on the trunk, buttock, or extremities. **c** Lipemia retinalis. A *milky* appearance of the retinal vessels and *pink* retina can be seen when plasma TG >35 mmol/L. **d** Tuberous xanthomas. Skin lesions filled with foam cells that appear as *reddish* or *orange*, and often shiny nodules up to 3 cm in diameter, which are usually moveable and nontender, usually on extensor surfaces, and are found in patients with familial dysbetalipoproteinemia (FDL; HLP type 3). **e** Palmar crease xanthomas. Skin lesions filled with foam cells that appear as *yellowish*, deposits within palmar creases, and are pathognomonic for FDL. (Figure from [1])

to be due to deposition of a large amount of lipid of chylomicron origin in the tissue, which overwhelms the clearance capacity of the macrophages, resulting in lipid accumulation [12]. This free lipid acts as a catalyst for the inflammation cascade that leads to the development of the eruptive xanthomas often seen in familial chylomicronemia patients [12].

Lipemia retinalis is the term used to describe retinal vessels that appear whitish-pink on fundoscopic (Fig. 11.1) examination due to the presence of chylomicron-rich serum [13]. This condition is a physical sign only and does not affect vision [13]. Hepatosplenomegaly is rapidly reversible with correction of serum TG levels [13].

Patients with familial chylomicronemia are at lifelong risk of developing recurrent acute pancreatitis [14]. This risk increases when TG >10 mmol/L (>900 mg/dL) and is greatest with TG levels >20 mmol/L (>1800 mg/dL) [15].

Pancreatitis is often serious and can be fatal. Besides the acute abdominal discomfort, severe chronic complications include the development of chronic pancreatitis, pancreatic insufficiency, pancreatic necrosis, pancreatic abscess, or pancreatic pseudocyst [10, 13]. The pathophysiology underlying HTG-induced pancreatitis is not entirely understood but is thought to be due to increased activity of pancreatic lipase-mediated hydrolysis of circulating or infiltrating TG into their component fatty acids in the pancreas [16]. These unbound fatty acids are thought to be toxic to the pancreatic acinar cells, leading to the premature activation of trypsinogen and autodigestion injury of the surrounding pancreatic tissue [16]. Increased levels of chylomicrons themselves are also thought to worsen the pathophysiology by causing capillary plugging and local ischemia [16].

Cardiovascular disease (CVD) risk is inconsistently associated with familial chylomicronemia. Earlier observations suggested that younger patients with chylomicronemia are less prone to CVD than are patients with other lipid disorders [17, 18]. Likewise, autopsy studies on this patient population failed to show any significant burden of atherosclerosis [18], presumably because chylomicrons are too large to penetrate the endothelial surface [18]. In addition, LDL cholesterol concentrations are lower than normal in patients with familial chylomicronemia [18].

Small prospective case studies have suggested that some patients with familial chylomicronemia can develop premature atherosclerosis, despite LDL cholesterol concentrations <1.6 mmol/L [18]. This was thought to be due to a pro-atherogenic effect of some smaller subspecies of chylomicron remnants, particularly after modifications such as oxidation [18]. HDL cholesterol also tends to be very low in these patients, which could impair reverse cholesterol transport and other potential benefits of HDL [18]. It has also been proposed that, irrespective of its catalytic activity, lipoprotein lipase (LPL) itself may act to retain LDL and VLDL in the arterial intima, promote their adherence to the extracellular matrix and enhance macrophage uptake of lipoproteins and the development of foam cells [18]. It has also been proposed that these functions of LPL may be preserved even in patients with deficient LPL hydrolysis, as long as the size of the molecule remains relatively intact, as is the case for many patients with familial chylomicronemia [18]. However, the controversy regarding the risk of atherosclerosis in familial chylomicronemia has not yet been definitively resolved.

Laboratory Features Plasma drawn from individuals with familial chylomicronemia appears turbid (lipemic) and milky (Fig. 11.1) [10]. If left to settle and refrigerated overnight, it will develop a creamy supernatant above a clear infranatant [1–3]. Fasting serum TG is generally >10 mmol/L (>900 mg/dL), and sometimes can exceed 100 mmol/L (9000 mg/dL) [19]. Concomitant lipid abnormalities include a modest elevation in serum total cholesterol, and decreases in LDL and HDL cholesterol [1–3].

Molecular Basis Mutations in five different genes cause familial chylomicronemia (Table 11.3), of which, by far, the most common is *LPL* encoding LPL. In earlier times, a diagnosis of LPL deficiency was established biochemically by the absence of LPL activity in plasma collected after intravenous heparin injection [19, 20]. Presently, the diagnosis is made more commonly by DNA sequence analysis showing the presence of mutations on both *LPL* alleles leading to complete LPL deficiency [18]. Reported mutations in LPL associated with severe HTG are shown in Fig. 11.2. LPL normally hydrolyses TG transported in TG-rich lipoproteins to liberate free fatty acids for TG resynthesis and storage in adipose tissue or beta-oxidation in skeletal muscle and heart [19, 20]. In total, >118 homozygous or compound heterozygous *LPL* mutations have been shown to cause LPL deficiency in patients [20] (Fig. 11.2).

Interestingly, the four other genes associated with monogenic HTG all play a role in activity, assembly or transport of LPL (see Table 11.3). Apo C-II is an essential LPL coactivator absolutely required for TG-rich lipoprotein hydrolysis [4–6, 21], thus homozygous mutations in *APOC2* cause apo C-II deficiency and monogenic HTG

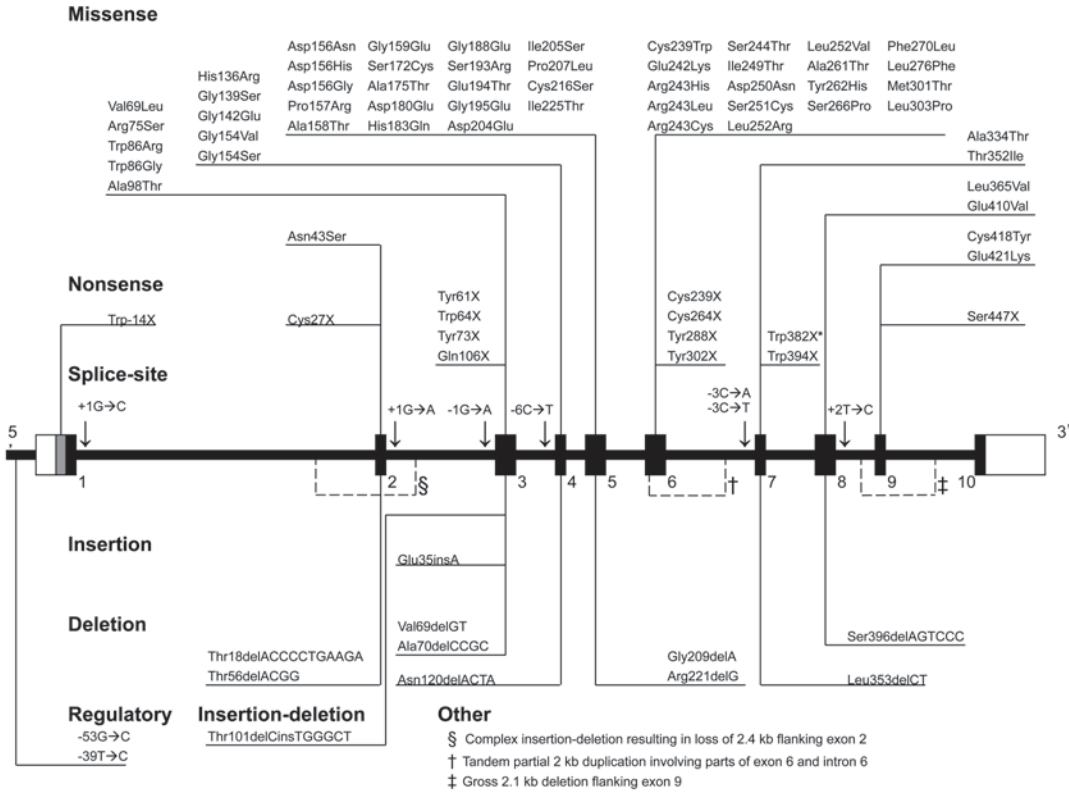

Fig. 11.2 Lipoprotein lipase (LPL) deficiency-causing mutations in the LPL gene. *Black boxes* denote exons, *gray box* denotes 27 codon signal sequence, and *white boxes* denote untranslated regions. (Figure from [20])

Table 11.3 Genes associated with familial chylomicronemia

Gene	Disease frequency	TG levels (mmol/L)	Onset	Genetic inheritance	Molecular basis
Lipoprotein lipase (*LPL*)	1 in 1 million (95% of cases)	>40–120	Infancy or childhood	Autosomal recessive	Severely reduced or absent LPL enzyme activity
Apolipoprotein C-II (*APOC2*)	<20 families described	>40–120	Adolescence to adulthood	Autosomal recessive	Absent or non-functional apo C-II, a key cofactor for LPL
Glycosyl-phosphatidyl-inositol-anchored HDL-binding protein (*GPIHBP1*)	<15 families described	<155	Infancy to Late adulthood	Autosomal recessive	Absent or deficiency in GPIHBP1, a CM anchoring protein and facilitator of LPL activity
Apo A-V (*APOA5*)	<5 families described	<130	Late adulthood	Autosomal recessive	Absent or defective Apo A-V, and facilitator of LPL activity
Lipase maturation factor-1 (*LMF1*)	<5 families described	<130	Late adulthood	Autosomal recessive	Defective or absent LMF1, a chaperone protein for LPL

Abbreviations: as in Tables 11.1 and 11.2

Table 11.4 Treatment strategies for hypertriglyceridemic states

Condition	Lifestyle modifications	Risk factor control	Medications	Experimental/ Other
Familial chylomicronemia	Low-fat diet (<20–50 g/d)	Avoid alcohol, obesity, exogenous estrogens, and steroids	Fibrates may be helpful in patients with partial LPL deficiency	Plasmapheresis for pancreatitis treatment and prophylaxis
		Improved control of diabetes, hypothyroidism		Gene therapy with virus-recombinant LPL
Dysbetalipoproteinemia	Weight loss	Improved control of diabetes, hypothyroidism	Fibrates, Statins, Niacin and omega 3 fatty acids may have some utility	
	Reduced fat diet			
Simple primary hypertriglyceridemia	Appropriate caloric intake	Improved control of diabetes	Fibrates	
	Decreased fat/ saturated fat intake	Improved control of cardiovascular risk factors (hypertension, obesity, smoking)	Niacin	
	Reduced carbohydrate intake		Omega-3 fish oils	
	Increased physical activity			
Mixed hyperlipidemia	Dietary fat restriction	Reduce alcohol intake	Fibrates	
	Weight control	Improved control of diabetes, hypothyroidism	Niacin	
			Omega- 3 fish oils	
			(Statins)	

LPL lipoprotein lipase

[4–6, 21]. *APOC2* mutations (defined at the amino acid level) were the first human mutations reported in patients with any dyslipidemia [21]. Apo A-V is also required for efficient lipolysis of TG-rich particles by LPL [22], although its precise mechanism of action is unknown. Homozygous mutations in *APOA5* causing apo A-V deficiency cause severe HTG [22]. Homozygous mutations in genes that are required for efficient assembly and transport of LPL, including *GPIHBP1* [23] and *LMF1* [24], were also recently shown to cause monogenic HTG.

Treatment Strategies The treatment of patients with severe HTG due to familial chylomicronemia follows the general principles for treating HTG outlined below, including dietary and lifestyle interventions, control of secondary factors, and pharmacological therapies (Tables 11.4 and 11.5). Unfortunately, current pharmacologic therapies that are effective for milder HTG states are less effective for familial chylomicronemia [1, 15]. In addition, because of the severe elevation of HTG and imminent risk of pancreatitis, further special treatment is indicated for patients with familial chylomicronemia, starting with significant fat restriction.

The current recommended targets for dietary management of familial hyperchylomicronemia are variable and range from the most liberal advice of <50 g of dietary fat intake per day, or <25% of daily caloric intake, to <20 g per day, or <10% of total daily caloric intake [1–3]. Unfortunately, these extreme dietary restrictions are usually difficult for patients to follow, and consequently success has been variable. Avoidance of triggers or causes of secondary HTG is also of utmost importance in these pa-

Table 11.5 Medications for hypertriglyceridemia (HTG)

Medication	Effect on lipid profile	Mechanism of action	Side effects
Fibrates	↓TG 30–50%	Increase LPL activity and synthesis	GI intolerance
	↑HDL cholesterol by up to 20%	Decrease hepatic VLDL production through PPAR-α	Increase risk of cholesterol gallstones
	Variable LDL effects		Interaction with statins
Niacin	↓TG 10–30%	Decrease fatty acid flow to liver and VLDL production	Flushing/lightheadedness/ Pruritis
	↑HDL 10–40%	Increase LPL activity	Worsen glucose intolerance
	↓LDL 5–20%		Can cause hyperuricemia and worsen gout
Omega 3 fish oils	↓TG 20–50% ↑HDL 5%	Reduced hepatic TG synthesis	Fishy taste, burping

TG triglyceride, *HDL* high-density lipoprotein, *VLDL* very low-density lipoprotein, *GI* gastrointestinal, *PPAR*-α peroxisome proliferator-activated receptor-alpha, *LPL* lipoprotein lipase

tients. These include alcohol, obesity, exogenous estrogens, and certain other medications such as corticosteroids and retinoids [1]. Pregnancy, hypothyroidism, diabetes, and chronic renal failure are also conditions which can worsen HTG and put patients at greater risk of developing pancreatitis [1].

Case reports suggest that plasmapheresis and direct removal of serum TG from patients experiencing acute pancreatitis may be of some clinical utility [25]. While the procedure seems to be very well tolerated with no major complications reported [25], it is expensive and requires specialized equipment and knowledgeable staff [25]. Further, in our experience, patients with severe chylomicronemia who are treated in hospital with cessation of all oral intake of calories and fluid replacement show just as rapid improvement in their plasma TG levels (reduction by half every 48–72 h) as patients who are treated with plasma exchange or plasmapheresis.

Finally, recent efforts have focused on the potential of gene therapy as a long-term cure for familial chylomicronemia patients. Expression of a virus-recombinant human gain-of-function LPL mutant S447X has shown promise in restoring LPL function in murine models [26]. Early clinical trials in human subjects using intramuscular injections of recombinant LPL were successful at inducing local LPL expression and resulted in a transient reduction in plasma TG levels and reduced incidence of pancreatitis [27]. This treatment (trade name Glybera) was recently approved by the European Medicines Agency for the treatment of HLP type 1 due to LPL deficiency [28].

Polygenic HTG: Common Genetic Basis for Complex HTG Phenotypes

Molecular genetic studies in our lipid clinic patients suggest that HLP types formerly known as 2B, 3, 4, and 5 all have a similar multigenic or polygenic background. Polygenic HTG has a complex genetic etiology consisting of common small effect variants and rare heterozygous large-effect variants in genes associated with plasma TG concentration [4–7]. We suggest that the disorder formerly known as HLP type 4 is the foundational HTG phenotype, and it results from the accumulation of both common and rare genetic variants that contribute to susceptibility to raised TG levels. Patients with the clinically more severe HLP type 5 have the same genetic predisposition as HLP type 4, with an additional burden of alleles or additional secondary or metabolic stress. Most patients with HLP type 3 are essentially HLP type 4 patients with the overlaid contribution of one additional genetic variant, namely the *APOE* E2/E2 genotype [8]. Finally, the overlay of common LDL-associated alleles on polygenic HTG susceptibility pushes the clinical phenotype in the direction of HLP type 2B [7, 8].

Table 11.6 Top ten common DNA polymorphisms associated with hypertriglyceridemia (HTG)

CHR	Gene	SNP	Risk allele	OR (95% CI)	P-value
11	*APOA5*	rs964184	G	3.43 (2.72–4.31)	1.12×10^{-25}
2	*GCKR*	rs1260326	T	1.64 (1.36–1.97)	1.97×10^{-7}
8	*LPL*	rs12678919	A	2.21 (1.52–3.22)	3.5×10^{-5}
8	*TRIB1*	rs2954029	A	1.50 (1.24–1.81)	3.8×10^{-5}
1	*ANGPTL3*	rs2131925	T	1.51 (1.23–1.85)	1.0×10^{-4}
7	*MLXIPL*	rs7811265	A	1.63 (1.25–2.13)	3.3×10^{-4}
4	*KLHL8*	rs442177	T	1.36 (1.13–1.64)	1.5×10^{-3}
10	*CYP26A1*	rs2068888	G	1.29 (1.08–1.55)	5.9×10^{-3}
19	*CILP2*	rs10401969	T	1.72 (1.16–2.54)	6.8×10^{-3}
2	*APOB*	rs1042034	T	1.28 (1.02–1.61)	0.032

CHR chromosome, *SNP* single nucleotide polymorphism, *OR* odds ratio for hypertriglyceridemia per risk allele, *CI* confidence interval, *APOA5* gene encoding apolipoprotein A-V, *LPL* gene encoding lipoprotein lipase, *TRIB1* gene encoding Tribbles homolog 1, *ANGPTL3* gene encoding angiopoietin-like protein 3, *MLXIPL* gene encoding MLX interacting protein-like 1, *KLHL8* gene encoding Kelch like protein 8, *CYP26A1* gene encoding cytochrome P450 26A1, *CILP2* gene encoding cartilage intermediate layer protein 2, *APOB* gene encoding apolipoprotein B (data from reference 31)

The gold-standard panel of replicable small-effect common variants that raise plasma TG levels are the 32 TG-associated loci from the Global Lipids Genetics Consortium [28]. The largest effects among these are at the *APOA5, LPL, GCKR,* and gene encoding apolipoprotein B (*APOB*) loci, for which the deleterious alleles raise TG levels by 0.05–0.20 mmol/L in the general population [28]. These same alleles increase HTG risk by two- to fourfold in lipid clinic patients [29]. A list of the top ten common single nucleotide polymorphism (SNP) alleles that are associated with HTG risk are shown in Table 11.6. A patient's total genomic burden of the risk alleles can be tallied to create a "genetic risk score" (GRS) for HTG susceptibility [28, 29]. GRSs can be raw (simple allele counts) or weighted, in which there is a further adjustment based on the degree of TG elevation caused by the specific risk allele: Alleles with larger effects on TG levels contribute more to the weighted GRS.

Groups of HTG patients have significantly higher mean GRS than normolipidemic patients [28, 29]. However, there is a very wide range of GRS around these means and considerable overlap of GRS between individual HTG and normolipidemic subjects. The GRS discriminates well between HTG and normolipidemic subjects at the extremes of the distribution but there is substantial overlap through the middle of the distribution [4–7]. Nonetheless, the potential diagnostic utility of the GRS is less important than the principle that the HTG population has a higher burden of small-effect common genetic polymorphisms, which form the basis of genetic susceptibility to most HTG states [4–7].

In addition to common small effect variants, patients with HTG also have a higher burden of rare large effect variants [30, 31]. Again, these are significantly more prevalent in the pool of HTG patients, but they are not diagnostic for the development of HTG in any particular individual. Further, the variants generally do not cosegregate with TG levels in family pedigrees. Individuals who carry a higher burden of variants are relatively rare even in the population of HTG patients—frequencies of such individuals approximate those of carriers of mutations for rare Mendelian diseases [30, 31].

Simple Primary Hypertriglyceridemia (HLP Type 4)

We suggest the term "simple primary HTG" for the disorder formerly known as "familial HTG" or HLP type 4. This relatively common phenotype is characterized by high TG levels due to an isolated elevation of VLDL particles, which results from both overproduction and decreased elimination of these particles [4–7]. Susceptibility to simple HTG results from a heterogeneous

group of mechanisms that cause elevations in VLDL [4–7, 31].

Epidemiology TG levels elevated to between 3.4 and 9.9 mmol/L due to an isolated elevation of VLDL particles is seen in up to 5% of adults [1, 3].

Clinical Features Simple primary HTG is associated with an increased risk of CVD, obesity, insulin resistance or frank diabetes, and is associated with hypertension and hyperuricemia [1]. With an additional metabolic stress, simple HTG patients can deteriorate into mixed hyperlipidemia (HLP type 5), with fasting chylomicronemia. Generally, the TG levels resulting from VLDL excess in simple primary HTG are not high enough to cause pancreatitis [15].

Laboratory Features Patients with simple HTG have moderately elevated plasma TG levels, on the order of 3.3–9.9 mmol/L [1]; these are frequently associated with depressed HDL cholesterol [1]. At the higher end of the TG range for this condition, serum may also appear turbid on examination due to the presence of large VLDL particles [1].

Molecular Basis The molecular basis for simple primary HTG follows the polygenic architecture for most "familial" HTG states, as described above, sometimes with the presence of one or more secondary factors that can force expression of the phenotype in a genetically susceptible person [4–7, 31]. The fundamental genetic susceptibility component, as described above, is an increased burden of common, small effect variants that individually raise TG levels by a fraction of a mmol/L in studies conducted in the general population. Such common variants tend to cluster in families, but the combinations of variants, because they are on different chromosomes, segregate independently and thus the susceptibility to HTG does not pass from parent to child in a clear Mendelian fashion [30, 31]. In addition, occasional heterozygous rare variants are seen at increased frequency in the pool of HTG subjects, but these also do not clearly cosegregate with HTG in family pedigrees.

Treatment Strategies Treatment of simple HTG follows the general strategy outlined below.

Dysbetalipoproteinemia (HLP Type 3)

Dysbetalipoproteinemia, also known as HLP type 3 or remnant removal disease, is characterized by increased serum TG and cholesterol rich lipoprotein remnants—essentially IDL and chylomicron remnants, sometimes collectively called beta-VLDL particles [1, 32]. These particles are usually rich in apo E. Dysbetalipoproteinemia is mainly caused by homozygosity for binding-defective apo E2 isoform on a background of genetic susceptibility to HTG that resembles HLP type 4 [32].

Epidemiology Dysbetalipoproteinemia affects ~1 in 10,000 people [1, 32]. The condition generally does not present until adulthood for men and in the postmenopausal years in women, and is more common in men overall [1, 32].

Clinical Features Nowadays, patients with dysbetalipoproteinemia tend to be identified early biochemically and then treated, so few of them have the classical physical stigmata (Fig. 11.3). Patients in the fourth decade of life or older who have not been treated can present with tuberous or tuberoeruptive xanthomas on the extensor surfaces of the extremities, such as on the elbow and knees and occasionally the buttocks [1, 32]. Planar or palmar crease xanthomas are also noted [32]; these appear as orange lipid deposits seen in the crease areas of the palm and are pathognomonic of familial dysbetalipoproteinemia, however they are not present in all individuals with the condition [1].

Patients with dysbetalipoproteinemia have increased risk of both coronary artery disease (CAD) and peripheral vascular disease (PVD) [1, 32]. Remnant and IDL particles are atherogenic, so that even in the context of reduced LDL cholesterol, dysbetalipoproteinemia patients are elevated risk of CAD and PVD [32]. Dysbetalipoproteinemia often requires secondary factors for overt disease expression. These include ad-

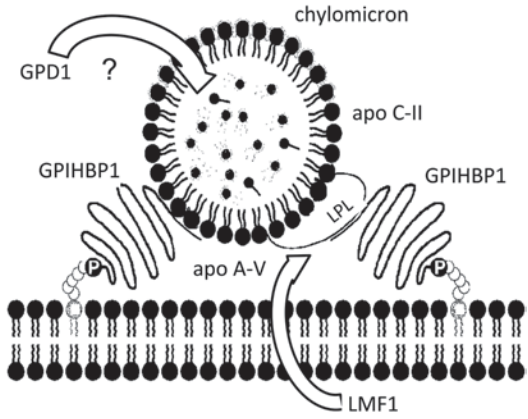

Fig. 11.3 Proteins with naturally occurring mutations that can affect the function of LPL and lipolysis, leading to familial chylomicronemia. The circulating triglyceride (TG)-rich chylomicron is shown in the *center* of the figure. Lipoprotein lipase (*LPL*) is the key enzyme that is involved in hydrolysis of TG within chylomicrons, and is shown tethered to the endothelial cell surface by glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (*GPIHBP1*). Among the apolipoprotein constituents of chylomicrons are apolipoproteins C-II and A-V (apo C-II and apo A-V, respectively), which enable the normal functioning of LPL. LPL undergoes maturation within cells with chaperone protein lipase maturation factor 1 (*LMF1*) which is responsible for bringing LPL to the endothelial cell surface. Mutations on both alleles of *LPL, GPIHBP1, APOC2, APOA5,* or *LMF1* genes that lead to loss-of-function of the respective gene products can lead to impaired lipolysis of chylomicrons and fasting chylomicronemia. Cytosolic glycerol-3-phosphate dehydrogenase (GPD1) is an NAD+-dependent enzyme that reduces dihydroxyacetone phosphate to glycerol-3-phosphate. The mechanism whereby homozygous mutations in the *GPD1* gene lead to chylomicronemia is currently unknown. (Figure adapted from Young SG, Davies BS, Fong LG, Gin P, Weinstein MM, Bensadoun A, Beigneux AP. GPIHBP1: an endothelial cell molecule important for the lipolytic processing of chylomicrons. Curr Opin Lipidol. 2007;18:389–96)

ditional genetic susceptibility variants, or other hormonal or environmental factors, such as the presence of disorders such as obesity, type 2 diabetes or hypothyroidism [32].

Laboratory Features Patients with dysbetalipoproteinemia typically present with elevated total cholesterol levels, generally between 6–11 mmol/L (240–450 mg/dL) with elevated TG also in the range of 3–10 mmol/L (250–900 mg/dL) [1, 32]. The levels of total cholesterol and TG are generally roughly equally elevated [1]. When directly measured, LDL cholesterol is typically low due to disrupted processing of VLDL to LDL [1, 8, 32]. The major component of circulating TG is in the form of IDL [32], but other remnant subfractions are increased. VLDL particles also tend to be cholesterol-enriched, which can be determined by ultracentrifugation of isolated VLDL particles [1].

Molecular Basis Similar to other HTG states, dysbetalipoproteinemia is a polygenic trait. These patients have a similar background of increased genetic susceptibility seen in simple HTG (HLP type 4). But in addition, dysbetalipoproteinemia patients have additional genetic variants or mutations that affect the normal function of apo E [1, 32]. Usually, dysbetalipoproteinemia patients are homozygous for the apo E2 allele variant or protein isoform, which binds abnormally to cell surface receptors, such as the LDL receptor [8, 32]. The common apo E allele is the apo E3 isoform, which differs from E2 by the presence of an arginine at residue 158 in the receptor-binding domain, while E2 contains a cysteine at this position [8, 32]. Less commonly—<5% of dysbetalipoproteinemia patients—will have rare dominant mutations in *APOE* [1, 8]. Such rare *APOE* mutations may not require secondary causes to express the dysbetalipoproteinemia phenotype [8]. The *APOE* mutations in dysbetalipoproteinemia result in elevated serum β-VLDL particles patients through impaired hepatic uptake of apo E-containing lipoproteins, such as CM remnants and IDL particles, and also cause a reduction in the conversion of VLDL and IDL to LDL particles [1].

APOE mutations are necessary for the expression of dysbetalipoproteinemia, but are not sufficient on their own to elicit the phenotype [8, 32]. In fact, <10% of homozygotes for the binding-defective E2 isoform develop dyslipidemia [32]. Therefore, additional factors, which we now believe to be the burden of HTG susceptibility arising from accumulation of common and rare HTG-associated alleles, or secondary factors, such as diabetes, hormonal disturbances

or obesity, are usually required for phenotypic expression of the phenotype [1, 32]. Two of the most replicated susceptibility variants are within the *APOA5* gene, namely S19W and −1131T > C, which are also the most strongly associated SNPs seen with other HTG-containing phenotypes [7, 33].

Diagnosis Dysbetalipoproteinemia is suggested in patients who have equimolar elevations of total cholesterol and TG [32]. When fractionation methods, such as ultracentrifugation and electrophoresis are available, the presence of a broad beta band or of IDL, are both suggestive of this phenotype. Another diagnostic test is an elevated ratio of VLDL-cholesterol to total TG; again this requires specialized biochemical testing methods that are becoming less commonly available. An elevated VLDL cholesterol to total TG ratio (> 0.3) along with apo E2/E2 homozygosity or another rare *APOE* mutation are pathognomonic for dysbetalipoproteinemia [1, 32].

Treatment Strategies Treatment of dysbetalipoproteinemia follows the general strategy for HTG outlined below. In addition, some of these patients are quite sensitive to weight loss, reduced fat diets and alleviation of secondary conditions, such as type 2 diabetes and hypothyroidism [1]. In our experience, these patients are also quite responsive to a wide range of pharmacological treatments, including fibrates, niacin, fish oil, and statins.

Mixed Hyperlipidemia (HLP Type 5)

Mixed hyperlipidemia, or HLP type 5, is, like HLP type 1, also characterized by the pathologic presence of chylomicrons in the serum after 12–14 h of fasting [1]. But in addition, HLP type 5 has elevated levels of VLDL particles, like HLP type 4. The phenotype is essentially a more extreme form of HLP type 4, in which chylomicrons accumulate during fasting.

Epidemiology Mixed hyperlipidemia has a population prevalence of ~ 1 in 600 [1–3]. A key distinguishing feature between mixed hyperlipidemia and familial chylomicronemia is the age of onset of presentation. Patients with familial chylomicronemia typically present in childhood or adolescence, whereas mixed hyperlipidemia patients typically present in adulthood [1, 4–7]. Inheritance pattern is variable, with the phenotype thought to be triggered in patients with an underlying genetic susceptibility coupled with the influence of environmental and hormonal exposures [1, 4–7].

Clinical Features These are similar to those seen in familial chylomicronemia, with eruptive xanthomata, lipemia retinalis, hepatosplenomegaly, and a greatly increased risk of developing pancreatitis [1]. Other features include neurological symptoms, such as the inability to concentrate [3], although this feature is variable and the underlying mechanism is not understood.

Laboratory Features The laboratory findings in primary mixed hyperlipidemia are similar to those seen with familial chylomicronemia, with an elevated fasting serum level of chylomicrons, typically > 10 mmol/L (> 900 mg/dL), together with elevated levels of VLDL particles [1, 8]. Plasma appears turbid, and develops a creamy supernatant when allowed to stand overnight [1]. Patients with primary mixed hyperlipidemia also have associated elevations in total cholesterol, and often other lipoproteins, particularly VLDL, which are not present in familial chylomicronemia [1, 8].

Molecular Basis HLP type 5 shares much of the same genetic susceptibility from common and rare TG-associated alleles that have accumulated in the genomes of affected individuals [1, 8]. We have observed that patients with HLP type 5 carry a greater burden of the common susceptibility alleles than patients with HLP type 4 [31].

Treatment Strategies Treatment of mixed dyslipidemia follows the general strategy for HTG outlined below, including weight loss, restriction of total calories, simple carbohydrates, trans and saturated fats, and alcohol. In addition, treat-

ments for primary mixed hyperlipidemia are focused on reversing or controlling any potential triggers for the condition, such as eliminating any medications known to worsen the condition, and maintaining optimal control of hypothyroidism and diabetes [3, 34]. Pharmacological management is also an option for these patients. Medications that can be useful in treatment of these patients include fibrates, nicotinic acid and fish oils, as discussed below [3, 34]. The chylomicronemia in this condition places patients at risk of pancreatitis; if this develops, the principles of management are similar to those discussed above for familial chylomicronemia.

Combined Hyperlipidemia (HLP) Type 2B)

Although this condition is the topic of another chapter, it is worth reemphasizing here the concept that the genetic architecture of this phenotype is determined by polygenic susceptibility to both HTG and high LDL cholesterol levels, through accumulation of SNP risk alleles for both biochemical disturbances based on higher GRS for each trait. There are a few monogenic forms of combined hyperlipidemia due to single gene effects that appear to segregate in families and have been replicated [7, 31]. But by and large, as with the other complex HTG states, there is no clear monogenic determinant of this common and complex phenotype.

General Treatment Approaches for HTG (Tables 11.4 and 11.5)

Nonpharmacological Treatment

There are certain common elements for management of all HTG states [1]. The cornerstone of treatment for HTG patients is diet, weight loss, reduction of alcohol intake and control of secondary metabolic factors, such as hyperglycemia. Treatment is focused on dietary control including monitoring of caloric intake, reduced fat, especially saturated fat, consumption, as well as reduction in carbohydrate intake, especially in the form of high glycemic or high fructose foods [34]. Alcohol raises TG levels in susceptible people, and reduction or elimination of alcohol intake is also an important component of treatment in patients with HTG. Increased levels of physical activity have also been shown to be helpful to lower TG levels [35]. Control of risk factors and other underlying conditions is also helpful in these patients. Improved glycemic control in diabetes, control of other cardiovascular risk factors, such as obesity and hypertension, and discontinuation of smoking are all helpful in HTG patients [1].

Fibrates

Fibrates, such as gemfibrozil, bezafibrate, and fenofibrate, reduce TG levels by 30–50%, and can also raise HDL cholesterol by up to 20% [1, 3, 17, 36]. The effect of fibrates on serum LDL is variable, in some HTG patients fibrates can increase LDL cholesterol [1, 3, 17, 36]. Fibrates are considered important as prophylactic treatment against pancreatitis [1, 3, 17, 36]. There have been no studies, however, that have shown a definitive reduction in cardiovascular outcomes or total mortality in this population [1, 15].

Fibrates act by increasing fatty acid oxidation through LPL, increasing LPL synthesis, and reducing apo C-III expression, which acts to decrease VLDL production and increase LPL-mediated breakdown of TG-rich particles [36]. Fibrates have been shown to act in the liver on the peroxisome proliferator-activated receptor-alpha (PPAR), which acts to lower hepatic production of VLDL [36].

Side effects of fibrates include gastrointestinal intolerance, and a slight increased incidence of cholesterol gallstones [1, 36]. There are also rare reports of fibrate use leading to hepatitis and myositis [36], especially for gemfibrozil when used in combination with statins that are metabolized through the CYP 3A4 pathway [36]. In contrast, fenofibrate can be safely combined with statins [36].

Nicotinic Acid (Niacin)

Although nicotinic acid, or niacin, has been shown to reduce TG levels by only 10–30%, it has been shown in some studies to reduce the risk of cardiovascular events and the development of atherosclerosis [31]. However, more recent studies, including the randomized controlled AIM-HIGH trial, reveal no further benefits beyond those gained from a statin alone [37]. Nicotinic acid also increases HDL cholesterol by ~10–40%, and lowers LDL cholesterol by ~5–20% [37].

The lipid-lowering mechanism of action of niacin is unknown: the dogma that it reduces the flux of fatty acids to the liver and thus reduces VLDL secretion by the liver has been called into question recently [38]. Niacin also acts in adipose tissue by increasing the sensitivity of LPL for TG and in the liver by inhibiting diacylglycerol acyltransferase-2 (DGAT-2) leading to decreased VLDL secretion [39]. It also stimulates the production of apo A1 in the liver, which results in a modest HDL cholesterol increase [39].

Unfortunately, niacin has use-limiting side effects, such as flushing, lightheadedness, and pruritis, which can occur shortly after administration of the drug and can last from 15–30 min [39]. Rarely, niacin can also cause hepatotoxicity or elevation of liver enzymes [1]. It can also worsen glucose intolerance, and should be used with caution in prediabetics, or overt diabetes with poor glycemic control [39]. It can also raise levels of uric acid in the blood, which may precipitate or worsen gout [39].

Statins

Statins, or 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG CoA) reductase inhibitors, are most often used to treat elevated levels of total of LDL cholesterol, and are not typically used as first line agents with TG levels >5 mmol/L [1]. However, there is copious evidence in support of statins reducing risk of CVD endpoints [40]. Statins are generally well tolerated but can occasionally cause myopathy and rarely rhabdomyolysis [40]. Statins can also interact with certain fibrates, especially gemfibrozil, so this particular combination should be avoided [36]. Statins may be considered for use in patients with HTG who may be at risk for CAD, in order to improve their CVD risk.

Omega-3 Fatty Acids

Omega 3 fatty acids, especially in the form of eicosapentaenoic acid (EPA) have been shown to reduce serum TG levels by 20–50% [41]. They also modestly raise HDL cholesterol by ~5% [41]. There is some evidence that reduced TG levels are associated with increases in low-density lipoprotein cholesterol (LDL-C), and no trials have yet demonstrated the effectiveness of omega 3 fatty acids in improving CVD outcomes [41]. The mechanism of action of omega 3 fatty acids is unclear [41]. Side effects associated with omega 3 fatty acids are minimal, and include fishy taste and burping (eructation) [41].

Conclusion

HTG is a commonly encountered clinical phenotype that is relevant because: (1) modestly elevated TG are associated independently with increased risk of CVD; (2) severely elevated TG are associated with increased risk of pancreatitis; and (3) HTG is often associated with other metabolic disturbances that are associated with increased cardiometabolic risk. Both genetic and nongenetic factors contribute to the development of HTG. However, the only truly monogenic form of HTG is HLP type 1 or familial chylomicronemia, which is associated with mutations in at least five separate genes. The other HTG states are polygenic or multigenic and typically require additional environmental, genetic, lifestyle or hormonal influences to manifest themselves clinically. The mainstay of treatment currently for all HTG states is control of risk factors, diet, and lifestyle choices to ensure maximal health for HTG patients, medication can also be useful in select populations. Ongoing research, especially

into gene therapy for familial chylomicronemia, may lead to long-term improvements in quality and length of life for patients with this rare monogenic phenotype.

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Genetic Disorders of HDL Metabolism

12

Jessica Sparks Lilley, MacRae F. Linton and Sergio Fazio

Introduction

High-density lipoprotein (HDL) was first identified in 1929 as an alpha globulin precipitated from horse serum, and the suggestion of its inverse relationship with cardiovascular risk many decades later created a new way of thinking in the pathogenesis of atherosclerosis and for the therapeutic possibilities to prevent and reduce myocardial infarctions via extraction of cholesterol from the [1]. HDL is seen as the carrier responsible for reverse cholesterol transport, the antiatherogenic mechanism by which lipids are collected from peripheral cells and transported to the liver for disposal through multiple pathways. This crucial role, along with its anti-inflammatory, antioxidant, and endothelial actions, strongly positions HDL front and center in the struggle to maintain arterial wall homeostasis. This chapter focuses on inherited causes of HDL-level variations.

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The influence of genetics on HDL-cholesterol (HDL-C) levels is stronger than that on other lipoproteins, with about 12.1 % of HDL-C variation in population studies explained by variation at known loci [2]. However, genetic causes of HDL derangements are often masked by environmental factors and difficult to discern in adult free-living populations. Although genes determine constitutional HDL levels, HDL function in general, and the effectiveness of reverse cholesterol transport, factors such as age, sex, diet, body habitus, comorbidities, medications, alcohol, smoking, and physical activity all affect HDL levels and function in ways that can overcome the direct genetic effect.

High levels of HDL-C have been shown in multiple epidemiologic studies to be protective against atherosclerosis [3–5], whereas low HDL-C levels are an independent and common risk factor for cardiovascular disease [6], with almost a quarter of Americans having low HDL-C (below 40 mg/dL in men, below 50 mg/dL in women) [7].

Though low HDL-C is extremely common, defined discrete mono and polygenic causes for this phenotype are rare. Strong evidence from animal and population studies led to the now questionable dogma that the association between low HDL-C and atherosclerosis is causal [8], but these observations are difficult to reproduce in individual patients or in family studies, given the multiple effectors of vascular regulation at play under common circumstances. Similarly, higher HDL-C levels have been associated with cardiac protection in large population studies,

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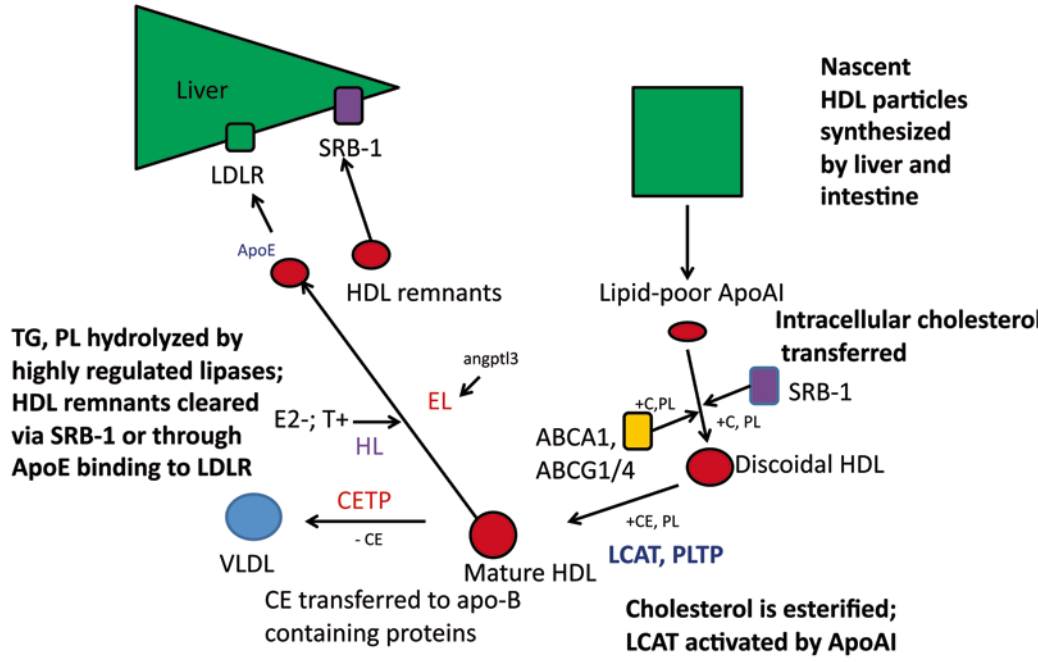

Fig. 12.1 Reverse cholesterol transport

but subjects with high HDL-C may also develop rampant atherosclerotic disease, suggesting that HDL particles can become proatherogenic and that HDL function is a determinant of cardiovascular risk with predictive power independent of HDL-C levels [9]. Although reverse cholesterol transport is the clearest mechanism for the cardioprotective effects of HDL, it is now accepted that this lipoprotein is capable of exerting its influence through many additional functions, made possible by its unique protein and lipid composition, and by a small size that makes it a natural nanoparticle capable of penetrating the plaque [10].

HDL Structure and Metabolism

HDL particles are heterogeneous in size, composition, and cargo. There are over ten subtypes of HDL particles [11], with more than 50 associated proteins [12], dozens of biologically active microRNAs [13], and hundreds of lipids that contribute to its tremendous heterogeneity. HDL has been classified based on shape, density, size, protein content, and mobility on gel electrophoresis [11]. HDL particles are small and globular and contain more protein than lipid. Most of the protein is apoAI and apoAII, with minor contributions from apoAIV, apoAV, apoD, apoJ, apoLI, apoE, apoM, and the C apoproteins (apoCI, apoCII, and apoCIII) [12, 14].

An overview of HDL metabolism is diagrammed in Fig. 12.1. HDL begins as lipid-poor apoAI secreted from the liver and intestine into the circulation, followed by prompt acquisition of cellular lipids via regulated transfer derived from interaction with the ATP-binding cassette transporter A1 (ABCA1). ABCA1 mediates the transfer of phospholipid and free cholesterol to lipid-poor apoAI to form nascent discoidal HDL particles. In peripheral cells, ABCG1 and scavenger receptor BI (SR-BI) also transfer lipids to the maturing HDL. SR-BI mediates net influx in the liver, whereas ABCA1 and ABCG1 drive

net efflux from peripheral cells, including macrophages [15].

The discoidal HDL grows its core via acquisition of cholesterol esters (CE) and phospholipids by the action of lecithin-cholesterol acetyltransferase (LCAT) and phospholipid transfer protein (PLTP), respectively. These enzymes are expressed in the liver and are carried by the HDL. By trapping esterified cholesterol in the center of the lipoprotein, LCAT promotes the concentration gradient of unesterified cholesterol between HDL and the cells, which favors flow of more free cholesterol from the cell into the HDL particle. ApoAI is the main activator of LCAT. The transfer of phospholipids from triglyceride-rich lipoproteins to HDL via PLTP impacts the size and maturation of HDL. The CE in mature HDL may be transferred to apoB-containing lipoproteins or delivered to the liver or to steroidogenic tissues [16].

The final step in reverse cholesterol transport requires delivery of CE from HDL to the liver. The hepatic HDL receptor SR-BI mediates selective uptake of CE from HDL, but does not regulate holoparticle internalization. SR-BI is also expressed in the brain as well as in the adrenals and gonads, where CE taken up from HDL contributes to the synthesis of steroid hormones [17]. Holoparticle internalization of HDL can occur via apoE-mediated binding to the LDL receptor. ApoE is the ligand for the clearance of remnants by the liver. However, a substantial amount of plasma apoE resides in the HDL and favors its expansion in size, leading to the formation of HDL particles that compete with apoB-containing lipoproteins for receptor-mediated removal. The amount of apoE in HDL is controlled by factors such as dietary cholesterol and appears to be isoform dependent (apoE2 > E3 > E4). Furthermore, the apoE content of HDL also affects its function, and patients with cardiovascular disease have apoE-enriched HDL [8, 18].

In humans, spherical HDL particles exchange CE for triglyceride and phospholipids with apoB-containing lipoproteins, such as VLDL and remnants, via interaction with cholesteryl ester transfer protein (CETP), resulting in less stable, triglyceride-enriched HDL that undergoes hydrolysis by action of both endothelial lipase (EL) and hepatic lipase (HL). HL is found in hepatic sinusoidal space as well as within the adrenals, ovaries, and testes; its activity is suppressed by estrogens and upregulated by androgens [19]. Angiopoietin-like 3 protein (ANGPTL3) is a regulator of lipoprotein lipase and EL, and function-altering mutations in this protein result in increased catabolism of lipoproteins giving rise to a pan-hypolipidemic phenotype including low HDL levels [20].

Since multiple proteins in different compartments must work in concert for the HDL-C cycle, there are numerous opportunities for mutations to affect HDL concentration and function. The understanding of the complex HDL proteome is evolving; in addition to the classic apoproteins, there are molecules with roles in innate immunity, complement regulation, thrombolysis, and other functions, furthering the recognition of HDL's role in protecting the cardiovascular system from inflammation and atherosclerosis [12].

Low HDL-C Syndromes

Low HDL-C levels increase the risk for atherosclerotic disease even in subjects with optimal LDL-C control [3], and the National Cholesterol Education Program considers an HDL-C < 40 mg/dL as an independent risk factor for heart disease [21]. HDL dysfunction and impaired reverse cholesterol transport may be responsible for some of the residual risk following cardiovascular events after LDL-C goals are reached [22].

Furthermore, low HDL levels have been associated with worse outcomes in cancer, diabetes complications, and infections, particularly in critically ill patients [23–25]. These observations are supported by the varied composition of HDL, and particularly the presence of proteins that play a crucial role in innate immunity and endothelial homeostasis [12]. While lifestyle modulates HDL levels in every individual, genetic predisposition to low HDL arises from mutations in the many genes whose products are necessary for HDL structure, transport, and function.

Though lower HDL levels have been considered to increase risk for cardiovascular disease,

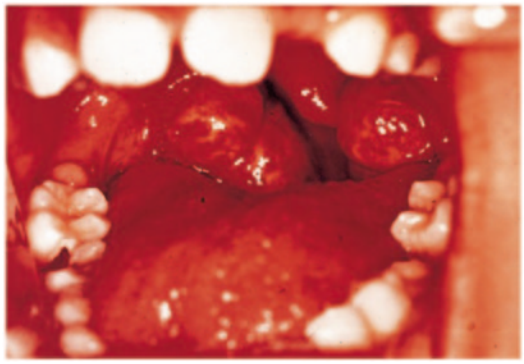

Fig. 12.2 Orange, enlarged tonsils characteristic of Tangier disease; similar deposits may be found throughout the reticulo-endothelial system, causing lymphadenopathy and splenomegaly

the implications of genetically determined low HDL presentations are not as straightforward. The polygenic condition familial combined hyperlipidemia (FCH) clearly increases the risk for cardiovascular disease, but other features like increased LDL and TG certainly are at play. Consistent with the hypothesis that low HDL-C levels are associated with cardiovascular risk, the complete inability to express apoAI or ABCA1 is apparently related to early development of atherosclerosis. On the other hand, whether heterozygous mutations in ABCA1, LCAT, and apoAI cause increased cardiovascular risk remains controversial. Aside from the obvious concern for atherosclerotic disease, abnormal reverse cholesterol transport is important clinically to recognize, as intracellular cholesterol buildup and amyloidosis can cause multisystem pathology.

Tangier Disease

The importance of the ABCA1 transporter on chromosome 9q31 in the biogenesis of HDL was established by the discovery that its absence is the molecular cause of Tangier's disease, a very rare inherited disorder of lipid metabolism characterized by extremely low plasma HDL-C levels and nearly absent apoAI [26–29]. In the absence of proper lipid transfer from cells to apoAI particles, HDL is not formed and apoAI is rapidly degraded by the kidney.

First described by Fredrickson in 1961 and named after the Chesapeake Bay island where the two probands lived [30], Tangier disease is inherited in an autosomal recessive fashion and results in near complete HDL and apoAI deficiency in homozygotes, with modest amounts of apoAI detected only in the lipid-poor nascent HDL [31]. Fewer than 100 families worldwide have been reported with such mutations [32]. Affected individuals have characteristic enlarged, orange tonsils (Fig. 12.2), hepatosplenomegaly, thrombocytopenia, corneal clouding, early atherosclerotic cardiovascular disease, and amyloidosis related to the reduced delivery of cellular phospholipids and free cholesterol to nascent apoAI-containing particles and HDL, leading to problematic intracellular cholesterol buildup. The yellow–orange tonsils are a result of intracellular accumulation of lipophilic retinyl esters and carotenoids. This unusual pigmentation deposition may be seen in the rectal mucosa as well. Affected individuals have HDL-C levels less than 5 mg/dL. More than half of adult patients present with symptoms of peripheral neuropathy. No therapy is currently available [32], although enlarged tonsils in children may require tonsillectomy. Other findings include hypertriglyceridemia and low LDL-C. Whether people who are heterozygous for ABCA1 mutations are at risk for increased heart disease is unclear since the phenotype is variable and the presence or absence of cardiovascular risk factors or comorbidities greatly influences clinical findings. However, HDL-C levels in affected subjects are usually 50 % lower than those of unaffected family members [33].

Familial LCAT Deficiency/Fish-Eye Disease

Familial LCAT deficiency (FLD) is inherited in an autosomal recessive fashion. Patients harbor mutations in the *LCAT* gene on chromosome 16q22 [34]. Reported LCAT mutations are dispersed throughout the gene, leaving little ability to predict phenotype based on the location of the variant. True LCAT deficiency affects fewer than one in 1 million individuals; [35] only 60 isolated cases and about 70 families with partial or com-

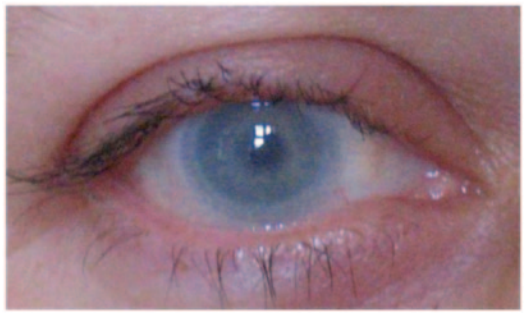

Fig. 12.3 Corneal clouding as seen in fish-eye disease and familial lecithin-cholesterol acetyltransferase (LCAT) deficiency

plete LCAT deficiency have been described following the first report of three sisters in Norway by Norum and Gjone in 1967 [36, 37].

Patients with LCAT deficiency have very low levels of CE-poor HDL. LCAT activity is not exclusive to HDL (alpha activity), and substantial activity is associated with LDL and VLDL (beta activity) [35]. Inability to esterify cholesterol leads to elevated levels of lipoprotein X (LpX), an LDL-type particle enriched in free cholesterol formed nonspecifically when circulating levels of free cholesterol or phospholipid are elevated [38]. Homozygote subjects with complete loss of LCAT have FLD, which manifest with corneal clouding (Fig. 12.3), moderate hemolytic anemia, and progressive renal insufficiency due to glomerulopathy [39]. In mouse models, this glomerulonephropathy is linked to the deposition of LpX [39], though pathologic studies have implicated apoE uptake into the mesangium in the absence of normal LCAT activity [40]. A milder form of LCAT deficiency, "fish-eye disease," is due to abnormal alpha-LCAT activity and results in low HDL levels and corneal clouding but no LpX formation or renal involvement. It is not certain that patients with LCAT deficiency have increased risk for early cardiovascular disease or asymptomatic atherosclerosis [41–44].

A study of four patients with LCAT-associated renal insufficiency showed lipid deposition in the glomerular basement membrane, lipid accumulation in the mesangium, and glomerular abnormalities; [45] these patients also have abnormally large LDL particles, linking the observation that patients with abnormalities in both alpha- and beta-LCAT activity are at risk of renal disease, while patients with only alpha-LCAT deficiency are not.

ApoAI Deficiency

Primary low levels of apoAI may result from mutant versions of the protein, such as apoAI Milano (apoAIm), or from reduced production of the native protein, which in homozygosity can cause near complete absence of apoAI [46]. The most severe form, complete deficiency of apoAI, was first described by Schaefer in 1982 and is associated with increased risk of cardiovascular events [47], whereas other variants, such as $apoAI_{Milano}$, cause low HDL levels but reduced risk for cardiovascular events. Other lesser known variants of apoAI that cause lower HDL levels but do not increase heart disease rates include apoAI Marburg, apoAI Giessen, apoAI Munster, and apoAI Paris [48]. Fewer than 20 families have been reported in the literature with complete apoAI deficiency [49]. Individuals with complete apoAI deficiency have unmeasurable plasma HDL-C levels. Physical findings include corneal clouding, arcus corneae, xanthomas, and xanthelasmas. Cerebellar ataxia has been reported in some cases, and atherosclerosis appears to be accelerated.

ApoAI variants can additionally induce lower LCAT activity and promote amyloid deposition [50]. These mutations are quite rare: one large population study showed a 0.27% prevalence of apoAI mutations associated with low HDL, and a 0.41% prevalence of apoAI variants associated with amyloidosis [51]. The fibrils in apoAI-based amyloidosis contain N-terminal fragments of apoAI, and the majority of amyloidogenic apoAI variants have an extra positive charge in the N-terminal region [52], with some exceptions [53]. Thirteen of the known apoAI variants are associated with amyloidosis; the amyloid deposition causes a characteristic green birefringence that can be detected in the intestines, heart, kidneys, larynx, liver, ovaries, uterus, and pelvic lymph nodes [54].

The apoAI Milano mutation is perhaps the best representative example of the complexity of

HDL system [55]. This variant was found in 3.5% of the population of a small village in northern Italy. Despite having low HDL-C and high triglycerides, affected individuals had low rates of cardiovascular disease. Propelled by promising animal studies, one clinical trial reported beneficial vascular effects after infusion of recombinant apoAI Milano-phospholipid complexes to patients with a recent acute coronary syndrome. After 5 weekly injections, atheroma volume was reduced significantly as measured by intravascular ultrasound [56]. Efforts to develop a synthetic form of this protein for the treatment of people with known atherosclerotic disease have been underway, but lack of follow through from these initial observations raises suspicions on the viability of this therapeutic approach.

Familial Combined Hypolipidemia

Low HDL-C levels may result from an underlying genetic cause that affects both apoB- and apoAI-containing lipoproteins. Mutations in the *ANGPTL3* gene on chromosome 1p31 (which produces ANGPTL3, a lipase inhibitor) can reduce HDL-C by 80% in homozygous individuals and also drastically reduce levels of all other lipoproteins. Adverse clinical sequelae have not been described in these individuals despite very low HDL-C levels. This syndrome in heterozygotes can be mistaken for hypobetalipoproteinemia, a condition that arises from mutations in the *ApoB* gene or in microsomal triglyceride transfer protein, resulting in reduced VLDL secretion from the liver, resulting in low LDL-C and hepatic steatosis and sometimes elevated HDL-C [20, 57]. Loss of function mutations in the *PCSK9* gene are also a cause of hypobetalipoproteinemia [57].

Familial Combined Hyperlipidemia

Contrary to the previously discussed rare variations in discrete loci, the underpinnings of the most common cause of inherited low HDL-C are poorly understood. FCH is characterized by hypercholesterolemia, hypertriglyceridemia, and low HDL-C levels in different degrees and combinations. FCH is inherited in an autosomal dominant fashion and is associated with premature atherosclerotic cardiovascular disease [59]. This polygenic dyslipidemia affects 1 in 200 Americans [60]. The genes involved have not been identified and the pathogenesis involves the loss of the apoB degradation mechanism used by the liver and intestinal cell to regulate the secretion of apoB-containing lipoproteins. The dysregulated overproduction of lipoproteins rich in triglycerides stimulates an exaggerated activity of CETP with resultant loss of cholesterol from the HDL compartment. The mechanism of inheritance is not straightforward, the presentations are varied, and the interplay with environmental factors such as diet, exercise level, alcohol consumption, and changes in body weight causes a wide range of phenotypes. However, its diagnosis is important: 2.7 million Americans are affected by this disorder or its phenocopies [60], and the condition causes a tenfold increase in risk for myocardial infarction [61].

Secondary Causes of Low HDL-C

The most common cause of low HDL-C in the USA is the metabolic syndrome, a constellation of findings driven by insulin resistance and a proinflammatory state that increases the risk for cardiovascular disease and type 2 diabetes. Though definitions vary among expert panels, the most commonly used criteria include hypertension, elevated fasting glucose levels, abdominal obesity, and a dyslipidemia characterized by low HDL and high triglycerides [62]. Certain polymorphisms in the CETP and apoE genes link the association between low HDL and other features of the metabolic syndrome, including abdominal obesity [63]. In addition to the metabolic syndrome, weight gain, sedentary lifestyle, smoking, and consumption of *trans* fats and refined carbohydrates are accompanied by low HDL-C. These associations may reflect direct mechanistic links between risk factors and low HDL-C. For instance, *trans* fats increase CETP activity and therefore lead to lower HDL-C [64], whereas insulin resistance is associated with

increases in both CETP and HL activity, which cause low HDL-C [65]. Certain medications, including anabolic steroids, androgens, benzodiazepines, beta-blockers, and progestins, also have the ability to lower HDL-C. Disease states such as fatty liver, hypertriglyceridemia, diabetes, autoimmune conditions, systemic inflammation, and infections also depress HDL-C. The interplay of HDL-C levels and environment is influenced by several genes, including genetic polymorphisms associated with obesity [66]. The reason for discussing secondary causes within a chapter on genetic HDL abnormalities is that in most cases the presentation of low HDL is the result of environmental pressure on a genetic substrate (e.g., FCH, LCAT defects, and others). This is particularly evident in obese children, where genetic susceptibility to dyslipidemia is heightened by insulin resistance to create a lipid phenotype similar to FCH, while dietary control readjusts plasma lipids to near normal levels.

Genetic Causes of High HDL-C Levels

Though much research has focused on causes of low HDL-C due to the assumption that elevated HDL-C was uniformly beneficial, recent efforts to exploit mechanisms to raise HDL-C with pharmaceutical agents have been disappointing. Better understanding of the environmental and genetic causes of hyperalphalipoproteinemia may reconcile recent clinical trial results with prior observations from large epidemiological studies. Higher HDL-C levels have been correlated with longevity and lower cardiovascular morbidity in multiple cohorts [67–70 ,10]. Based on these observations, estimates were made that for every 1 mg/dL rise in HDL-C, the risk for coronary atherosclerotic disease would decline 2% in men and 3% in women [71]. This categorical knowledge is currently being revised since the field is at an impasse after the negative cardiovascular results of clinical trials with medications that raise HDL-C levels [72–74]. The importance of this subject is crucial, and thus a small section on clinical trial design and results is presented below to put this new knowledge in the right context. Despite this, attempts are still ongoing to raise HDL levels as therapeutic maneuver to reduce cardiovascular risk.

CETP Deficiency

The gene encoding for cholesteryl ester transferase protein (*CETP*) is located on the long arm of chromosome 16 and spans 16 exons [75]. Compared to other genes known to influence plasma lipids, *CETP* has the strongest effect on HDL-C levels [76]. Decreased CETP-mediated exchange of CE and triglyceride between HDL-C- and apoB-containing lipoproteins leads to increased HDL-C levels and formation of large, triglyceride-enriched HDL particles.

Decreased CETP activity as a cause of elevated HDL-C was first described in Japanese siblings in 1985 [77], and the molecular basis for the CETP deficiency was elucidated in 1989 [78], a mutation at a splice donor site in intron 14 resulting in a truncated mRNA and no production of CETP protein from the affected allele. True CETP deficiency is fairly common in individuals of Japanese descent (prevalence of 1–7%), but rare in Caucasians [79, 80]. However, several common small nucleotide polymorphisms leading to decreased CETP activity, including the *Taq*IB, I405 V, −C29C >A, D442G, −631C > A, and R451Q variants, have been described [81]. People homozygous for the D442G variant have only partial CETP deficiency with mean HDL-C of 96 mg/dL, while homozygotes for the deletion mutation in intron 14 have complete CETP deficiency with extremely elevated HDL levels (mean, 167 mg/dL) [82]. Alternative splicing may serve as an additional mechanism for variation in CETP activity levels [83].

Although the levels of HDL-C are strikingly elevated by CETP deficiency, evidence that this decreases the risk for cardiovascular disease is lacking. A study of Japanese men in the Hawaiian Heart Study showed that those with CETP deficiency due to the *Taq*IB or D442G variants actually had higher risk for cardiovascular disease than people with normal HDL-C [9]. The relationship between CETP variants and

cardiovascular risk appears to be variable. For example, the *TaqI*B effect on cardiovascular disease changes with gender [84] and environmental influences [81, 85]. The I405-V variant is associated with longevity [68] but is also linked to unfavorable response to diet high in saturated fat [64] and to higher cardiovascular disease risk in the setting of hypertriglyceridemia [86].

Primary Hyperalphalipoproteinemia

Primary hyperalphaproteinemia, classically defined as HDL above the 90th percentile, encompasses the condition of high HDL-C in the absence of CETP deficiency or known secondary causes, and includes rare forms of elevated HDL levels, such as familial HL deficiency and EL deficiency.

Familial Hepatic Lipase Deficiency

This extremely rare autosomal recessive condition is due to mutations in the HL gene on chromosome 15q21 and presents with high HDL-C levels, corneal opacities, and variable predisposition to atherosclerosis, which can be enhanced in the setting of an independently acquired atherogenic dyslipidemia [87, 88]. Affected individuals have large, triglyceride-rich HDL particles and can present with hypertriglyceridemia, high remnant levels, and low levels of typical LDL. Although low levels of HL have been shown to be protective in familial hypercholesterolemia, considerable controversy exists on whether the elevated HDL caused by HL defects is protective or deleterious for coronary heart disease.

Familial Hyperalphalipoproteinemia Associated with Variant apoCIII

One family has been reported with a mutation in apoCIII (Lys58→Glu); [89] affected heterozygotes had decreased apoCIII levels and elevated HDL, though the mechanism for hyperalphalipoproteinemia was not determined. HDL particles were large and enriched with apoE. It is not clear whether inhibiting apoCIII (a strategy currently in development: http://www.isispharm.com/Pipeline/Therapeutic-Areas/Cardiovascular.htm#ISIS-APOCIIIRx) causes elevated HDL and whether this effect would be cardioprotective.

Endothelial Lipase Deficiency

EL, encoded by the *LIPG* gene on chromosome 18q21, is a glycoprotein with a good degree of homology to both lipoprotein lipase and HL. It is synthesized by endothelial cells and primarily shows *sn*-1-phospholipase activity, particularly toward HDL. Different mutations and variants affecting function have been reported to cause elevated HDL, but no association has been found between activity, HDL levels, and cardiovascular disease rates.

As previously mentioned, large epidemiological and observational studies have yielded important conclusions about plasma lipids and cardiovascular disease, but confounding factors are difficult to overcome when analyzing the contribution of a single component. One approach to decrease confounding is to use Mendelian randomization in population studies, in which a subject's genotype is randomly grouped into a carrier or noncarrier cohort. This instrumental variable is similar to randomization in a clinical trial and strengthens inferences that a particular genotype may be causal for an outcome. LIPG is an obvious target of study since its influence is solely on HDL-C. A large recent Mendelian randomization study analyzed one LIPG SNP, LIPG Asn 396Ser, and found that variations had no association with cardiovascular outcomes despite significant differences in HDL-C levels [90]. This suggests that manipulating LIPG is unlikely to be a successful approach to reduce cardiovascular risk. The results of this Mendelian randomization study should not be over-interpreted to suggest that HDL does not have antiatherogenic properties.

SR-BI Deficiency

Defective or absent SR-BI is expected to increase HDL concentration due to decreased unloading of HDL lipids by the liver. SRB-I knockout mice have high levels of HDL-C but also show accelerated atherosclerosis. No human examples of SR-BI mutations causing high HDL have been reported. Interestingly, an intronic SNP within the

SCARB1 gene on chromosome 12q24 has been associated with subclinical atherosclerosis and incident cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis (MESA); however, this effect was independent of HDL-C levels [91].

Clinical Challenges with HDL Therapeutics

Given the inverse relationship between cardiovascular disease and HDL, there has been a longstanding implicit assumption that raising HDL levels would be protective for the vasculature. However, individuals with very high HDL levels may have increased atherosclerosis, and mounting evidence supports the notion that abnormal HDL function may be responsible for this paradox [92]. Discouraging clinical trial results have created an aura of impotence around the concept of HDL manipulation.

The ILLUMINATE trial, in which the CETP inhibitor torcetrapib produced a 60% increase in HDL-C levels, was terminated early due to increased cardiovascular outcomes among those taking the atorvastatin and torcetrapib compared to those taking only atorvastatin. This negative effect was largely attributed to off target changes in serum aldosterone and electrolyte levels caused by the experimental drug [77]. Other CETP inhibitors were developed that did not share this off target effect. A trial with one of them (dalcetrapib) has recently been halted due to lack of efficacy [74]. Two other molecules, anacetrapib and evacetrapib, have thus far shown good efficacy and absence of obvious serious adverse effects [93–95] and are being evaluated in large cardiovascular outcome trials (http://clinicaltrials.gov/show/NCT01687998) [96]. Niacin has a long history of use as an HDL-raising agent, and a recent meta-analysis has suggested that the drug reduces cardiovascular events [97]. Unfortunately, a recent cardiovascular outcome trial with extended-release niacin failed to show benefits when added to simvastatin and was stopped prematurely [98]. A more recent larger trial has also failed to show any benefit of extended-release niacin while showing higher incidence of serious adverse events [99].

A feature in common among subjects with CETP deficiency and individuals treated either with a CETP inhibitor or with niacin is the presence of large HDL particles. This observation may be a starting point in determining why most genetic causes of elevated HDL do not provide cardiac benefit. It is also important to remember that while LDL is atherogenic by virtue of simply getting stuck in the artery wall, HDL must perform active functions to slow or halt atherosclerosis development. Thus, the new concept of HDL functionality is likely to become the critical keyword in the development of new therapeutics. We know that HDL loses functionality in subjects with coronary artery disease [100] and chronic kidney disease [101], among other patient groups, but it is still uncertain which test will become standard and whether interventions that improve HDL function will have an easier time to show benefits on cardiovascular health.

Conclusions

High HDL is a strong negative predictor of cardiovascular disease [4], but the mechanism for HDL elevation may be key [100]. Lifestyle maneuvers that raise HDL may be particularly effective, but improved cardiovascular health may be related to other effects of a healthy stance that also cause higher HDL levels. On the other hand, genetically determined changes in HDL-C do not support a direct role of this lipoprotein lipid in arterial homeostasis. For example, EL gene defects raise HDL but do not affect the prevalence of myocardial infarction [90], and ABCA1 mutations decrease HDL but are not associated with higher prevalence of ischemic heart disease [102]. In one large genome-wide association study, a polymorphism in the *LCAT* gene was found to have the strongest association with isolated low HDL-C [2], but carriers had normal risk for cardiovascular disease [103]. Outside of its role in classifying rare monogenic disorders, genetic testing is not endorsable for practical purposes since gene–environment interactions are more important for management than is the knowledge of the exact genetic background, and heritability of HDL function has not been proven.

A revolution in our understanding of HDL abnormalities and of management of the cardiovascular risk associated with them will take place when the molecular basis of HDL dysfunction is elucidated and after defining whether the loss of normal HDL function is a heritable trait that can nonetheless be therapeutically modulated.

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Sitosterolemia and Other Rare Sterol Disorders

13

Shailendra B. Patel

Introduction

Sterols are an integral part of the lipoprotein, as well as all cell membranes. Cholesterol is the single most abundant mammalian species representing > 99 % of body sterols. Cholesterol is synthesized de novo in the body, and it is also absorbed from the diet. In nonmammalian organisms, other sterols can be used to fulfill similar functions; ergosterol is the primary sterol in yeast and fungi, sitosterol and campesterol (as well as a host of many other phytosterols) fulfill these functions in plants and there are organisms, such as shellfish, crustaceans, etc., which utilize a mixture of different sterols species, including cholesterol. Humans, being omnivorous, are exposed to dietary cholesterol, as well as these xenosterols (sterols that are not made by the mammalian body). Thus, an understanding of how these sterols are handled physiologically is of importance. Disruption of pathways regulating sterol metabolism (synthesis, transport, and breakdown) can lead to dyslipidemia, but in many cases, the astute clinician is led astray as the standard lipid test (which does not discriminate between these sterols) may not offer clues to allow these conditions to be identified. This chapter focuses on disorders that can affect sterol trafficking, sterol synthesis, and sterol breakdown, using one disease entity in each group to highlight the key points that allows for better diagnosis and management.

Sterol Trafficking Disorders

Clearly, any disorder of lipoprotein trafficking *per se* will also affect sterol trafficking, since sterols are a necessary constituent of these particles. Unfortunately, while sterol trafficking disorders lead to human disease, many such disorders do not result in dyslipidemia, as judged by blood lipid analyses. Thus, Niemann–Pick C (NPC) disease is a progressive neurological disorder caused by defects in one of the two genes, *NPC*1 or rarely *NPC*2, and involve a failure of release of the lysosomal sterols into the cells for further metabolism and transport [1]. Or the loss of cholesterol transport from the outer to the inner mitochondrial membrane, mediated by steroidogenic acute regulatory (StAR) protein, results in congenital lipoid adrenal hyperplasia, an endocrine disorder, but does not result in dyslipidemia [2].

This section focuses on sitosterolemia that specifically results in disruption of whole body sterol trafficking and may be more relevant to the practicing lipidologist. Sitosterolemia, also known as phytosterolemia, results in failure to traffic sterols, xenosterols as well as cholesterol.

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Sitosterolemia/Phytosterolemia

History

In a review in 2007, we wrote, "The history of how mammals can distinguish between dietary non-cholesterol sterols and cholesterol is intertwined with the history of cholesterol itself; whether cholesterol could be synthesized by the body or was wholly absorbed from the diet, whether the body degraded cholesterol, what determines its absorption and biliary secretion, and whether cholesterol was involved in the process of atherosclerosis are all questions that have involved or continue to involve non-cholesterol sterols. Investigations of such observations led to the discovery that plant sterols were excluded by the body, but could compete with bulk cholesterol for entry into the micelles formed during digestion, thus preventing dietary absorption of cholesterol." [3]. Historically, these concepts were considered and investigated from the early parts of the last century [4, 5]. The average human diet consists of about 250 mg of cholesterol and 300 mg of plant sterols a day, yet plant sterols are barely detectable in human blood or tissue. For almost a century, the molecular and physiological pathways responsible for these observations were never fully explored. Conventional wisdom held that plant sterols were essentially unabsorbable, and, thus, this was the main reason for their exclusion. How then cholesterol from the diet absorbed remained essentially ignored? In a classical paper, Bhattacharyya and Connor [6] reported two sisters who had signs and symptoms suggestive of familial hypercholesterolemia, FH, with arthralgia and tendon xanthomas, yet did not have elevated plasma cholesterols and their parents were not hypercholesterolemic either [7]. Examination of the blood sterols by gas chromatography led to the discovery that these two sisters had massive elevations in plant sterols. They named the disease β-sitosterolemia, after the most abundant plant sterol detected (this disease is more accurately phytosterolemia, as all xenosterols accumulate and some argue the term xenosterolemia is a better term) [7, 8]. This report prompted the investigations of two subsequent families with sudden atherosclerotic cardiac death of teenagers, where familial hypercholesterolemia was suspected, but did not have elevated cholesterols [6]. One of these families was Amish, and an extended pedigree analyses confirmed that sitosterolemia behaved as an autosomal recessive condition, and thus a single locus was involved [9]. I posited that a single gene product was responsible for regulating dietary cholesterol, with a working hypothesis that this protein worked as a pump, to pump cholesterol in and noncholesterol sterols out. With the help of colleagues from across the globe, we assembled pedigrees with sitosterolemia, mapped the disease locus, *STSL*, to chromosome 2p21 and positionally cloned and identified the genetic defect responsible for this condition [10–13]. Helen Hobbs and coworkers also independently cloned the sitosterolemia genes [14]. To our surprise, not one, but two genes, *ABCG5* and *ABCG8,* comprised the *STSL* locus, and complete mutations in either gene resulted in the disease. These genes belong to the ATP-binding cassette transporters, family G. Current work suggests that ABCG5 and ABCG8 work as obligate heterodimers; they are expressed on the apical surfaces of the hepatocyte and enterocyte, and are responsible for pumping cholesterol and plant sterols out into the biliary lumen or intestinal lumen, respectively [14–16]. These pumps have a preference for non-cholesterol sterols, but in the absence of the latter, are bona fide cholesterol exporters. Although these transporters have been described as "defenses against cholesterol" [17]as well as defenses against xenosterols [18], the former may be relevant to the majority of people with variant forms of ABCG5/ABCG8, as opposed to the rare individuals with severe mutant forms that lead to xenosterolemia and disease.

Epidemiology

The true prevalence of sitosterolemia is unknown, but based on all families described and reported in the literature, this disease is not more common than 1 in 1,000,000. As for any rare disease, the true prevalence is always likely

to be higher because of under-detection; since plant sterol accumulation leads to some clinical manifestation, chances of underreporting are minimized (see Clinical Findings).

Etiology and Pathogenesis

Mutations in one of the two *A*TP *b*inding *c*assette transporters belonging to the *G* family, ABCG5 or ABCG8, cause the disease [11, 13, 14]. Both copies of ABCG5 or both copies of ABCG8 have to be mutant, and this was the first indication suggesting that they likely worked together, or in tight tandem for regulation of xenosterols. The Hobbs group has shown definitively that these proteins act as obligate heterodimers and that they need to be expressed at the apical surface for normal function [15, 16, 19, 20]. Figure 13.1 shows a depiction of the gene structure of the *STSL* locus and Fig. 13.2 shows mutations in ABCG5 and ABCG8 that lead to disease, as well as natural variants found in "normal" humans. The normal ABCG5/ABCG8 heterodimer needs to fold correctly in the endoplasmic reticulum (ER) to allow its progression through to the Golgi and then to the apical surface. There, in conjunction with proteins that export bile acids (ABCB11/BSEP) and phospholipids (MDR3), ABCG5/ABCG8 facilitate the extrusion of sterols from the outer membrane into the lumen, though the exact mechanism(s) has not been defined. The mutational spectrum encompasses missense mutations, null mutations as well as microdeletions, and ones that affect transcript stability. As a rule, failure to express one of the two proteins results in mis-folding of the other subunit and its degradation in the ER of the hepatocytes and the enterocytes.

Clinical Findings

The most important step in making a diagnosis of sitosterolemia (and this principle applies to any rare disease) is considering the possibility of this diagnosis. The "classical" presentation would be a person who presents in a fashion similar to familial hypercholesterolemia (arthralgia, tendon xanthomas), but where the standard lipid tests show that the LDL-C is less than 200 mg/dL. While this was the case with the presentations of the earliest cases, a compilation of the presenting features of other subjects with sitosterolemia shows that these features (xanthomas) are not as frequent [8]. Table 13.1 lists all of the possible presenting features. One could consider the presence of premature atherosclerotic disease

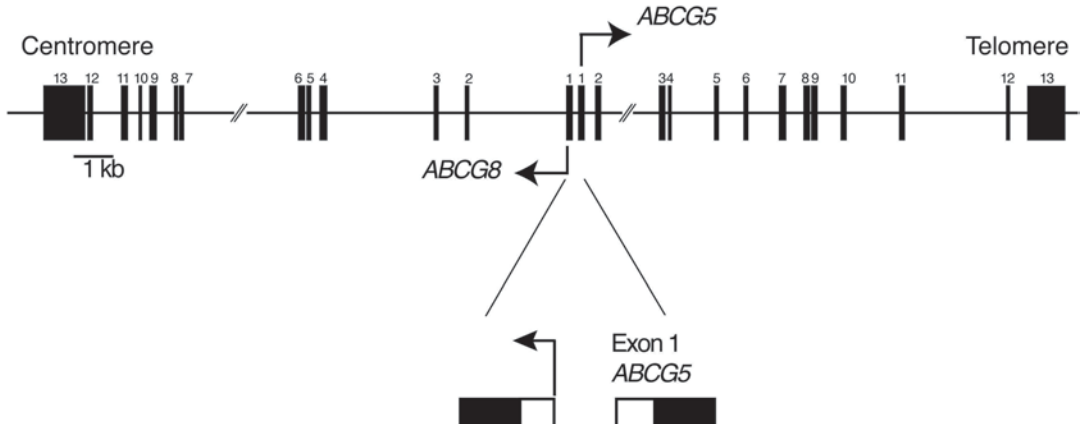

Fig. 13.1 Genetic organization of the sitosterolemia locus. The intron–exon structure of the STSL locus, containing the genes ABCG5 and ABCG8, is as shown. The genes likely arose as a result of gene duplication and reside on opposite strands of the DNA, being transcribed in opposite direction. Note that the promoter region separating the two genes is exceedingly small, suggesting non-conventional regulation

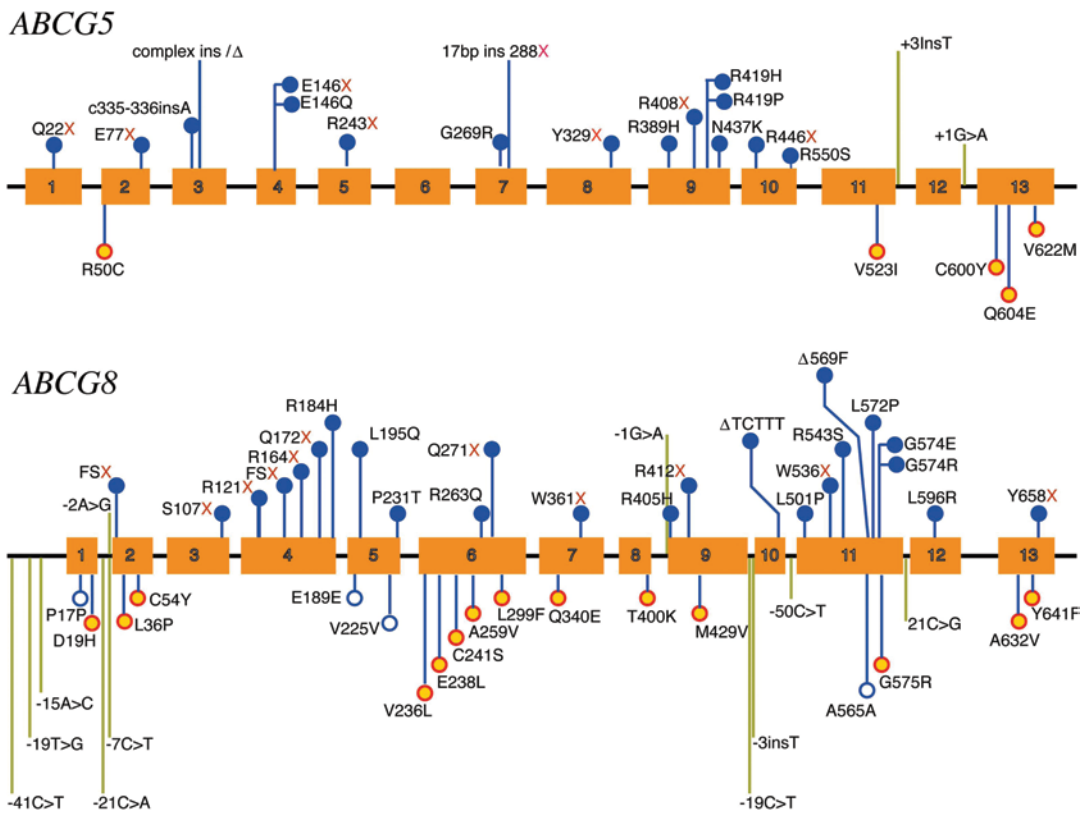

Fig. 13.2 Known mutations and common variations in ABCG5 and ABCG8 genes. Mutations in ABCG5 and ABCG8 that cause sitosterolemia are depicted *above* the gene structure, whereas polymorphic variants identified in normal humans are depicted *below* the gene. Some of the normal variants are very rare and their relevance is not established. Others have been shown to be associated with increased propensity to form gallstones [66]

Table 13.1 Presenting features of sitosterolemia

Signs and symptoms	Relative frequency[a]
Arthralgia	Common
Tendon or tuberous xanthomas	Common
Mildly elevated total cholesterol	Common
Mild anemia	Common
Thrombocytopenia	Common
Elevated liver enzymes (<3 ULN)	Common
Valvular thickening	Infrequent
Carotid bruits	Infrequent
Premature coronary artery disease	Infrequent
Severe hypercholesterolemia	In childhood only
Sudden death <40 years of age	Rare
Endocrine insufficiency	Very rare
Progressive liver disease	Very rare

[a]Based upon clinical observations reported only, no formal study available. Based upon the ezetimibe studies, we define common as ~30%, infrequent as <10%, rare as ~1%, and very rare as <1%. (Adapted from reference [8])

in the face of seemingly normal lipid profiles (e.g., total cholesterol <200 mg/dL or LDL-C <160 mg/dL), yet these two phenotypes are still much more frequent than sitosterolemia, and will lead to many more futile tests than uncover this disease. Some notable features should lead to increased suspicion. With the permission of Dr. Goldstein and Dr. Brown, we were able to re-contact some children they had investigated for FH, presenting with massively elevated LDL-C levels, but did not have any defects involving the LDL receptor. These children were deemed to have "pseudohomozygous" FH. We were able to redraw their blood, and showed that they had diagnostically elevated plant sterol levels (see Fig. 13.3). Why children go through a phase where plasma cholesterol levels are so high is not well understood. Prospective study of any child who may present with such high levels of cholesterol and does not have homozygous FH may shed light on the mechanism(s). Another feature that was also known early on was hemolysis, with some teenagers developing splenomegaly and therefore coming to the attention of hematologists. The hematological aspects have now gained more attention since the discovery that the only presenting feature may be macrothromobocytopenia (see Fig. 13.4), a condition initially reported in association with stomatocytosis [21]. Mutations affecting ABCG5 or ABCG8 have now been shown to cause this disease and the reported subjects did not seem to manifest any tendon xanthomas or arthralgias. Interestingly, mouse models of sitosterolemia have been reported to show these hematological features [22, 23], though the "pseudohomozygous FH" phase has not been reported. As with any rare disease, cases are now reported where for several years the correct diagnosis has not been made or delayed until this possibility is considered. A case of liver failure [24], a case of adrenal and ovarian failure [25], and the presence of valvular heart disease [26, 27] have all been described. Since the manifestation of any of these clinical features is depend upon exposure to xenosterols, it is important to consider the dietary components; an Iranian girl remained asymptomatic in her native country, where intake of plant-based foods was relatively minimal, but when she moved to Europe and started a "healthier" diet, she developed

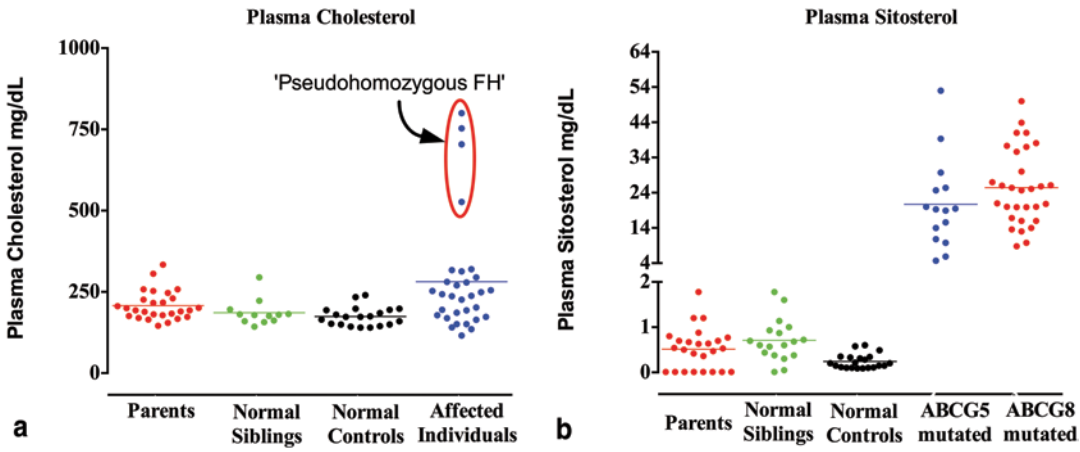

Fig. 13.3 Sterol profiles in families with sitosterolemia. The cholesterol profiles, determined by GC-MS, in parents (obligate carriers), normal siblings, sitosterolemic subjects, or random controls are shown. As can be seen, in general the cholesterol values are indistinguishable, except in four subjects who had massively elevated cholesterols. All four were previously labeled as "pseudohomozygous FH" and are only noted when subjects are children; adults with this pattern have not been reported. The panel on the *right* shows the plasma sitosterol levels, this time the affected subjects are segregated by whether they have mutations in ABCG5 or ABCG8. As can be seen, this does not affect the level of phytosterolemia. Additionally, the pseudohomozygous FH pattern has been seen in subjects with mutant ABCG5 and ABCG8

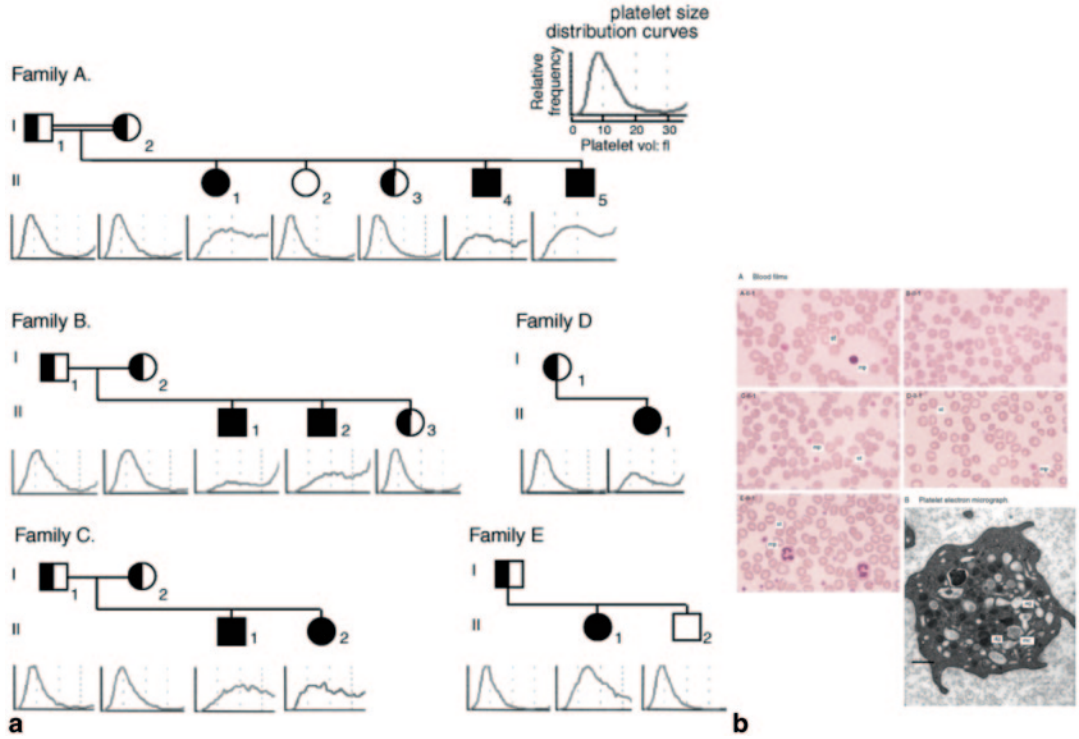

Fig. 13.4 Macrothrombocytopenia and stomatocytosis as presenting features of sitosterolemia. The panel on the *left* shows the platelet volume profile plotted versus the relative frequency (*y*-axis), in each of the family members. The filled symbols indicate sistosterolemic subjects; obligate carriers are in half-filled symbols and normal are unfilled symbols. As can be seen, sitosterolemia leads to reduced and larger platelets in all of the affected subjects. The panel on the *right* shows the blood films from affected subjects and shows the presence of macroplatelets, as well as stomatocytes and an electron micrograph shows that the platelet is very large, but does not have any abnormal structures or granules. (Reproduced from reference [21], with permission from John Wiley and Sons)

many of the features that led to the diagnosis of sitosterolemia [28]. This also highlights another key feature, namely the key xenosterol(s) that is pathological has not been established. The various different xenosterols in any food component will vary significantly, not only between different types of plant sources (Brassica foods compared to starches, etc.). While sitosterol is clearly the most abundant plant sterol, in vitro other plant sterols, such as avenosterol, fucosterol, stigmasterol, etc., seem to be more potent at activation of the transcriptional factor LXR [29]. These latter sterols are also less abundant and no study has correlated levels of these relatively harder to measure sterols and whether they are causatively related to the clinical features.

Laboratory Tests

The diagnostic test is to measure the plant sterol in the plasma or serum (or tissue) [7]. Elevated plant sterols are diagnostic for sitosterolemia and no other disease condition has been shown to mimic this [30]. Conventional "cholesterol" tests are performed using enzymatic assays that measure all sterols, and thus do not distinguish between xenosterols and cholesterol. However, under normal circumstances, more than 99% of the sterols in normal humans is cholesterol, thus the utilization of this assay is valid. However, to detect plant sterols, one needs to utilize methods that can separate and distinguish between these different sterols. This is accomplished using

either gas chromatography or high-performance liquid chromatography, and can be aided by using mass spectroscopy in tandem. In the USA, a limited number of centers perform these analyses for clinical diagnostic use and this can be ordered via all local laboratories as a send-out test. Normal humans have plant sterols that are typically <0.5 mg/dL, although rare normal individuals with levels as high as 1–2 mg/dL have been reported. All sitosterolemia subjects typically have plants sterols that are >10 mg/dL, making this diagnosis definitive [18]. Molecular diagnostic testing for mutations in ABCG5 or ABCG8 can also be used, but are not necessary, as the elevation of plant sterols is diagnostic.

Differential Diagnosis

Tendon xanthomas, in the presence of normal to moderately elevated cholesterol levels, may also suggest a diagnosis of cerebrotendinous xanthomatosis (CTX; see below). However, the plasma/serum plant sterol levels are diagnostic for sitosterolemia. Many other conditions can also result in macrothrombocytopenia, though most of these are also relatively rare. In cases of thrombocytopenia where no cause has been identified definitively, we would recommend a single determination of blood plant sterol levels. As these conditions are so much more prevalent than sitosterolemia, this diagnosis should be considered where the definitive diagnosis is absent and where other associated factors have been identified (e.g., presence of large platelets, >12 fl, or thrombocytopenia and valvular heart disease, or liver disease, or premature atherosclerosis, etc.).

Complications

Untreated sitosterolemia has been shown to be fatal [6, 26] with sudden cardiac deaths, premature atherosclerotic disease, and hematological disease that does not improve until a correct diagnosis has been made. The commonest complications of this disease are premature atherosclerotic disease and macrothrombocytopenia. However, rare cases of liver, adrenal, and ovarian failure have been reported as well as fatal and nonfatal valvular disease.

Clinical Course and Treatment

Limiting the intake of foods that contain xenosterols would seem to be a reasonable strategy, except this is exceptionally difficult to achieve, as a balanced diet for healthy living requires a varied diet. Additionally, ABCG5/ABCG8 are also important players for biliary cholesterol excretion, and a diet only containing animal products may result in increased propensity to atherosclerosis. Therapy is therefore aimed at preventing xenosterol absorption. Bile acid resin therapy formed the mainstay, until the discovery of the dietary sterol-blocking agent, ezetimibe (Zetia®). Prior to the approval of this drug as a cholesterol-lowering agent, the mechanism of action was not known and ABCG5/ABCG8 were considered a potential target. In order to evaluate this hypothesis, subjects with sitosterolemia were enrolled in a short-term study with daily ezetimibe, and to the surprise of the investigators, lowered plant sterols by 21–24% [31]. An extension study showed maximal sitosterol reductions of 44% at 52 weeks on 10 mg of ezetimibe [32]. These data led to the approval of ezetimibe as a specific therapy for sitosterolemia and is a unique instance where a billion dollar drug was codeveloped for the benefit of a very rare condition prior to approval. The inventors of ezetimibe went on to identify the true target of this drug, and showed that the molecule NPC1L1, which is now known to function as a key molecule that allows dietary sterol entry into the enterocyte [33], and that ABCG5/ABCG8 allow for sterol exit. As this is a rare condition, while case reports suggest benefit at individual levels who are treated with ezetimibe, it is not clear if this will be translated to blocking all features of this disease. Prior to this, bile acid sequestrants were the mainstay of therapy, although these reduced plant sterols by only 10–15% (G. Salen, *pers commun*), but intolerance to these agents is high (mainly constipation or diarrhea).

Sterol Synthesis Disorders

At the turn of the last century, it was assumed that cholesterol was a preformed molecule we absorbed from our diets, and it was not until the elegant work by Schoenheimer who showed that cholesterol not only was synthesized in the mammalian body but also could be destroyed by the mammalian body [5]. However, the identification of sterol synthesis disorders (as defined by affecting any sterol synthetic pathway beyond squalene, the first committed sterol synthesis intermediate) affecting humans is a very modern discovery. A number of human sterol synthesis disorders are now known; the Smith–Lemli–Opitz syndrome, demosterolosis, lathosterolosis, Conradi–Hunermann syndrome, CHILD congenital hemidysplasia with ichthyosiform erythroderma and limb defects) syndrome, CK syndrome, and sterol C4 methyloxidase deficiency [34]. These disorders are all congenital disorders and present as malformation syndromes, not dyslipidemia. However, all of these conditions do exhibit lower than normal cholesterol profiles, typically in the lower 5th centiles. Since these disorders do not typically present with dyslipidemia, their discussion is curtailed herein. The reader is directed to a recent review of this topic [35].

Smith–Lemli–Opitz Syndrome

History

A new dysmorphology syndrome, termed RSH syndrome, was described in 1964 by three astute clinical geneticists [36]. Their clinical definition led to more cases with similar dysmorphological findings identified (see Fig. 13.4), but the genetic basis for this syndrome remained elusive. The insight into this came when two other clinical geneticists, Drs. Mira Irons and Ellen Elias, collaborated with Dr. G. Stephen Tint's group and showed that a low serum cholesterol, but a very high precursor sterol, 7-DHC, was a strong marker for this condition [37, 38]. They proposed that the Smith–Lemli–Optiz syndrome (SLOS, now an accepted term after the discoverers) was actually a disease caused by a defect involving an enzyme, dehydrocholesterol Δ7 reductase (DHCR7), that catalyzes the conversion of 7-DHC to cholesterol. This was viewed very skeptically by the clinical geneticist community as no dysmorphology syndrome had been known previously to be caused by a defect in a metabolic enzyme. Over the ensuing years, the biochemical test of 7-DHC became a diagnostic test for this disease and garnered greater acceptance of this hypothesis. This was solidified when Drs. Glossmann, Utermann, and their colleagues cloned the gene for DHCR7 and demonstrated mutations on a cohort of subjects diagnosed with Smith–Lemli–Opitz syndrome [39, 40], a finding verified by several other groups [41–43].

Epidemiology

The incidence of SLOS varies by region with an estimated 1:40,000 live births in the USA, but increasing to 1:20,000 in Eastern Europe. SLOS is much less common in Asia and Africa [44].

The true incidence may be masked for this autosomal recessive condition, as loss of pregnancy early on may be caused by this condition, but the family would not be investigated further if a subsequent normal pregnancy results. If only when a live birth with dysmorphology, or an abortus with dysmorphology is detected would this diagnosis be entertained.

Etiology and Pathogenesis

Genetic defects of the enzyme dehydrocholesterol Δ7 reductase (DHCR7) are responsible for causing this condition. The reduction of the Δ7 bond in the sterol molecule is absolutely necessary for cholesterol synthesis, whether the synthesis follows the Bloch or the Kandutsch–Russell pathways. Failure to do so results in the accumulation of the immediate precursor 7DHC, which can spontaneously isomerize to 8DHC. Despite the knowledge of the enzymatic defect, the pathophysiological mechanisms that then lead to the highly specific dysmorphology syndrome

remains a challenge. Cholesterol fulfills a host of protean functions, ranging from its structural role in membranes, lipoproteins, in specialized membranes such as myelin, as part of the skin barrier, as a substrate for bile acid synthesis, or steroid hormones and even as esoteric as modifiers of protein structure, such as a covalent tail for the hedgehog proteins, etc. In addition, cholesterol metabolites have important regulatory roles. Each of these roles has been examined in the pathogenesis of SLOS, and while some evidence for each of these pathways being potentially disrupted has been accumulated, a unifying pathway linking these has not been easy to forge. A recent summary of potential mechanisms can be found in reference [45].

Clinical Findings

This condition is very strongly associated with dysmorphology, thus the presentation will be typically at the neonatal and pediatric stages [35]. It is highly unlikely to present to most general internists. However, as with all relatively rare diseases, knowledge about this disorder may alert consideration of this condition as a "missed" diagnosis. The presentation of SLOS ranges from a severe dysmorphology and intrauterine death and spontaneous abortion, to being born with a number of developmental defects (Fig. 13.4). These range from external characteristic facial dysmorphology (flat face, micrognathia or retrognathia, short palpebral fissues, low set ears, short nose with concave nasal ridge and anteverted nostrils, cleft palate), bifid uvula, cataracts, polydactyly, 2–3 syndactyly, hypospadias and ambiguous genitalia in males, together with internal organ developmental defects ranging from midline CNS malformations (holoprosencephaly, absent corpus callosum, cerebellar hypoplasia, etc.), hypotonia, congenital cardiac defects (almost all kinds), renal agenesis and cysts, pulmonary hypoplasia, to intestinal malformations and Hirschsprung disease [44]. Developmental cognitive defects are evident as these children age, with mental retardation and behavioral issues as very common sequelae. However, for the internist, rare cases of SLOS have been described where there are almost no structural defects, may have the mildest of 2–3 syndactly and no mental retardation [46]. In these cases, the diagnosis is suspected based upon the above clinical features and a consideration of the diagnosis, confirmed by biochemical testing (see below). In all of the cases, the "dyslipidemia" is a low plasma cholesterol level [38]. Thus for the internist facing a child, or a young adult in whom there are subtle signs of mild cognitive defects, with a remote history of some corrected midline organ defect, and an examination showing 2–3 syndactyly, one could suspect the diagnosis of SLOS.

The genetic basis of SLOS is an autosomal recessive genetic defect involving the enzyme dehydrocholesterol Δ7 reductase that is responsible for reducing 7-DHC to cholesterol [47]. This enzyme also reduces 7-dehydrodesmosterol to desmosterol (which is further reduced by 24-dehydrocholesterol reductase to form cholesterol). Failure to do so results in failure to synthesize cholesterol, with the diagnostic accumulation of the precursor sterol, 7DHC, in the blood and tissues.

Laboratory Tests

The diagnostic test is the determination of 7DHC in the blood, and requires the use of high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS), but is available as a send-out laboratory test. Combined with clinical features, a low plasma cholesterol and an elevated 7DHC is diagnostic for SLOS. Very rarely, mild elevations in 7DHC can be seen in CTX, but the clinical features are very different (see below). Molecular diagnostic testing for mutations in *DHCR7* gene can also be used, especially as three mutations account for >70% of the mutations causing SLOS. Molecular testing may aid cases where the clinical features are very mild, the plasma 7DHC levels are minimally elevated, but SLOS is strongly suspected. Figure 13.5 shows depiction of some of the mutations of DHCR7 that cause loss of enzyme activity. A newer compendium of more than 150 mutations has been assembled by Waterham and Hennekam [47].

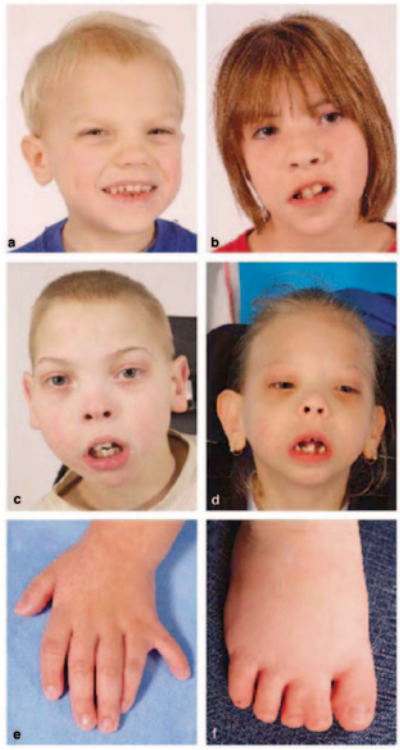

Fig. 13.5 Dysmorphological features commonly seen in SLOS. Panels a–d shows typical features of Smith–Lemli–Optiz syndrome (SLOS) patients; microcephaly, ptosis, broad nasal bridge, upturned nose, and micrognathia. Panel e shows a short proximally placed thumb, clinodactyly, and postaxial polydactyly, with syndactyly (the commonest SLOS finding) of the second and third toes (f). (Reproduced from reference [45], with permission from Nature Publishing Group)

Differential Diagnosis

As stated above, a defect at almost any of the postsqualene sterol synthesis defect could mimic SLOS [35], although the sterol diagnostic determination allows for clear distinction. However, in the mildest of SLOS cases, where the elevation of 7-DHC may not be dramatic and the presence of any major structural defects absent, one may have to consider genetic analyses of the DHCR7 locus. Any condition that leads to partial inhibition of DHCR7 activity, as seen in untreated CTX, may lead to very mild elevations in 7DHC. However, these two conditions are sufficiently different to allow for distinction (Fig. 13.6).

Clinical Course and Treatment

The commonest clinical issues are the major structural defects involving almost any organ system that may require surgical repair or amelioration. Additionally, mental retardation, significant cognitive defects, as well as seizures, behavioral issues, hyperstimulation, and autism-like neurological issues are frequently described. The accumulation of 7-DHC in the skin is also thought to lead to skin photosensitivity in some cases. Cases of adrenal insufficiency have been reported.

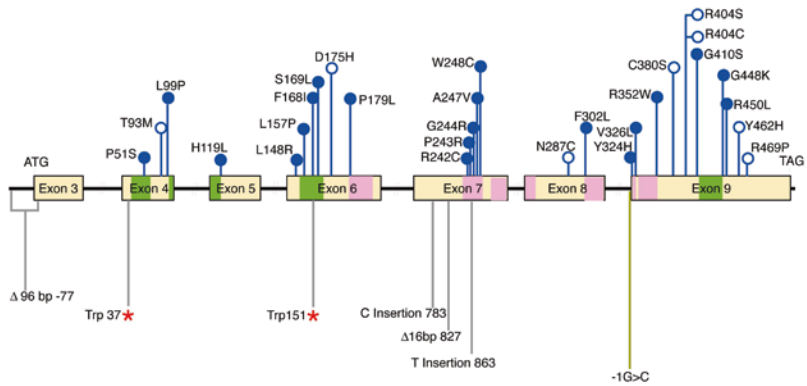

Fig. 13.6 Mutational spectrum of the DHCR7 gene in Smith–Lemli–Optiz syndrome (SLOS). The distribution and variety of mutations observed of the DHCR7 gene is shown. As can be seen, these affect almost any part of the gene, although five gene mutations are highly prevalent, namely IVS8-1G>C (c.964-1G>C), R404C, T93M, W151X, and V326L. However, now more than 150 unique mutations have been reported (see ref. [47])

There are few well-designed randomized clinical studies to direct treatment [48]. Since the defect involves a failure to synthesize cholesterol, the standard approach is to increase dietary cholesterol with supplementation using purified products. Once the enterohepatic bile acid pools are restored, there is adequate absorption of cholesterol from the diet to reduce the plasma levels of 7-DHC. However, it is not clear if this affects any of the longer term consequences, such as behavioral issues or mental retardation, as controlled studies testing this have been difficult to conduct [49]. One approach, that to inhibit cholesterol synthesis by use of statin drugs (in conjunction with cholesterol supplementation), has not led to any meaningful conclusions to be drawn, despite improved biochemical changes [48]. Thus, long-term management is aimed at expectant management, or correction of any structural defect.

Sterol Breakdown Disorders

The major pathway by which the body can rid itself of cholesterol is to excrete cholesterol into the intestinal lumen (via biliary secretion, major, or directly via the intestine) or by breakdown of the cholesterol molecule via the bile acid synthesis pathway and excretion via the biliary system. There are a number of genetic or acquired defects involving the bile acid/biliary secretion pathway that can lead to dyslipidemia. For example, severe hyperlipidemia can be seen under conditions of cholestasis. This section focuses only on the genetic pathways that lead to bile acid synthesis defects using CTX as an example. The reader is directed to some excellent reviews on a wider aspect of genetic disorders that can affect bile acid secretion.

Cerebrotendinous Xanthomatosis

History

In 1937, Von Bogaert, Scherer, and Epstein described a case that manifested progressive motor and cognitive neurological deterioration (having been very normal) and manifested juvenile cataracts, and tendon xanthomas [50]. In the subsequent years, many more similar cases were reported and a distinct clinical entity was formulated that consisted of the above, but with added observations that histological analyses showed increased cholesterol, but more importantly cholestanol deposits in brain samples [51–55]. Despite the presence of tendon xanthomas, the plasma cholesterols were not always elevated. The source of the cholestanol not well understood at that time, but it was felt that this was the primary reason for the neurological issues. With increasing cases, it became clear that this was a recessive trait, as the parents were normal, and so a genetic cause was suspected. The key observation by Salen that the bile acid amount was reduced by 50 % allowed him and his colleagues to demonstrate that chenodeoxycholic acid (CDCA) was almost completely absent in CTX subjects and that there was an accumulation of bile alcohols, and an inability to detect C-26 hydroxylated intermediates (now renumbered to be C27-hydroxylated intermediates) [56, 57]. Ensuing work from a number of other groups also confirmed a putative defect in CYP27A1, establishing that CTX was caused by a defect in this key bile acid synthesis enzyme necessary for the synthesis of CDCA (but not cholic acid, which is relatively normal in CTX). The next breakthrough came when Russell and his colleagues cloned the gene and characterized the enzyme for CYP27A1 and showed that this was not only responsible for the biochemical defects observed by Salen and his colleagues on the bile acid pathway but mutations of this enzyme were responsible for causing CTX [58, 59].

Epidemiology

The prevalence of CTX is probably very close to that of sitosterolemia. In the USA, there are somewhere between 60 and 80 subjects known. Thus, this is a truly rare disease. There are pockets of this disease, based upon isolated populations where an increased incidence is noted (such as Jews of North African origin). The disease is worldwide and is present on all five continents.

Etiology and Pathogenesis

The condition is inherited as an autosomal recessive condition and is caused by mutations affecting CYP27A1, encoding cholesterol 27-hydroxylase. The biochemical defect prevents the synthesis of CDCA, but not cholic acid (Fig. 13.7). The liver is the only organ that has all of the necessary enzymes for the synthesis of bile acids (see Fig. 13.7). These enzymes are also located in different compartments within the hepatocyte, and CYP27A1 is a mitochondrial enzyme. Under normal circumstances, 5β-cholestane-3α,7α,12α-triol is transported to the mitochondrion for further oxidation of this by CYP27A1 [60]. In its absence, this bile acid intermediate is transported back to the microsomes, where it is acted upon by 25-hydroxylase, part of which results in the synthesis of cholic acid but microsomal metabolism also leads to generation of tetrol, pentol, hexol bile alcohols, which are likely the major pathogenic molecules resulting in the pathogenesis of CTX. This microsomal pathway cannot generate CDCA, without 27-hydroxylation of the side chain. Bile acid alcohols are glucuronidated and can be found in significantly increased amounts in the blood, urine, and feces of untreated CTX subjects. Additionally, CTX subjects have increased generation of cholestanol. The bile alcohols are toxic, and lead to disruption of the blood–brain barrier, allowing increased accumulation of cholestanol, as well as disruption of CNS sterol metabolism, resulting in progressive neurological damage. This damage may also occur to peripheral nerves. It is this progressive damage and accumulation of sterols in the CNS that is responsible for the

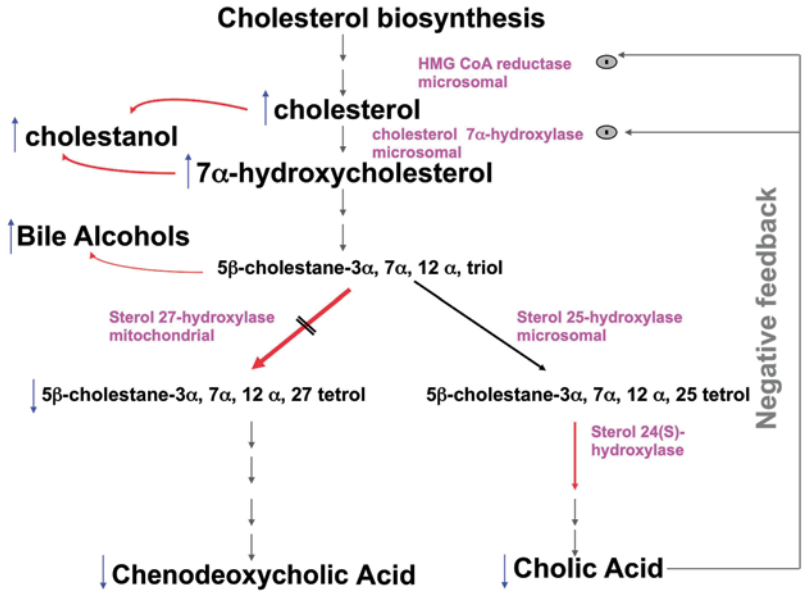

Fig. 13.7 Aberrant bile acid synthesis in cerebrotendinous xanthomatosis. Normal bile acid synthesis starts with 7α hydroxylation, which is quantitatively the most important pathway. Following the generation of 5β-cholestane-3α, 7α, 12α triol in the microsomal compartment, transfer of this sterol to the mitochondrion results in the production of 5β-cholestane-3α, 7α, 12α 27 tetrol but the action of CYP27A1, which is necessary for the synthesis if chenodeoxycholic acid (CDCA) but also of cholic acid (not shown for simplification). In cerebrotendinous xanthomatosis (CTX), where CYP27A1 is deficient, the block shown of the *bold red arrow,* leads to increased microsomal triols, where, through the action of CYP25, 5β-cholestane-3α, 7α, 12α 25 tetrol can be synthesized and thus cholic acid, but this pathway cannot lead to synthesis of any CDCA. The production of cholic acid remained diminished though. The lack of enough CDCA leads to reduced feedback inhibition of both the synthesis of cholesterol, as well as CYP7α, increasing flux via this pathway, thus further compounding the build-up of the intermediaries, resulting in accumulation of cholestanol, as well as bile alcohols

neurological defects. CYP27A1 is an enzyme also present in many other sites in the body, including macrophages. Accumulation of both cholesterol and cholestanol in macrophages is likely responsible for the pathogenesis of xanthomas, though the precise mechanisms have still to be defined. Additionally, this enzyme is also present in osteoclasts, and although osteoporosis is also a feature of CTX, the mechanism of this is also undefined at present.

Clinical Findings

The clinical presentation of CTX starts very early in life. There are two clinical presenting features that warrant highlighting; intractable diarrhea associated with failure to thrive in the first 3–4 years of life, and the development of juvenile cataracts, where there are no identifiable precipitating factors (such as steroid use, radiation, known genetic diagnosis, etc.). Many CTX patients report having had diarrhea that would not easily subside when they were very young, and cataracts by the age of 21 years seem to be almost universal in untreated cases. Other presenting features in childhood include psychomotor retardation and neurological damage early on may show signs of pyramidal as well as cerebellar damage. Rare cases of hepatitis have also been reported as presenting features in this age category. CTX, however, remains a misdiagnosis and the majority of cases continue to be diagnosed later in adult life, when they present with tendon or tuberous xanthomas (in almost all cases, the Achilles tendon is invariably involved), and neurological features that range from long tract signs, pyramidal paresis, bulbar palsies, cerebellar dysfunction, dystonia, and movement disorders (including signs of Parkinson's), peripheral neuropathies and progressive psychomotor and cognitive deficiencies. Thus, this diagnosis should be entertained in anyone who has juvenile cataracts removed, has any neurological signs and symptoms and especially if their Achilles tendons look bigger than normal. Other reported clinical manifestations include premature atherosclerotic disease, epilepsy, and osteoporosis.

Laboratory Tests

The laboratory tests for CTX include the determinations of plasma cholestanol levels, typically using a GC-MS, or HPLC techniques, looking for elevated cholestanol levels. The standard lipid test will not show major abnormalities, beyond perhaps some mild hyperlipidemia. Sterols need special testing. However, since cholestanol can be elevated under many other conditions (including sitosterolemia), the diagnostic tests include the determinations of bile alcohols in the plasma, urine, or feces of affected individuals. Molecular diagnostic testing for mutations in CYP27A1 can also be used. In isolated communities with a high rate of CTX, mutational screening is not only feasible, it may be cost-effective as therapy can be initiated as early as possible [61]. For the majority of CTX cases, this may not be feasible. Figure 13.8 shows a depiction of the mutations that have been reported by us, and how these map onto putative model of CYP27A1 [62]. However, the clinical presentation, together with the bile alcohol determinations, is usually all that is necessary to make an accurate diagnosis. In rare cases, where clinical signs suggest CTX, but no other features are helpful and diagnostic tests are equivocal, one can provoke accumulation of bile alcohols but depletion of body pools of bile acids using ingestion of bile acid resins for 48 h before the plasma and urine are analyzed. The loss of bile acids in the intestine leads to upregulation of the bile acid synthesis pathway and should exacerbate any enzymatic block in the pathway.

Differential Diagnosis

In any patient who presents at an early age with only tendon or tuberous xanthomas, the differential diagnosis includes familial hypercholesterolemia (plasma cholesterol is only moderately elevated CTX and is thus "diagnostic" excluder), and sitosterolemia. The test that measures cholestanol will also detect plant sterols, thus examination of the chromatogram allows for this distinction.

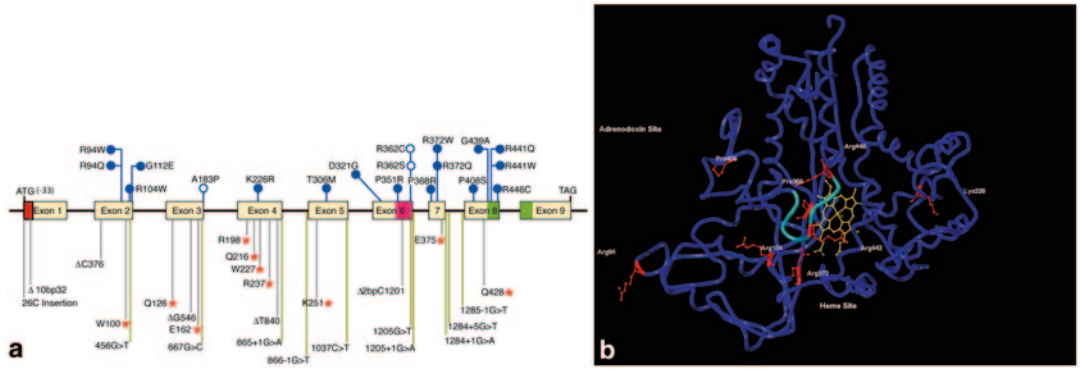

Fig. 13.8 Structural mapping and mutational spectrum affected in CYP27A1 in cerebrotendinous xanthomatosis (CTX). The positions of known mutations affecting CYP27A1 are shown on a depicted intron–exon structure of CYP27A1 in the panel on *right*. All of these are known to be pathogenic, except three (shown in *open circles*) which may be normal variants. Despite the "scatter" of these mutations, mapping these onto a model of CYP27A1 (*left-hand panel*) shows that almost all of these affect the critical heme-adrenodoxin binding domain of CYP27A1 [62]. This domain is critical for enzymatic activity

Clinical Course and Treatment

With the institution of early and adequate replacement with oral CDCA, almost all of the CTX disease manifestations (except xanthomas) are greatly ameliorated and prevented [63–65]. CDCA therapy aims to suppress the bile acid synthesis pathway in the liver, greatly diminishing the generation of bile acid intermediaries and thus the bile alcohols. This allows for any damaged blood–brain barrier to heal and thus prevent any neurological damage from accumulation of cholestanol (and likely other toxic products) in the CNS. Restoration of CDCA into the enterohepatic circulation also improves digestion and absorption of fat and fat-soluble vitamins with an increase in weight. As this disease is rare, there are no large long-term prospective studies that have accumulated enough subjects treated very early on with CDCA to document the outcome. However, retrospective studies show that initiation of CDCA can result in significant reversal of signs and symptoms in many subjects, with consolidation of these gains with continued therapy. The addition of a statin, to suppress cholesterol synthesis, has also been shown to improve biochemistry and some clinical features in anecdotal case reports. The key to successful therapy is not only to lower the cholestanol levels (frequently used as a marker of the disease) but also to ensure that all bile alcohols have been cleared from the blood (or urine), as the latter are more directly an indicator of disease activity. Cataract development may not be affected by early therapy, though this aspect has not been well studied. Finally, the development of xanthomas, especially at sites of repeated trauma may also continue to be an issue, despite good biochemical control. Presumably, this is because the pathogenesis of xanthoma formation may be related more closely to the absence of CYP27A1 in the macrophages, than to the direct effects of the bile acid intermediaries on macrophage biology. Removal of xanthomas may further aggravate local xanthoma formation and is not recommended. To improve bone health, supplementation of Vitamin D is recommended, with monitoring of bone density where clinically necessary. Atherosclerotic heart disease has also been reported in CTX and thus lowering of plasma cholesterol with statins is also recommended in middle-aged adults and in women unlikely to be childbearing. Women with treated CTX have successfully carried pregnancy to term with no complications, suggesting fertility is not affected and there are no major defects of the endocrine system. Regular medical

monitoring is mandatory to ensure that no ongoing damage to the CNS accrues, as neurological damage is the most important comorbid factor for a good quality of life.

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Genetic Abetalipoproteinaemia and Hypobetalipoproteinaemia

14

Amanda J. Hooper and John R. Burnett

Introduction

Inherited diseases of lipoprotein metabolism may give rise to marked hypocholesterolaemia with low or absent levels of betalipoproteins, depending on the gene involved and mode of inheritance of the condition, together with the severity of the mutation or mutations present [1, 2].

The most extreme form of these disorders is abetalipoproteinaemia (Online Mendelian Inheritance in Man (OMIM) 200100), a very rare recessive disorder characterised by the absence of apolipoprotein (apo) B-containing lipoproteins in plasma, leading to a variable clinical phenotype that presents in early childhood with fat malabsorption, steatorrhoea, and failure to thrive, and may include progressive neurological and ophthalmological abnormalities as the patient ages [3]. The molecular basis of this disorder is the inheritance of two mutations in the microsomal triglyceride transfer protein gene (*MTTP*), a chaperone protein critical for the assembly and secretion of apoB in the formation of very low-density lipoprotein (VLDL) and chylomicrons.

Low plasma concentrations of low-density lipoprotein (LDL)-cholesterol and apoB are observed in familial hypobetalipoproteinaemia (OMIM 107730), a codominant disorder of lipoprotein metabolism caused by the inheritance of a mutation in *APOB,* usually giving rise to a truncated apoB protein [4]. Patients are generally asymptomatic, but may be at increased risk of fatty liver disease. Inheritance of two such mutations in *APOB* is known as homozygous familial hypobetalipoproteinaemia and is clinically indistinguishable from abetalipoproteinaemia.

Chylomicron retention disease (OMIM 246700) is characterised by the selective absence of apoB-48 containing particles. Instead of being incorporated into chylomicrons, lipid droplets accumulate within the enterocytes [5]. Clinical findings include fat malabsorption, diarrhoea, abdominal distension, vomiting, and failure to thrive. Patients with chylomicron retention disease inherit two defective copies of secretion associated, Ras related GTPase 1B (*SAR1B*), the product of which is critical for the intracellular trafficking of chylomicron particles.

In recent years, additional causes of inherited hypobetalipoproteinaemia have been found in the proprotein convertase subtilisin kexin 9 (*PCSK9*) and the angiopoietin-like 3 (*ANGPTL3*) genes, although these have not been associated with any clinical symptoms. Loss-of-function mutations in *PCSK9* result in reduced concentrations of LDL-cholesterol in a gene dose-dependent

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manner, leading to a lifetime low risk of cardiovascular disease [6]. Mutations in *ANGPTL3* are associated with recessive familial combined hypolipidaemia, characterised by a reduction in all plasma lipids, including high-density lipoprotein (HDL)-cholesterol [7, 8].

This chapter reviews the molecular basis, pathogenesis, and clinical aspects of these disorders of apoB production and catabolism, focusing on abetalipoproteinaemia, familial hypobetalipoproteinaemia, and chylomicron retention disease.

History

Abetalipoproteinaemia was originally named Bassen–Kornzweig syndrome, after the two physicians who in 1950 described the clinical association of peripheral blood acanthocytosis, atypical retinitis pigmentosa, and ataxia [9]. An autosomal recessive mode of inheritance was suggested, but it was not until 1958 that the observation of low levels of serum cholesterol was made, and in 1960 the absence of beta-migrating lipoproteins by electrophoresis (betalipoproteins) described, leading to the name of the disease being changed to abetalipoproteinaemia [10]. The distinction was then made between abetalipoproteinaemia and homozygous familial hypobetalipoproteinaemia, whereby carriers of familial hypobetalipoproteinaemia have marked hypocholesterolaemia while carriers of abetalipoproteinaemia do not [11].

In 1987, a truncated apoB was found in the plasma of a family with familial hypobetalipoproteinaemia [12], leading to a four-nucleotide deletion being identified in the *APOB* gene [13]. The finding of absent MTTP in hepatic and intestinal microsomes in 1992 suggested that defects in this protein were the cause of abetalipoproteinaemia [14], with mutations subsequently identified in the *MTTP* gene the following year [15, 16].

Chylomicron retention disease was originally named Anderson's disease, after the physician who in 1961 described an infant with fat malabsorption and fat-laden enterocytes on histology, in whom chylomicrons were absent from plasma after meals, and plasma lipids and fat-soluble vitamin levels were low [17]. It was subsequently shown that in Anderson's disease, the enterocytes reacted intensely to monoclonal antibodies to apoB-48, but not to those selectively reactive with apoB-100 [18]. In 1991, the *APOB* gene was excluded as a cause of Anderson's disease [19]. In 2003, mutations in the *SAR1B* gene were identified in patients with Anderson's disease and chylomicron retention disease, revealing that they were, in fact, the same disease [20].

Epidemiology

Data from the Framingham Offspring Study identified persistent hypobetalipoproteinaemia in 1.9% of over 3800 individuals, and a truncated apoB species causing familial hypobetalipoproteinaemia in only one of these subjects [21]. This gives an estimated prevalence of the condition at ~1 in 3000, taking into account that the immunoblot testing method used does not detect circulating plasma apoB of a size less than 30% of the full-length protein. The prevalence of abetalipoproteinaemia and chylomicron retention disease is unknown, but these conditions appear to be extremely rare (<1 in 1 million).

Heterozygous *PCSK9* nonsense mutations can be found in ~2% of Africans and African-Americans [22, 23], which predicts homozygosity in ~1 in 10,000 in these populations.

Etiology and Pathogenesis

Abetalipoproteinaemia

Patients with abetalipoproteinaemia carry two defective copies of the *MTTP* gene on chromosome 4q22–24. The role of MTTP in this disorder was first implicated in 1992, when its activity was not detected in intestinal biopsies of patients with abetalipoproteinaemia [14]; mutations in the *MTTP* gene were subsequently described [16]. *MTTP* encodes an 894 amino acid protein, which forms a heterodimer with the ubiquitous endoplasmic reticulum enzyme protein disulfide isomerise (PDI) [24]. The function of the MTTP heterodimer is to facilitate the transfer of lipids to nascent apoB by a shuttle mechanism [24]; lack of MTTP activity results in insufficient lipidation

of nascent apoB and targets the apoB to a degradation pathway [25], preventing the secretion of triglyceride-rich lipoproteins. Mutations in *MTTP* associated with abetalipoproteinaemia may disrupt production of the normal MTTP protein, disrupt its binding with the PDI subunit, or affect MTTP's lipid transfer activity [26–28].

Familial Hypobetalipoproteinaemia

In familial hypobetalipoproteinaemia, mutations in the *APOB* gene on chromosome 2p23–24 either abolish the expression of apoB or interfere with the translation of full-length apoB leading to formation of prematurely truncated apoB forms [1, 30–32]. These apoB truncations have traditionally been named according to the centile system that also gave the name to apoB-48. The majority of mutations are nucleotide substitutions and deletions in exon 26, which at over 7500 nucleotides is one of the largest exons in the human genome. Several missense mutations in the N-terminal βα1 domain of apoB causing familial hypobetalipoproteinaemia have also been described [33–35]. The mutation R463W was shown to cause impaired secretion of VLDL by impaired endoplasmic reticulum exit and enhanced binding of the mutant protein to MTTP [33]. It is worth noting that missense mutations in the LDL-receptor-binding domain in the carboxyl-terminus of apoB cause familial ligand-defective apoB-100, a form of familial hypercholesterolaemia [36].

In patients with familial hypobetalipoproteinaemia, truncated forms of apoB are produced at lower rates (about 25%) than apoB-100 [37]. The secretion rate was shown to be reduced by about 1.4% for each 1% of apoB truncated [38]. However, for every 10% decrease in apoB length, there is a 13% reduction in the lipoprotein core volume, indicating that the lipid content of secreted apoB-containing lipoproteins is decreased as apoB is shortened [39]. Truncated apoB species shorter than apoB-30 are not detectable in plasma; this appearing to be the minimum length of apoB that is required for MTTP-dependent lipoprotein assembly. Shorter apoB species are unable to acquire sufficient lipid, leading to intracellular degradation rather than secretion [40]. In addition, clearance of the truncated apoB species is faster than the clearance of apoB-100, particularly for the longer truncations such as apoB-89 that contain the LDL-receptor-binding domain, resulting in enhanced LDL-receptor-binding [41].

Linkage analysis in families with familial hypobetalipoproteinaemia without mutations in *APOB* has identified chromosomes 3p21.1–22 and 10q25.1–10q26.11 as susceptibility loci, but the genes responsible remain to be identified [42, 43].

Chylomicron Retention Disease

Mutations in *SAR1B,* a member of the Sar1-adenosine diphosphate (ADP)-ribosylation factor family of small GTPases that control the intracellular trafficking of proteins, are the cause of chylomicron retention disease [20]. SAR1B is needed for the fusion of the intestine specific pre-chylomicron transport vesicle to the Golgi apparatus, allowing transport of chylomicrons through the cellular secretory pathways [44]. Mutations in *SAR1B* result in the inability to secrete chylomicrons, resulting in the accumulation of lipid droplets within the enterocytes.

Other Molecular Causes of Hypobetalipoproteinaemia

PCSK9 is an important regulator of plasma LDL-cholesterol concentrations [45]. It is a protease that binds to the LDL-receptor and targets it for lysosomal degradation within hepatocytes. Gain-of-function missense mutations in *PCSK9* can cause a severe autosomal dominant form of hypercholesterolaemia [46]. In contrast, nonsense and loss-of-function *PCSK9* missense mutations increase the number of LDL-receptors on the cell surface and, therefore, the number of LDL particles able to be internalised by the cell, reducing both circulating LDL-cholesterol concentrations and coronary heart disease risk (Fig. 14.1).

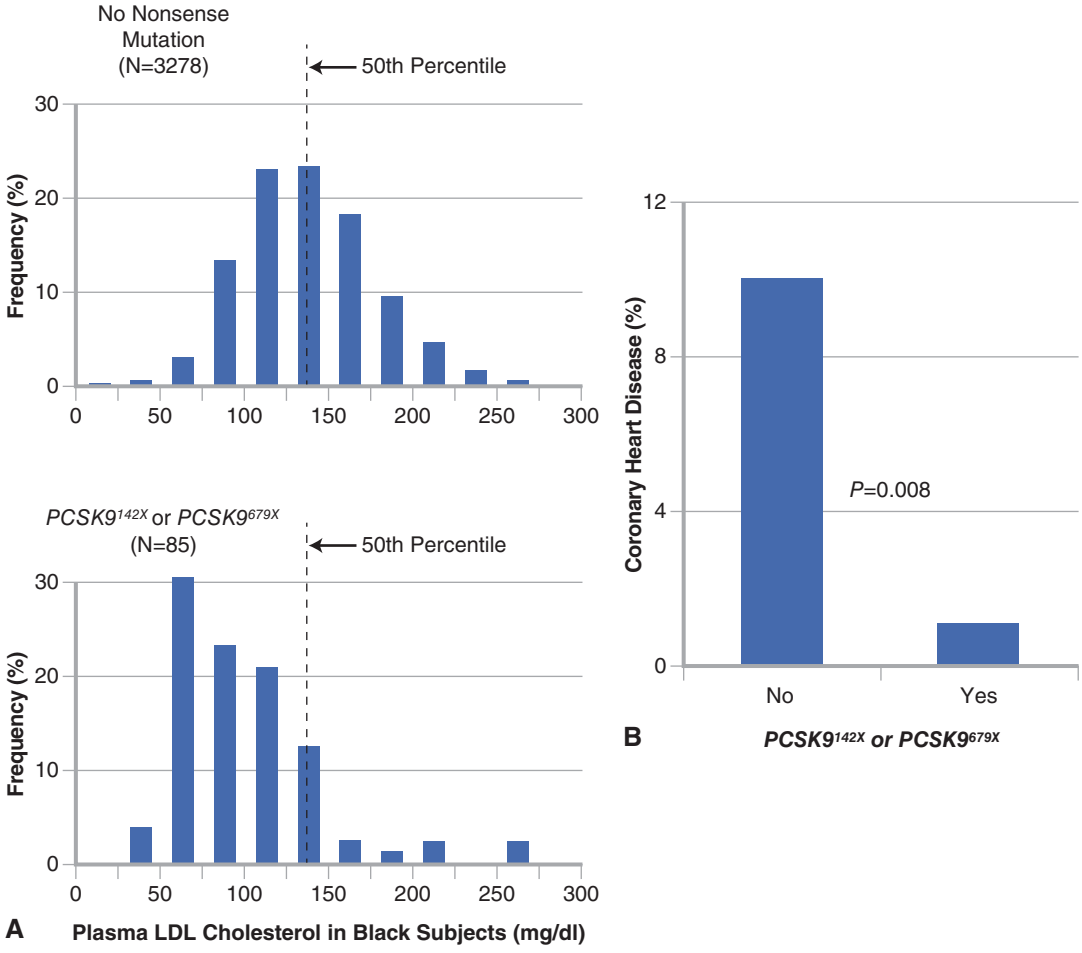

Fig. 14.1 Distribution of plasma LDL cholesterol levels and incidence of coronary heart disease, according to the presence or absence of a *PCSK9*^{142X} or *PCSK9*^{679X} allele. **a** The distribution of plasma LDL-cholesterol levels at baseline among 3278 black subjects who did not carry a *PCSK9* nonsense mutation (*top*) compared to the 85 subjects who carried either *PCSK9*^{142X} or *PCSK9*^{679X}. **b** The percentage of participants from these two groups who had no evidence of coronary heart disease (CHD) at baseline and in whom CHD developed during 15 years of follow-up. *LDL* low-density lipoprotein, *PCSK9* proprotein convertase subtilisin kexin 9. (Adapted from, Cohen 2007) [6]

ANGPTL3 gene mutations are associated with a combined hypolipidaemia. The function of ANGPTL3 appears to be the reversible inhibition of lipase activity, involving endothelial lipase [47], lipoprotein lipase [48], or hepatic lipase [49]. The disruption of ANGPTL3 production would therefore increase lipolysis, enhancing clearance of lipoproteins and decreasing circulating lipid concentrations.

Classification

Lipoprotein disorders causing primary hypocholesterolaemia—abetalipoproteinaemia and hypobetalipoproteinaemia—are classified depending on the lipid biochemical phenotype, gene involved, and mode of inheritance of the condition, together with the severity of the mutation or mutations present (Table 14.1).

Table 14.1 Lipoprotein disorders causing genetic abetalipoproteinaemia and hypobetalipoproteinaemia

Lipoprotein disorder	Gene	Inheritance	Biochemical phenotype	Clinical phenotype
Abetalipoproteinaemia	*MTTP*	Recessive	Absence of LDL and chylomicrons	Variable; includes failure to thrive, steatorrhoea, progressive neurological and ophthalmological abnormalities
Familial hypobetalipoproteinaemia	*APOB*	Codominant	Heterozygous: LDL-cholesterol 30% levels of normal for age and sex Homozygous: absence or very low levels of LDL-cholesterol	Heterozygous: generally asymptomatic, may include fatty liver Homozygous: indistinguishable from abetalipoproteinaemia
Chylomicron retention disease	*SAR1B*	Recessive	Absence of chylomicrons LDL-cholesterol < 50% levels of normal for age and sex	Variable; includes failure to thrive, steatorrhoea, and progressive neurological abnormalities
Familial hypobetalipoproteinaemia	*PCSK9*	Codominant	~40% reduction in LDL per allele	None
Familial combined hypolipidaemia	*ANGPTL3*	Recessive	Reduced levels of all plasma lipoproteins	None

MTTP microsomal triglyceride transfer protein, *LDL* low-density lipoprotein, *APOB* apolipoprotein B, *SAR1B* secretion associated, Ras related GTPase 1B, *ANGPTL3* angiopoietin-like 3

Clinical and Physical Findings

Abetalipoproteinaemia

Abetalipoproteinaemia is associated with multisystem manifestations. Patients with abetalipoproteinaemia typically present in childhood with failure to thrive, growth failure, malabsorption of fat, acanthocytosis, and low plasma cholesterol and vitamin E concentrations [50]. Later in life, retinitis pigmentosa, spinocerebellar ataxia, and myopathy have complicated most of the cases.

Gastrointestinal manifestations of abetalipoproteinaemia include steatorrhoea and fat-soluble vitamin deficiency. Fat malabsorption is a central feature of abetalipoproteinaemia and is usually observed in the neonatal period with diarrhoea, vomiting, and failure to thrive. The severity of the intestinal symptoms relates to the fat content of the diet, and usually decreases with age, in part, due to the avoidance of dietary fat in these patients [51]. A yellow colour of the duodenal mucosa has been observed on endoscopy as a result of intestinal lipid accumulation [52]. A characteristic intestinal histology shows normal villi with enterocytes that are distended with lipid droplets (Fig. 14.2).

Haematological manifestations of abetalipoproteinaemia include acanthocytosis (Fig. 14.3) with acanthocytes comprising 50% or more of circulating erythrocytes [51]. Of interest, normal erythrocytes become acanthocytic after transfusion into abetalipoproteinaemia patients and circulate in plasma [50]. Acanthocytosis in abetalipoproteinaemia could be a result of either vitamin E deficiency or an altered membrane lipid composition. Other abnormalities include low erythrocyte sedimentation rates, decreased red cell survival [53], anaemia, hyperbilirubinaemia and haemolysis [54], and increased international normalised ratio due to vitamin K deficiency [55].

Hepatic manifestations of abetalipoproteinaemia include abnormal liver transaminases with hepatomegaly. Liver biopsies have shown marked hepatic steatosis that may or may not be associated with increased liver transaminase concentrations [50]. Steatosis can progress to steatohepatitis and fibrosis [56], and, importantly, cirrhosis has been reported in abetalipoproteinaemia [57].

Neuromuscular manifestations of abetalipoproteinaemia typically begin in the first or second decade of life, affecting both the central and peripheral nervous system, with either upper

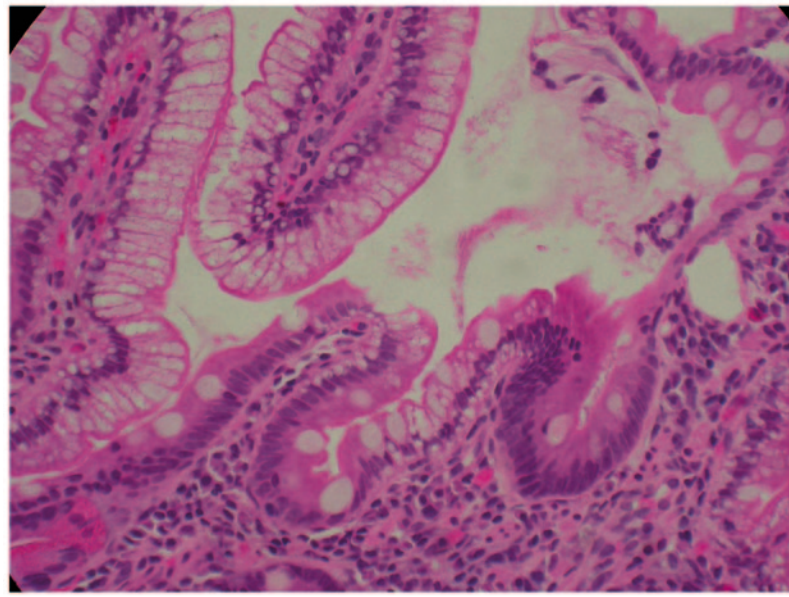

Fig. 14.2 Haematoxylin and eosin-stained light micrograph of the duodenal biopsy from a patient with homozygous familial hypobetalipoproteinaemia, showing marked cytoplasmic microvacuolization of enterocytes (magnification, ×400). (Reprinted with permission, Vongsuvanh [86])

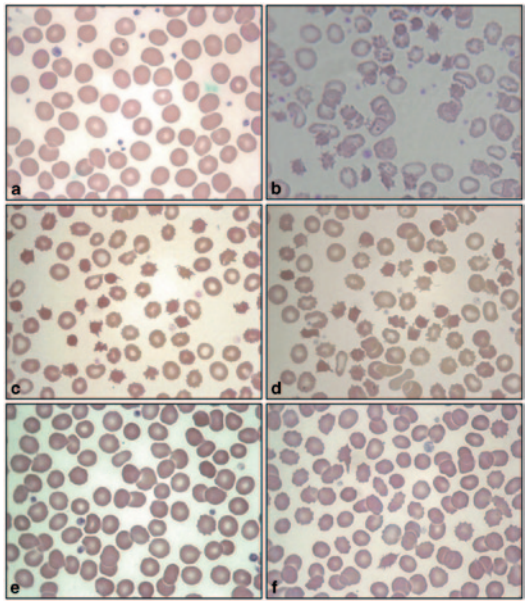

Fig. 14.3 Acanthocytes in abetalipoproteinaemia and familial hypobetalipoproteinaemia. **a** 'Normal' subject. **b** homozygous familial hypobetalipoproteinaemia. **c** and **d** abetalipoproteinaemia. **e** and **f** two different subjects with heterozygous familial hypobetalipoproteinaemia carrying apoB-6.9 mutation

or lower motor neuron abnormalities or both. Neurological signs related to the deficiency of vitamin E include the progressive loss of deep tendon reflexes, vibratory sense and proprioception, muscle weakness and, eventually, a Friedrich's-like ataxia [58].

Ophthalmological manifestations of abetalipoproteinaemia are variable with the most prominent being an atypical pigmentation of the retina characterised by small, irregularly distributed white spots on fundoscopy [59]. Although visual acuity can be affected during the first decade, many patients are asymptomatic until adulthood, with loss of night vision and/or colour vision occurring early in the course of disease. Patients develop annular scotomas with macular sparing that slowly enlarge with progression of the retinopathy. Without treatment, complete visual loss can occur.

Familial Hypobetalipoproteinaemia

Patients with heterozygous *APOB*-linked familial hypobetalipoproteinaemia are often asymptomatic; however, most develop fatty liver (Figs. 14.4 and 14.5), and mild acanthocytosis and fat malabsorption can occur [60, 61]. The clinical and biochemical features of familial hypobetalipoproteinaemia in homozygous and compound heterozygous form are, in general, indistinguishable from those of abetalipoproteinaemia [62]. How-

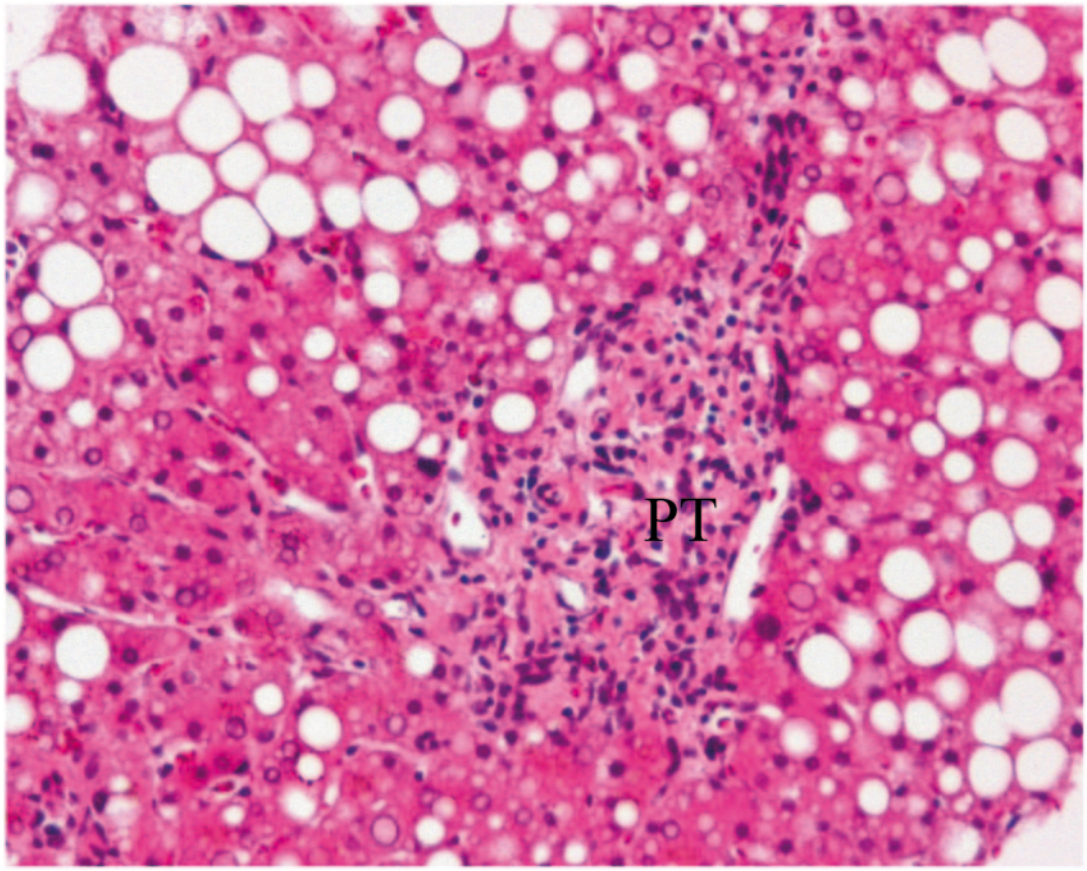

Fig. 14.4 Liver biopsy showing severe macrovesicular steatosis from a patient with heterozygous familial hypobetalipoproteinaemia (H&E, 100×). *PT* indicates portal tract

ever, the clinical features appear to depend on the apoB truncation length, with longer truncations (i.e., greater than apoB-48), as well as those with rare missense mutations who may be asymptomatic, having a milder phenotype.

Familial hypobetalipoproteinaemia might represent a longevity syndrome and be associated with cardiovascular protection due to resistance to atherosclerosis due to a lower lifetime exposure to atherogenic apoB-containing lipoproteins [63]. However, the cardioprotective effects of familial hypobetalipoproteinaemia in humans have, to date, only been shown using the surrogate markers carotid intima-media thickness and distal common carotid arterial wall stiffness [64].

Chylomicron Retention Disease

Chylomicron retention disease presents shortly after birth with diarrhoea, fat malabsorption, and failure to thrive, with vomiting and abdominal distension often present [5, 65]. Acanthocytosis is rare and may be transient. Hepatomegaly and hepatic steatosis may develop in some patients, but do not correlate with liver transaminase levels, along with fat-soluble vitamin deficiencies and their corresponding manifestations. In contrast to abetalipoproteinaemia and familial hypobetalipoproteinaemia, cirrhosis has not been reported in chylomicron retention disease. A white colour of the duodenal mucosa has been observed on endoscopy, with histology like in

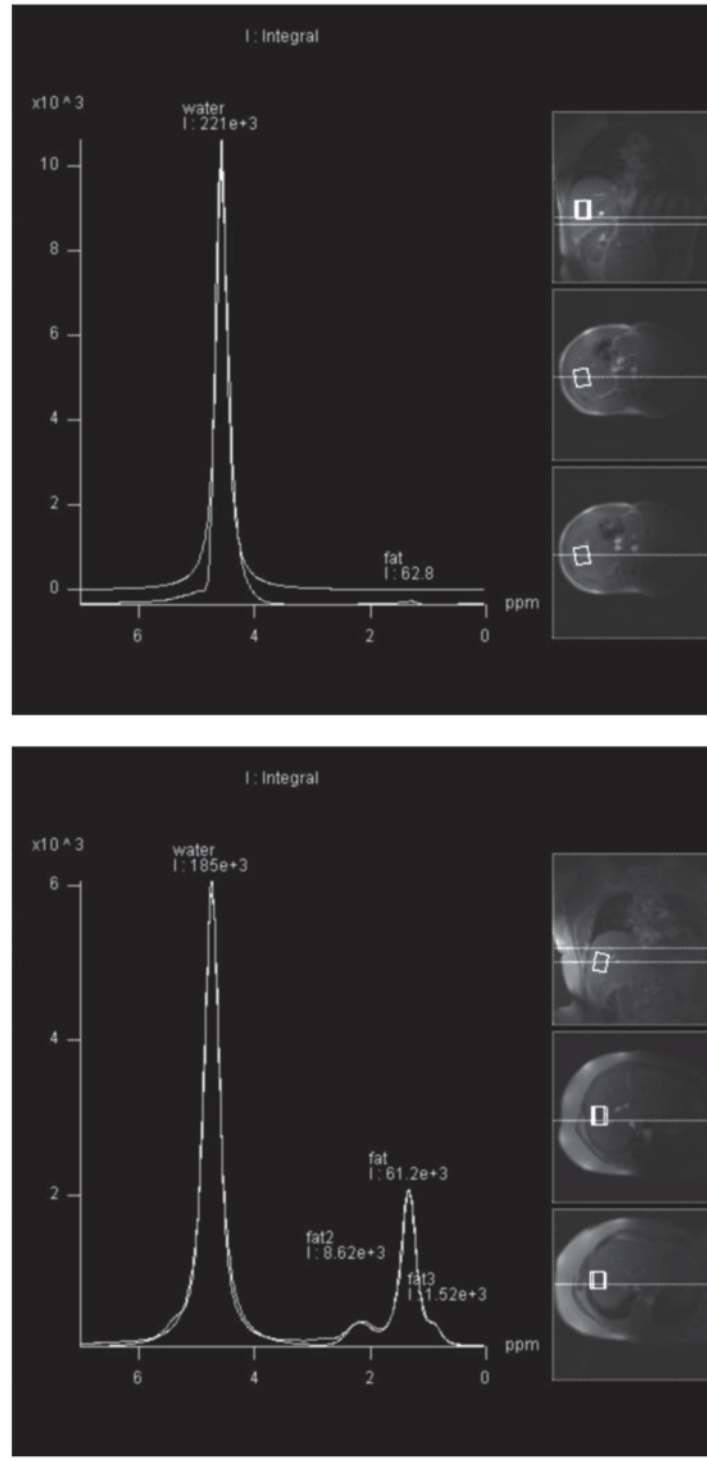

Fig. 14.5 Liver proton nuclear magnetic resonance spectroscopy (^{1}H MRS) in a normal and familial hypobetalipoproteinaemia subject. Liver ^{1}H MRS is shown for a normal subject (*upper panel*), and a heterozygous familial hypobetalipoproteinaemia subject with 28% liver fat (*lower panel*). The area for water and fat peaks are labelled as 'I:'. Percentage liver fat was calculated by 100*fat/(water + fat)

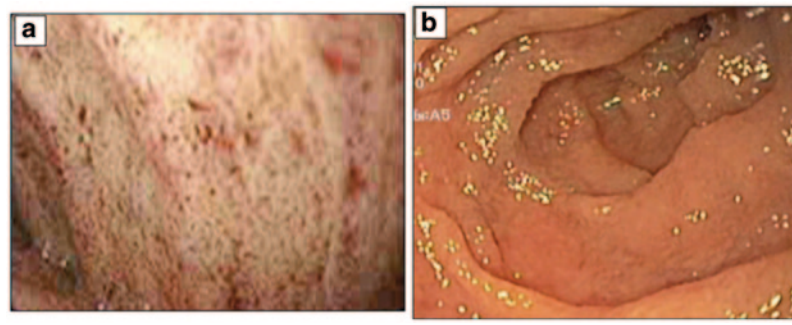

Fig. 14.6 Endoscopy of a chylomicron retention disease patient. Upper endoscopy reveals a white duodenal mucosa in **(a)** chylomicron retention disease compared with **(b)** normal subject (Reproduced with permission from Peretti et al. [5])

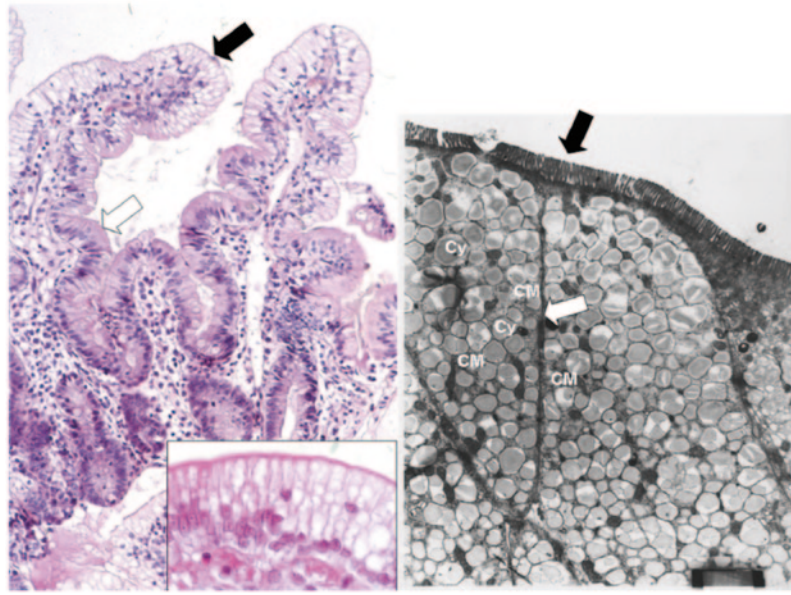

Fig. 14.7 Histology of a jejunal biopsy from a chylomicron retention disease patient. *Left panel*: photomicrograph of haematoxylin–eosin staining showing vacuolisation of enterocytes and well-preserved villous structure. The distribution of vacuolisation, which corresponds to lipid droplets, is heterogeneous: Fat filled enterocytes (*black arrow*) in the upper part of the villus are associated with normal enterocytes in the crypts (*white arrow*) (×20). *Inset*: Higher magnification (×100) of the same patient's biopsy. *Right panel*: Electronic microscopy. The pictures show the apical pole of the enterocytes exhibiting well-preserved microvilli (*black arrow*), numerous chylomicrons (CM) and fat droplets of homogenous size gorging the cytoplasm (Cy). The intercellular membranes demonstrate a complete juxtaposition of intercellular membranes where lipid particles are absent (*white arrow*) (×4000). (Reproduced with permission from Peretti et al. [5])

abetalipoproteinaemia showing vacuolisation of enterocytes in intestinal villi of normal structure (Figs. 14.6 and 14.7).

Approach to the Patient

The usual biochemical trigger for the investigation of genetic abetalipoproteinaemia and hypobetalipoproteinaemia is the finding of marked hypocholesterolaemia with plasma LDL-cholesterol and apoB concentrations below the fifth percentile for age and sex [1]. A personal and family history should be taken and a physical examination conducted. Secondary causes of hypobetalipoproteinaemia should be excluded (see differential diagnosis below) and clinical manifestations such as fat malabsorption, growth failure, fat-soluble vitamin deficiency, fatty liver disease, and neuro-ophthalmological dysfunction

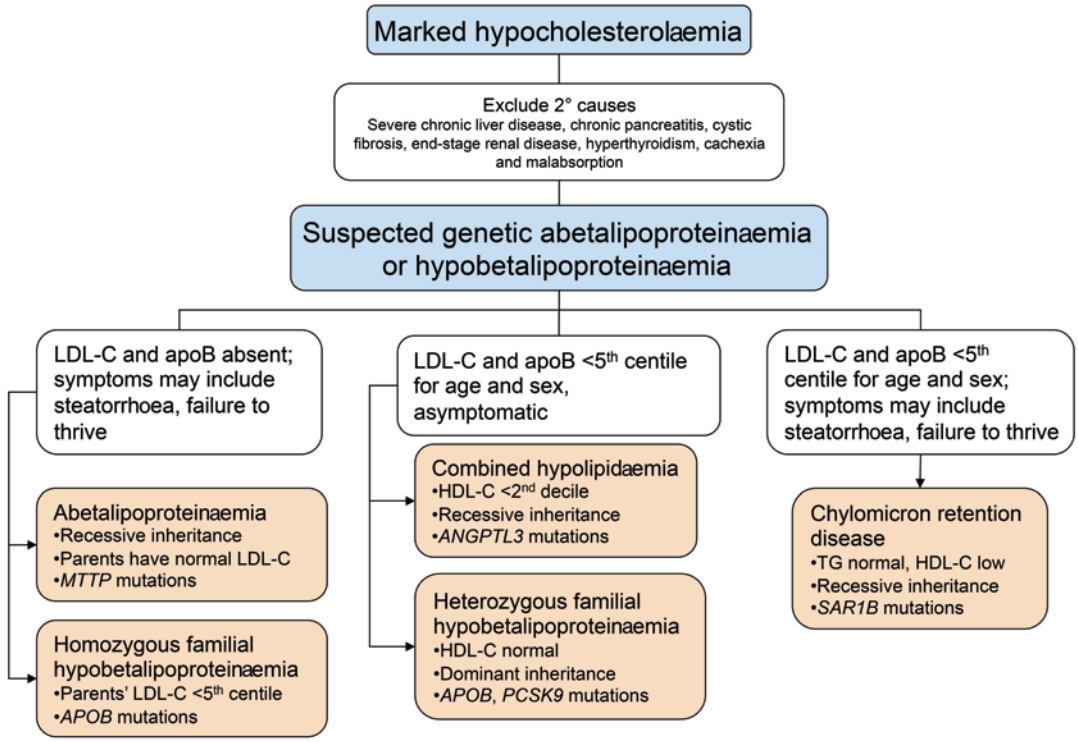

Fig. 14.8 The diagnosis of genetic abetalipoproteinaemia and hypobetalipoproteinaemia. *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *apoB* apolipoprotein B, *TG* triglyceride

sought. A suggested algorithm for the investigation of marked hypocholesterolaemia is shown in Fig. 14.8.

Laboratory Tests

A full fasting lipid profile including total cholesterol, HDL-cholesterol, triglyceride, LDL-cholesterol, and apoB should be performed where genetic abetalipoproteinaemia or hypobetalipoproteinaemia is suspected.

Patients with abetalipoproteinaemia or homozygous familial hypobetalipoproteinaemia will have very low plasma total cholesterol and generally low triglyceride concentrations. LDL-cholesterol, when measured by direct methods, and apoB, will be absent or very low. Vitamin E levels will also be very low, and acanthocytosis may be observed on peripheral blood smear (Fig. 14.3).

Patients with heterozygous familial hypobetalipoproteinaemia typically have plasma LDL-cholesterol and apoB concentrations that are one quarter to one third of normal. The reasons for these lower-than-expected levels may include decreased hepatic secretion of the apoB-containing lipoproteins, or the up-regulation of the LDL-receptor, resulting in an enhanced clearance rate for VLDL and LDL particles produced by the normal allele [37].

Subjects who carry a single *MTTP* mutation (i.e. abetalipoproteinaemia 'carriers') may have normal plasma lipids or may have LDL-cholesterol and apoB concentrations similar to those seen in heterozygous familial hypobetalipoproteinaemia [66].

In chylomicron retention disease, total cholesterol, LDL-cholesterol, and HDL-cholesterol are low, but triglyceride levels are generally normal. The low plasma LDL- and HDL-cholesterol are a consequence of low rates of apoB-100 and apoA-I production [67]. High creatine kinase (4–5x normal) may be seen from infancy, along with deficiencies in fat-soluble vitamins [5].

Molecular Tests

Molecular testing of *MTTP, APOB, SAR1B, PCSK9,* and *ANGPTL3* genes is available in specialist laboratories [68]. Target exonic regions in genomic DNA should be amplified by polymerase chain reaction, ensuring at least 20 base pairs of flanking intronic sequence is included in order to capture any potential splice site mutations. Sequencing should be bidirectional and, if possible, testing of the patient's parents is recommended to confirm that where two mutations are identified, that they originate from two different chromosomes.

In patients with abetalipoproteinaemia where the inheritance pattern is unclear or *MTTP* mutations unable to be identified, then the *APOB* gene should also be sequenced given the clinical and biochemical similarities with homozygous familial hypobetalipoproteinaemia. Likewise, in patients with homozygous familial hypobetalipoproteinaemia, the *MTTP* gene could be sequenced in the event where *APOB* mutations are unable to be found. Alternatively, high-throughput sequencing technology is emerging as a means for screening multiple genes for mutations; massively parallel sequencing could potentially analyse a panel of the genes associated with low cholesterol [69]. This may prove more cost-effective than traditional Sanger sequencing, particularly where multiple genes may need to be sequenced and when the *APOB* gene is involved, which needs over 40 primer sets to cover the whole coding region.

Western blotting can be used as a screening method for mutations in *APOB,* to detect truncated apoB species that are >30% of full-length protein size. DNA sequencing of the region where the mutation is estimated to occur can then be performed. However, truncated apoB species shorter than apoB-30 are not detectable in plasma, so if Western blotting fails to detect an apoB truncation, then sequencing of the first 30% of the *APOB* gene (exons 1–25) should be performed.

Differential Diagnosis

Illnesses and diseases associated with secondary causes of hypobetalipoproteinaemia include severe chronic liver disease, chronic pancreatitis, cystic fibrosis, end-stage renal disease, hyperthyroidism, cachexia, and malabsorption [32, 51]. A vegan diet is associated with ~50% of the general population levels of plasma LDL-cholesterol and triglyceride [70].

Prognosis, Clinical Course, and Complications

Abetalipoproteinaemia and Homozygous Familial Hypobetalipoproteinaemia

The impact on prognosis of age at diagnosis, commencement of a low-fat diet and vitamin replacement therapy, and genotype is variable in abetalipoproteinaemia and homozygous familial hypobetalipoproteinaemia [3, 4, 71]. Early treatment with high-oral doses of vitamin E and A, which are thought to bypass the intestinal chylomicron pathway via the portal circulation, can reduce the potential severity of neuropathy and retinopathy [71–75]. Patients need to be followed regularly for evaluation of symptoms, complications, and to monitor compliance with therapy. A relative paucity of data makes it difficult to predict clinical outcomes based on *MTTP* or *APOB* genotype.

Heterozygous Familial Hypobetalipoproteinaemia

Although the majority of familial hypobetalipoproteinaemia heterozygotes are asymptomatic, most have increased liver transaminases and hepatic steatosis, the long-term consequences of which are unknown [60, 61, 76–78]. Familial hypobetalipoproteinaemia heterozygotes have three- to fivefold greater liver fat content compared to control subjects with no difference in adiposity or insulin resistance [61, 76, 78, 79]. It would seem prudent to monitor biochemically and by imaging techniques the livers of these individuals given a potential increased risk of progression to cirrhosis, particularly in the presence of known risk factors, such as alcohol, caloric excess, and liver injury [32, 77, 80, 81].

Chylomicron Retention Disease

Patients with chylomicron retention disease get better within a few days or weeks with a low-fat diet [5]. No relationship has been found between liver transaminases, hepatomegaly, and hepatic steatosis. Neurological manifestations include hyporeflexia and loss of proprioception in teenagers through to ataxia, myopathy, and sensory neuropathy in adults.

Treatment

Abetalipoproteinaemia

The cornerstone of treatment for abetalipoproteinaemia is dietary modification and the replacement of fat-soluble vitamins [1, 50, 51, 71]. A low-fat diet eliminates steatorrhoea and allows absorption of other nutrients essential for growth and development. Oral vitamin E supplementation (100–300 mg/kg/day orally) in abetalipoproteinaemia is recommended to halt the progression of the neurological disease; however, despite this high dose, serum levels do not fully normalise [50, 51]. Most adult patients with abetalipoproteinaemia who have not received supplements exhibit neuro-ophthalmological complications [50]. Supplementation with a combination of vitamins E and A has been shown to be effective in reducing retinal degeneration [72]. Patients treated with very large oral doses of vitamin E from the age of 16 months do not develop neurological or retinal features, while progression is halted or sometimes even reversed in older patients who already show symptoms of neurological dysfunction [83]. Although serum vitamin E is usually undetectable in untreated abetalipoproteinaemia, supplementation results in trace concentrations, with normal levels in adipose tissue [84]. Erythrocyte and platelet vitamin E have also been used to assess tissue vitamin E status [85]. Oral supplementation of two to four times the recommended daily allowance of vitamin A normalises serum levels. Vitamin D deficiency is not a consistent finding; however, vitamin D replacement should be considered in abetalipoproteinaemia patients, along with other supplementary nutrients such as iron and folate.

There is a need for novel therapeutic approaches to abetalipoproteinaemia as vitamin therapy alone fails to completely control or cure this disease.

Familial Hypobetalipoproteinaemia

In familial hypobetalipoproteinaemia homozygotes, dietary fats should be restricted to prevent steatorrhoea. Long-term high-dose vitamin E and A supplementation should prevent or slow progression of the neuromuscular and retinal degenerative disease [51, 72]. Moderate-dose vitamin E supplementation in familial hypobetalipoproteinaemia heterozygotes with low serum vitamin E concentrations has been recommended to prevent neurological disease [30]. However, this recommendation has been called into question [85].

Chylomicron Retention Disease

There are no specific recommendations for follow-up or treatment of chylomicron retention disease, with therapeutic regimens based on those recommended for abetalipoproteinaemia. Vomiting, diarrhoea, and abdominal distension improve on a low-fat diet of polyunsaturated fatty acids and supplementation with fat-soluble vitamins, particularly vitamin E, can prevent neurological complications [5].

Conclusion

The monogenic hypocholesterolaemic lipid disorders are classified depending on the lipid biochemical phenotype, gene involved, and mode of inheritance of the condition, together with the severity of the mutation or mutations present. These disorders may or may not be associated with clinical manifestations such as fat malabsorption, growth failure, fat-soluble vitamin deficiency, fatty liver disease, and neuro-ophthalmological

dysfunction. We have reviewed the molecular basis, pathogenesis, and clinical aspects of these disorders of apoB production and catabolism, focusing on abetalipoproteinaemia, familial hypobetalipoproteinaemia, and chylomicron retention disease.

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Drug-Induced Dyslipidemia

15

Vinaya Simha

Introduction

Evaluation of patients with dyslipidemia must include a thorough investigation for secondary causes which may be exacerbating a primary lipid disorder. Lifestyle factors including diet, activity, and smoking, as well as comorbidities such as diabetes, hypothyroidism, liver, and kidney disease may contribute to elevated cholesterol and triglyceride levels. Similarly, medications used in the treatment of a variety of diseases may have adverse effects on lipid metabolism. The effects may vary from a thiazide-induced mild increase in serum cholesterol with unclear long-term consequences to a dramatic increase in serum triglycerides due to retinoid therapy leading to acute pancreatitis. A large group of medications have been identified to cause or worsen dyslipidemia, and since a growing number of patients are on polypharmacy, it is very important to be aware of the potential contribution of concomitant medications on hyperlipidemia. This brief chapter focuses on the magnitude, mechanisms, and management of lipid abnormalities induced by medications. The salient effects of some commonly used drugs on plasma lipids are summarized in Table 15.1.

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Antihypertensives

Beta-Adrenergic Blockers

Beta-adrenergic blockers are commonly used in the treatment of hypertension and coronary artery disease. Despite their proven efficacy in reducing cardiovascular morbidity and mortality, they are well known to cause unfavorable changes in lipid profile. Tanaka et al. [1] observed nearly 40 years ago that while acute administration of propranolol led to reduced free fatty acid levels, chronic treatment resulted in elevated serum triglycerides and reduced post-heparin-lipolytic activity, an effect that was reversed 10 days after drug withdrawal. Subsequent studies in the next decade showed a 24–46% increase in serum triglycerides with propranolol therapy [2, 3], a 18–36% increase with atenolol therapy [4, 5], and a 16% increase with metoprolol [6]. All these studies also showed a modest reduction in serum high-density lipoprotein (HDL) cholesterol, but no significant effect on total or low-density lipoprotein (LDL) cholesterol.

Nonselective beta-adrenergic blockers such as propranolol have generally been held to cause greater dyslipidemia than cardioselective (beta 1)-adrenergic receptor blockers [7, 8]. However, the new third-generation beta-adrenergic blocker, carvedilol, which is a nonselective beta 1- and beta 2-adrenergic receptor antagonist besides being a weak alpha 1-adrenergic receptor antagonist, is not associated with similar adverse effects

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Table 15.1 Effect of some commonly used medications on serum lipid levels

Drug	TC	LDL-C	TG	HDL-C
Beta blockers				
Atenolol, Metoprolol, Propranolol	N	N	↑	↓
Carvedilol	N	N	N	N
Diuretics				
Thiazides	↑	↑	↑-↑↑	↓
Loop diuretics	↑	↑	↑	N
Potassium sparing	N	N	N	N
Steroids				
Glucocorticoids	↑	↑	↑	↑-↑↑
Estrogens	↓	↓	↑-↑↑↑	↑
Tamoxifen	↓	↓	↑-↑↑↑	N-↑
Clomiphene	N	N	↑-↑↑↑	N
Progestogens	N-↑	N-↑	N-↓	N-↓
Androgens	N-↑	N-↑	↓	↓
Immunosuppressants				
Cyclosporine	↑-↑↑	↑-↑↑	↑-↑↑	↓
Tacrolimus	N-↑	N-↑	N-↑	N
Sirolimus	↑	↑	↑-↑↑↑	↓
Antineoplastic agents				
Retinoids	↑	↑	↑-↑↑↑	N-↓
Interferons	N-↑	N-↑	↑-↑↑↑	N-↓
Capecitabine, L-asparaginase	N-↑	N-↑	↑-↑↑↑	N-↓
Antipsychotics, atypical	N	N	↑-↑↑↑	↓
Antiepileptics	N-↑	N-↑	N	N-↑
Protease inhibitors	↑	N-↑	↑-↑↑↑	N-↓
Propofol	N-↑	N	↑-↑↑↑	↓

↑ increase, ↓ decrease, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *N* no change, *TC* total cholesterol, *TG* triglycerides

on lipid profile. A post hoc analysis of the Glycemic Effects in Diabetes Mellitus: Carvedilol-Metoprolol Comparison in Hypertensives (GEMINI) trial showed favorable lipid effects of carvedilol compared to metoprolol in over 1200 subjects with diabetes [9]. Sharp et al. [10] analyzed 12 carvedilol studies, 6 of which compared carvedilol with selective beta 1-adrenergic antagonists and found that carvedilol had either a neutral or a mild beneficial effect on serum lipids. This difference may be due to the alpha 1-antagonist action as alpha-adrenergic blockers such as prazosin have been consistently shown to reduce serum triglycerides and increase HDL cholesterol [2, 11, 12]. Alpha-adrenergic stimulation is known to inhibit lipoprotein lipase activity [13], and the reflex increase in alpha-adrenergic activity that occurs during beta-adrenergic blocker therapy may be responsible for impaired triglyceride removal and hypertriglyceridemia.

Interestingly, the adverse effects of beta-adrenergic blocker therapy are most evident in patients with baseline hypertriglyceridemia [14, 15], and recently, beta 2-adrenergic receptor polymorphisms have been shown to influence serum triglyceride levels during metoprolol treatment [16]. While future studies in this direction may help identify patients who are most prone to develop hyperlipidemia during beta-adrenergic blocker therapy, it would be prudent at this time to use these medications cautiously in patients with abnormal lipid levels, and consider using carvedilol in dyslipidemic patients who need beta-adrenergic blocker therapy.

Diuretics

Diuretics are one of the oldest and most commonly used medications for hypertension, and the Joint National Commission on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) recommends thiazide diuretics as the first line of therapy unless there are compelling indications for other drug classes [17]. However, thiazide diuretics including hydrocholorothiazide and chlorthalidone have been noted to increase both total and LDL cholesterol by 6–7%, increase triglycerides by about 15%, and cause a mild reduction in HDL cholesterol [5, 18–22]. A large meta-analysis of 474 trials involving more than 65,000 patients showed that diuretic therapy increased total cholesterol by an average of 0.29 mmol/L (11 mg/dL), LDL cholesterol by 0.24 mmol/L (9 mg/dL), and triglycerides by 0.35 mmol/L (31 mg/dL), and reduced HDL cholesterol by 0.02 mmol/L (1 mg/dL) [23]. Further, these effects were more pronounced in African Americans and at higher doses, but did not depend on the type of diuretic used. However, limited data from small studies suggest that loop diuretics and potassium sparing diuretics have only a mild or neutral effect [8, 24–26].

The mechanisms by which diuretics induce dyslipidemia are not very clear, but may involve a reflex activation of alpha-adrenergic activity and renin–angiotensin–aldosterone axis as a result of volume depletion [13, 27]. This could lead to increased lipolysis and increase in hepatic very low-density lipoprotein (VLDL) and LDL synthesis. Thiazides are also known to cause insulin resistance [19, 28] and glucose intolerance due to hypokalemia [29, 30] which may also contribute to lipid effects.

The clinical significance of thiazide-induced dyslipidemia is also not very clear. In the Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack (ALLHAT) trial, a randomized double-blind study of over 33,000 hypertensive patients, chlorthalidone therapy was associated with higher total cholesterol levels, but with lower cardiovascular events [31]. While this might suggest that the mild dyslipidemia induced by thiazides has no deleterious effects, it is also possible that the lipid effects might have decreased the overall benefits of blood pressure reduction. A safe strategy would be to use thiazides in low doses, especially in subjects prone to dyslipidemia.

Steroid Hormones

Glucocorticoids

Glucocorticoids are another commonly used group of medications which have generally been held to cause an adverse effect on lipid profile, including an elevation in both total and LDL cholesterol and triglycerides [8, 32]. However, it is often difficult to discern if the alleged lipid effects are due to the glucocorticoids or due to underlying disease and other concomitant medications. Studies in healthy volunteers have yielded inconsistent results, with one study reporting a 40% increase in serum triglycerides after 14 days of prednisone therapy [33], while others observed no significant effect on triglycerides, but the study duration was only 7 days [34]. However, both studies [33, 34] reported elevation in HDL cholesterol and no change in LDL cholesterol. A cross-sectional analysis of over 15,000 participants in the Third National Health and Nutrition Examination Survey showed that both oral and inhaled glucocorticoid use was associated with a higher HDL cholesterol level and lower total to HDL cholesterol ratio in subjects over age 60 years, but not with an adverse lipid profile [35]. Two other prospective studies have also shown a similar increase in HDL cholesterol levels with no change in LDL cholesterol or triglycerides [36, 37]. Other studies have shown that patients with systemic lupus erythematosus who are treated with glucocorticoids have a higher LDL cholesterol and serum triglycerides [38, 39]. A clear dose–response relationship between glucocorticoid dose and serum triglycerides and LDL cholesterol has been observed in hypopituitary patients on glucocorticoid replacement therapy [40]. Marked elevation in both total cholesterol and triglycerides is also noted in organ transplant recipients receiving glucocorticoid-inclusive

immunosuppression. This has been observed in patients undergoing renal transplantation [41, 42], cardiac transplantation [43, 44], and liver transplantation [45]. It must however be noted that these effects are not uniform, and many transplant recipients do not demonstrate any lipid abnormalities [46, 47]. Underlying medical conditions such as uremia and other concomitant medications such as cyclosporine and rapamycin may modulate the effect of glucocorticoids on serum lipids. Overall, it appears that the most consistent direct effect of glucocorticoids is to increase serum HDL cholesterol, sometimes by up to 20–40%, with a more variable effect on LDL cholesterol and triglycerides noted in only some patients.

The mechanism by which glucocorticoids raise HDL cholesterol is not well known, but may be secondary to an increase in lipoprotein lipase activity [34]. Further, most conditions requiring glucocorticoid therapy are associated with systemic inflammation and low HDL cholesterol, and glucocorticoids may raise HDL by their anti-inflammatory effect [48]. Glucocorticoids also increase lipolysis, hepatic steatosis, and insulin resistance [49] which can increase VLDL production and thus increase serum triglycerides and LDL cholesterol.

Estrogen and Related Compounds

The lower incidence of coronary heart disease in premenopausal women compared to men, and the increase in LDL cholesterol in women after menopause, suggests a beneficial effect of estrogens on the lipid profile. Indeed, unopposed estrogen administration does lead to reduction in total and LDL cholesterol and increase in HDL cholesterol [50, 51]. However, these and other studies [52–54] have also shown a 30–40% increase in serum triglyceride levels. The hypertriglyceridemic effect of estrogens is dose dependent, and most prominent in patients with baseline hypertriglyceridemia. In the large Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, about 1.5% of the subjects had serum triglycerides above 500 mg/dL [51], and there are many instances of estrogen-induced pancreatitis from hypertriglyceridemia in patients with underlying lipid disorders such as type I hyperlipoproteinemia [55, 56] and lipodystrophy [57]. Estrogens increase VLDL production [58] which accounts for the hypertriglyceridemic effect despite a direct modest increase in clearance of apolipoprotein B-containing particles. In subjects who already have impaired clearance of these particles (type I or type III hyperlipoproteinemia), or have increased VLDL secretion (lipodystrophy, metabolic syndrome), further increase in VLDL production leads to severe hypertriglyceridemia. Unlike oral estrogen, transdermal estrogens, which do not undergo first-pass metabolism in the liver, have only minimal effects on lipid levels [59].

Estrogens are often administered in combination with progesterone, such as in combined oral contraceptives, which modifies their effect on lipids. Both natural progesterone and its synthetic derivatives have a weak androgenic effect. When used alone in high doses, they increase LDL cholesterol and decrease triglycerides and HDL cholesterol [60], but low doses such as progesterone-only pill have minimal effects [61], while depot medroxy progesterone acetate preparations cause a 15–30% decline in serum HDL cholesterol levels [62, 63]. More recent long-term studies showed that the HDL-lowering effect of depot medroxy progesterone acetate was temporary and improved after 6 months even when the drug use was continued [64]. Progestogens, like androgens, are thought to decrease HDL cholesterol levels by increasing the activity of hepatic lipase leading to increased HDL catabolism [65]. The effect of combined oral contraceptives on lipids depends on the "androgenicity" of the progestogen being combined with estrogens. Oral contraceptives containing the older second-generation progestogens such as levonorgestrel and norethisterone, which have strong androgenic effects, increase LDL cholesterol and triglycerides and decrease HDL cholesterol [66, 67]. The newer third-generation progestogens such as desogestrel and gestodene are least androgenic and do not cause unfavorable effects on LDL and HDL cholesterol, but may cause hypertriglyceridemia [68–70]. Even the combined contracep-

tive vaginal ring containing ethinylestradiol and etonogestrel (NuvaRing) has been noted to increase serum triglycerides and apolipoprotein B levels in comparison with levonorgestrel containing combined oral contraceptives which increase LDL cholesterol [71]. In women at risk for severe hypertriglyceridemia, progesterone-only methods such as levonorgestrel intrauterine device, the etonogestrel implant, or progesterone-only pills containing desogestrel or levonorgestrel do not exacerbate the elevation in triglyceride levels [72–74]. Despite the mild increase in LDL cholesterol, combined oral contraceptives are unlikely to pose a significant cardiovascular risk, even in subjects with metabolic syndrome [75].

The selective estrogen receptor modulator tamoxifen also causes a modest reduction in total and LDL cholesterol [76], but may sometimes cause severe hypertriglyceridemia and acute pancreatitis [77–79]. Liu and Yang [80] sequentially followed 116 patients with breast cancer on tamoxifen therapy, and reported that 102 patients had clinically insignificant rise in serum triglycerides and there was improvement in 10 other subjects after dose reduction. Apolipoprotein E polymorphisms may influence triglyceride levels during tamoxifen therapy [81]. Raloxifene, another selective estrogen receptor modulator, has generally not been associated with severe hypertriglyceridemia, and has been even shown to reduce total cholesterol and apolipoprotein B levels in hypertriglyceridemic subjects [82]. Nonetheless, limited data suggest caution in using this drug also in patients who have experienced estrogen-induced hypertriglyceridemia [83]. Clomiphene is another synthetic estrogen analog which is structurally similar to tamoxifen and has been used to induce ovulation. Severe hypertriglyceridemia has been reported in three patients with polycystic ovarian disease during treatment with clomiphene, two of whom developed acute pancreatitis [84–86]. One of the patients was eventually diagnosed to have familial dysbetalipoproteinemia [85]. It is therefore important to screen patients for baseline dyslipidemia before starting this medicine. The aromatase inhibitors are not associated with hypertriglyceridemia, and though anastrazole has been reported to cause mild hypercholesterolemia, their effects on lipids is generally mild and clinically insignificant [87].

Androgens

Androgen replacement therapy in hypogonadal men has many beneficial effects including increase in lean body and bone mass, but is also noted to consistently lower HDL cholesterol by 10–20% [88–90]. The total and LDL cholesterol levels do not change much, or may decrease slightly. More dramatic changes are seen with administration of oral testosterone preparations with more than 50% decline in HDL cholesterol levels and a concomitant increase in LDL cholesterol, especially in athletes who abuse anabolic steroids [91–93]. Some studies have not shown significant change in HDL cholesterol when supraphysiologic doses of testosterone are administered parenterally in normal healthy men [94], but others have noted both a decrease in HDL cholesterol and an increase in LDL cholesterol [95, 96]. Supplementation of oral dehydroepiandrosterone (DHEA), a weak androgen, in both men and postmenopausal women has also been shown to reduce HDL cholesterol levels [97, 98]. Androgens have been shown to increase the activity of hepatic lipase and thus accelerate catabolism of HDL particles, besides decreasing apolipoprotein A1 synthesis, thus leading to low HDL cholesterol [99]. Single intramuscular dose of 500 mg of testosterone in healthy volunteers has been shown to increase the expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase enzyme and cause hypercholesterolemia [100]. It is important to closely monitor lipid levels in men on androgen replacement therapy, and to create awareness about these adverse effects among potential androgen abusers. Similarly, the use of leuprolide and other gonadotropin releasing hormone agonists for androgen deprivation therapy may cause elevation in total cholesterol, triglycerides, and HDL cholesterol besides other metabolic complications such as insulin resistance [101, 102].

Immunosuppressive Drugs

Recent advances in the development of new immunosuppressant drugs have greatly decreased acute rejection rates following solid organ transplantation. However, as the life span of organ transplant recipients improves, there is increasing awareness of long-term complications including dyslipidemia and accelerated atherosclerosis. Following cardiac transplantation, over 60% of subjects develop hyperlipidemia within a month, and the prevalence increases to over 90% by 10 years [47, 103]. Similarly, more than half of renal and liver transplant recipients also develop hyperlipidemia [104–107]. While multiple factors including underlying disease, comorbidities, diet, physical activity, and other host factors play a role in the genesis of posttransplantation dyslipidemia, immunosuppressive medications are probably the most important cause. Besides glucocorticoids, whose effects have already been discussed, cyclosporine and sirolimus are commonly associated with adverse lipid effects, while azathioprine and mycophenolate mofetil have only minimal effects.

Cyclosporine

The calcineurin inhibitors, cyclosporine and tacrolimus, suppress the transcription of inflammatory genes in the T cells by inhibiting the translocation into the nucleus of a critical transcription factor called nuclear factor of activated T cells (NFAT) [108]. They form the backbone of most immunosuppressive regimen, especially in renal transplant recipients. Cyclosporine can cause mild to moderate elevation in both total and LDL cholesterol and serum triglycerides [109–111]. Change in serum triglycerides and concomitant lowering of HDL cholesterol is less consistent than elevation in total and LDL cholesterol. Some studies have shown a correlation between lipid levels and cyclosporine levels and dosages, while others have not [104, 112–114]. Interestingly, cyclosporine-induced dyslipidemia has been observed to improve over time [115]. Elevation in total and LDL cholesterol has also been seen in non-transplant patients treated with cyclosporine [116, 117].

The exact mechanism by which cyclosporine increases cholesterol and triglyceride levels is not known, and may involve multiple pathways (Fig. 15.1). In vitro studies suggest that cyclosporine decreases LDL receptor activity [118, 119], and may also decrease the conversion of cholesterol to bile acids by inhibiting the enzyme cholesterol 27-hydroxylase [120, 121]. Inhibition of this enzyme and the resultant decrease in levels of its product 27-hydroxycholesterol can further worsen hypercholesterolemia as 27-hydroxycholesterol is involved in the negative feedback inhibition of HMG CoA reductase, and increased activity of HMG CoA reductase could account for decreased LDL receptor activity [122, 123]. Interestingly, it has been shown that this reduction in LDL receptor activity can be reversed by administration of HMG CoA reductase inhibitors in an in vitro cell culture model [124] which establishes a basis for statin therapy for cyclosporine-induced hypercholesterolemia. Clinical trials have indeed shown the efficacy of statin therapy in both reducing cholesterol levels and increasing survival in heart transplant recipients on cyclosporine-based immunosuppression [125–127]. However, cyclosporine can increase the serum levels of statins such as lovastatin, simvastatin, and atorvastatin by competing with the hepatic cytochrome CYP3A4 enzymes involved in their metabolism. Fluvastatin and pravastatin may be safer to use in combination with cyclosporine [128–130].

Tacrolimus is also a calcineurin inhibitor like cyclosporine, but with much less effect on lipid metabolism. Lower levels of total and LDL cholesterol and triglycerides are seen in patients on tacrolimus-based therapy compared to cyclosporine, and improvement in lipids are noted when cyclosporine is switched to tacrolimus [131–134]. Thus, patients with significant cyclosporine-induced dyslipidemia can be managed either with cautiously dosed statin therapy [135] or by switching to tacrolimus which does not affect the efficacy of immunosuppression.

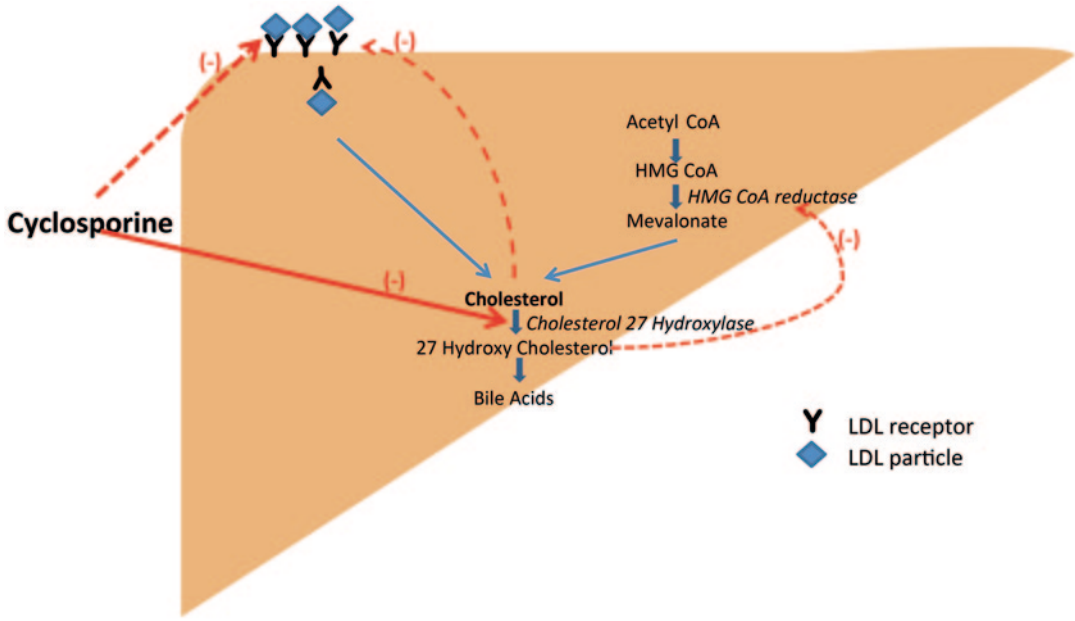

Fig. 15.1 Mechanisms of cyclosporine-induced hypercholesterolemia. Cellular cholesterol is derived from two sources, receptor mediated endocytosis of LDL particles and synthesis from acetyl CoA. Cholesterol is converted to bile acids in the hepatocytes for excretion. Cyclosporine interferes with the catabolism of cholesterol to bile acids by inhibiting the enzyme cholesterol 27-hydroxylase. In the process, it also decreases the production of 27-hydroxy cholesterol, which normally inhibits HMG CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. As a result of increased cellular cholesterol synthesis and decreased breakdown, there is inhibition of LDL receptor expression. Cyclosporine may also directly affect binding of LDL particle to the receptor

Sirolimus

Sirolimus or rapamycin is a newer immunosuppressive agent which is structurally similar to tacrolimus, but acts in a calcineurin-independent manner. It binds to the kinase enzyme, mammalian target of rapamycin (mTOR), leading to cell cycle arrest at the G1 to S phase of cell cycle, and subsequent inhibition of T cell activation and proliferation in response to cytokine stimulation [136]. Since it complements the action of calcineurin inhibitors, and has a different side-effect profile, it is often advantageous to combine low doses of these two classes of medications. However, use of sirolimus and other mTOR inhibitors such as everolimus and temsirolimus is also associated with many adverse effects [137] including hyperlipidemia. Dose-dependent elevation of serum triglycerides by up to 20% in 50–75% of renal and liver transplant patients on sirolimus has been reported [138–140]. In some patients, more marked serum triglyceride elevations up to 2000 mg/dL may be noted [141]. Reduction of sirolimus dosing and therapy with fibrates or statin may be helpful in some cases, while the medication had to be discontinued in other subjects to control the hypertriglyceridemia [141]. Mild increase in total cholesterol and reduction in HDL cholesterol may also occur. The mechanisms by which sirolimus causes dyslipidemia (Fig. 15.2) are not clear, but may involve both an increase in apolipoprotein B and VLDL synthesis and a decrease in triglyceride hydrolysis due to increase in apolipoprotein CIII levels [142–144]. Further, inhibition of mTOR has been shown to increase the expression of PCSK9 which leads to reduced LDL receptors and an increase in LDL/VLDL cholesterol [145].

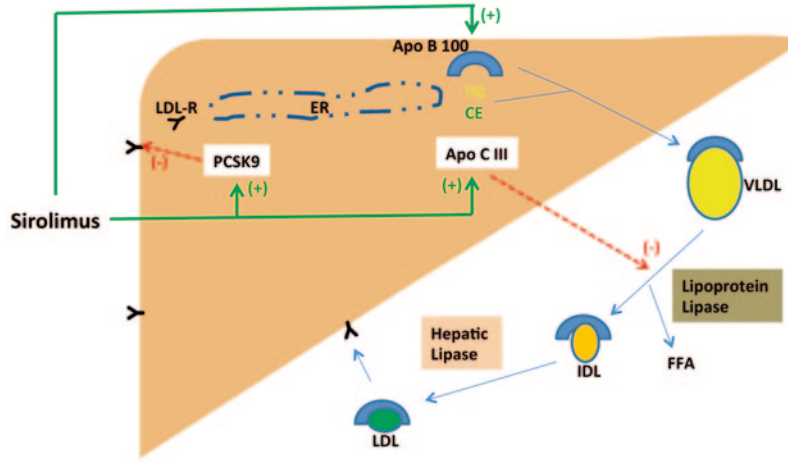

Fig. 15.2 Mechanisms of sirolimus-induced hyperlipidemia. Sirolimus increases *Apo B* levels and thus *VLDL* secretion. In addition, it increases production of *Apo C III* which inhibits the activity of lipoprotein lipase leading to decreased *VLDL* triglyceride hydrolysis. Both these actions cause hypertriglyceridemia. Further, it can increase *PCSK9* expression leading to increased destruction of LDL receptors (*LDL-R*), decreased clearance of *LDL* particles, and resultant hypercholesterolemia

Antineoplastic Agents

Retinoids

Retinoids have been used both locally and systemically in the treatment of a variety of skin disorders including acne, psoriasis, cutaneous T cell lymphoma and other hyperkeratotic disorders. They are vitamin A derivatives and include isotretinoin (13-cis retinoic acid), tretinoin (all-trans retinoic acid), acitretin, alitretinoin, and bexarotene.

Isotretinoin is a naturally occurring metabolite of retinol used in the treatment of severe acne vulgaris. Marked elevation in serum triglycerides and a mild elevation in serum total cholesterol may however occur with this therapy, and may also increase risk for diabetes mellitus or metabolic syndrome in the future [146–148]. A large retrospective analysis of over 13,000 patients with no baseline lipid abnormalities showed that 44 % of the subjects developed hypertriglyceridemia, 31 % had elevation in total cholesterol levels, and 11 % an increase in hepatic transaminase levels [149]. These abnormalities were however generally transient and reversible, but marked hyperlipidemia and acute pancreatitis can also occur [150]. Patients with baseline elevations of cholesterol and triglycerides are more likely to experience more severe elevations, and frequent monitoring of lipid levels is necessary in such patients if they are started on isotretinoin [151].

Acitretin is a synthetic derivative of retinoic acid used in the therapy of psoriasis which can also cause hyperlipidemia, but to a lesser degree [152, 153]. Bexarotene is also a synthetic derivative of retinoic acid which is used in the treatment of cutaneous T cell lymphoma. Over half of these patients have been reported to develop hypertriglyceridemia and central hypothyroidism [154, 155]. Interestingly, when bexarotene was used in a phase III trial of advanced non-small cell lung cancer, subjects who developed hypertriglyceridemia had longer survival which was associated with certain genetic polymorphisms [156].

The mechanism by which retinoids induce hyperlipidemia is thought to involve their interaction with nuclear receptors, Retinoid receptors (RAR) and retinoid X-receptor (RXR), and the nuclear transcription factor, FOXO1, which leads to decreased hepatic fatty acid oxidation and increased VLDL synthesis and secretion. There is also decreased triglyceride hydrolysis due to increased apolipoprotein CIII [157, 158]. Some of

these actions can be antagonized by peroxisome proliferator-activated receptor-α agonists which have opposing effects on fatty acid oxidation and VLDL secretion. Indeed, fibrates and fish oil supplementation have been reported to improve hypertriglyceridemia in patients treated with retinoids [159–161], but it would be best to avoid using them in patients with baseline hypertriglyceridemia.

Central hypothyroidism may also contribute to hyperlipidemia in bexarotene-treated patients, and it is important to check both thyroid-stimulating hormone (TSH) and free thyroxine levels in these patients. Among 27 patients with cutaneous T cell lymphoma on bexarotene therapy, 26 developed reversible TSH suppression, and 19 developed overt clinical hypothyroidism [162]. A single dose of bexarotene has also been shown to suppress TSH levels without any effect on other pituitary functions [163]. Data from mice studies suggest that this is due to direct suppression of transcription of TSH β subunit gene in the pituitary thyrotrophs [164]. Therefore, before employing lipid-lowering therapy in bexarotene-treated patients, adequate levothyroxine replacement should be given to maintain serum-free thyroxine levels near the mid-normal or even high-normal range.

Interferons

The use of interferon-α in the treatment of chronic hepatitis C infection, and as an adjuvant to chemotherapy and radiotherapy in certain malignancies is becoming increasingly popular. One of the adverse effects of interferon therapy is hypertriglyceridemia [165–168]. Serum triglycerides increased by nearly 70% during 1 year of interferon therapy for chronic hepatitis C, but returned to normal when the drug was discontinued [169]. While none of these patients developed significant hypertriglyceridemia, marked hypertriglyceridemia leading to acute pancreatitis has been reported [170]. Elevation in serum triglycerides is usually noted within 4 weeks of treatment initiation and is not dose dependent and can occur in patients with normal baseline triglycerides. Interferons have been shown to reduce hepatic triglyceride lipase activity which may be responsible for the hypertriglyceridemic effect [171, 172]. In vitro studies have also shown that they can stimulate hepatic triglyceride synthesis [173] which may also be contributing to elevated triglyceride levels. Patients with significant triglyceride elevations can be treated effectively with low-dose fibrates if lifestyle modification is not sufficient [174].

L-Asparaginase

L-Asparaginase (L-asp) is used in the treatment of hematological malignancies such as acute lymphocytic leukemia (ALL) where it works by reducing the availability of the essential amino acid L-asparagine to the malignant lymphoblastic cells. More than 60% of patients treated with this medicine develop mild hypertriglyceridemia which is usually benign and transient [175–177]. However, severe hypertriglyceridemia leading to acute pancreatitis or hyperviscosity syndrome causing neurological complications can also occur [178–182]. Some of these patients required plasmapheresis or intravenous insulin-dextrose infusion to correct the acute, severe hypertriglyceridemia, but many patients can be managed conservatively by fasting or low-fat diet [177]. Drug therapy using fibrates, omega-3 fatty acids, and acarbose have also been reported to be beneficial [177, 183–185]. Dexamethasone is often used along with L-asp and may exacerbate the hyperlipidemia. Avoidance of concomitant dexamethasone has been reported to improve serum triglyceride levels despite continuation of L-asp [186]. Interestingly, rechallenge with L-asp has been reported to have not led to recurrent hypertriglyceridemia in three children with ALL [187]. L-asp has been shown to inhibit lipoprotein lipase action which is responsible for the chylomicronemia and hypertriglyceridemia [188]. Some investigators have also shown an increase in ratio of apolipoprotein CIII to apolipoprotein CII before the onset of hypertriglyceridemia, thus suggesting that increased apolipoprotein CIII activity may also contribute to decreased triglyceride clearance [189].

Capecitabine

Capecitabine is a novel oral antineoplastic agent used in the treatment of colorectal carcinoma and other metastatic gastrointestinal and breast cancers. It is a prodrug which leads to increased levels of 5-flurouracil within the cells. While 5-flurouracil is itself not known to cause any adverse lipid effects, there have been about a dozen reports of severe hypertriglyceridemia due to capecitabine [190–196]. Serum triglycerides normalized after drug discontinuation and increased on rechallenge. A prospective study in over 200 patients on capecitabine showed that 3.7% of the patients developed clinically significant hypertriglyceridemia [197]. Most patients responded well to fenofibrate therapy without any need for drug discontinuation, and there were no cases of pancreatitis. However, capecitabine has been reported to cause pancreatitis without triglyceride elevation [198, 199]. The mechanism by which capecitabine leads to triglyceride elevation is not known, post-heparin plasma lipolytic activity has been reported to be normal [196].

Atypical Antipsychotics

The second-generation (atypical) antipsychotics have gained popularity over phenothiazines because of their better efficacy and less extra-pyramidal side effects. However, they are increasingly recognized to cause weight gain and lead to the metabolic syndrome [200–204]. Weight gain due to increased appetite may be mediated by the combined blockade of H1 histamine and serotonin 2C receptors on the hypothalamic neurons regulating feeding behavior [205, 206]. Dyslipidemia in the form of elevated serum triglycerides and low HDL cholesterol could be secondary to obesity and diabetes, but direct lipid effects independent of obesity are also thought to be at play. Increased transcriptional activity of lipid biosynthetic enzymes such as fatty acid synthase and steroyl CoA desaturase has been observed in peripheral blood cells of patients being treated with olanzapine [207]. In vitro studies have also shown the ability of atypical antipsychotics to directly impair insulin signaling and fatty acid uptake and release in cultured adipocytes [208]. The most consistent clinical effect noted is a slight increase in serum triglycerides and reduction in HDL cholesterol leading to increased ratio of LDL to HDL cholesterol, but severe hypertriglyceridemia and pancreatitis can also occur [209–211].

A pharmacovigilance study of pooled, spontaneously reported adverse events showed that, of the 192 patients who developed pancreatitis when on antipsychotic medications, more than 90% were on one of the three atypical antipsychotics, clozapine, olanzapine or resperidone, even though maximum patient exposure was to haloperidol [212]. Among the different antipsychotics, clozapine and olanzapine are associated with the highest risk for metabolic complications followed by resperidone and quetiapine, while ziprasidone and aripiprazole have the least risk [206, 213].

There is lot of interest in trying to identify genetic risk factors which predispose to dyslipidemia during treatment with these medications, and polymorphisms in apolipoprotein A5, leptin, and leptin receptor gene have been reported to increase the risk [214, 215]. While further studies in this direction may help identify at-risk patient, it is important at this time to monitor lipids at regular intervals in patients being treated with second-generation antipsychotics. Similarly, some of the selective serotonin receptor reuptake inhibitors used in the treatment of depression such as sertraline and paroxetine may rarely cause mild elevations in cholesterol and triglyceride levels, which need to be monitored.

Antiepileptic Drugs

The effect of chronic antiepileptic therapy on atherosclerotic risk factors and incidence of coronary heart disease is controversial. Some studies have reported a lower risk of death from coronary heart disease in patients on antiepileptic drugs [216], while others have shown an increased risk [217]. While many factors may play a role in determining the overall cardiovascular risk and thus explain these discrepancies, the effect of these med-

ications on lipid profile is also not very clear. In general, majority of studies have shown that carbamazepine and phenobarbital modestly increase total and LDL cholesterol, often accompanied by an increase in HDL cholesterol as well [218–222]. Most studies did not show significant effect on serum triglycerides, though some did report a significant increase with carbamazepine [223, 224].

The mechanisms by which these medications cause dyslipidemia are not clear. Since these drugs are metabolized by the hepatic cytochrome P450 enzymes, it has been proposed that they competitively interfere with the catabolism of cholesterol to bile acids which is also catalyzed by the same enzyme system [218, 225]. Hypercholesterolemia could also be augmented by the mild hypothyroidism often seen in association with these drugs [226].

Valproic acid and other newer antiepileptics such as topiramate and oxcarbazepine have minimal adverse effects on lipid profile, and some studies have even reported a modest improvement [218, 222, 224, 227]. Whether long-term antiepileptic therapy in children adversely effects cardiovascular risk is still not clear, but it may be advisable to use newer agents such as oxcarbazepine and topiramate in children with strong family history of cardiovascular disease [228].

Propofol

Propofol is an anesthetic agent used for long-term sedation in critically ill patients. It is administered in a lipid emulsion and has been associated with a moderate to severe triglyceride elevation [229, 230]. Pancreatitis has also been reported to occur, though it may be independent of hypertriglyceridemia [231]. A retrospective analysis of 159 intensive care patients treated with propofol infusion for over 24 h showed that 18% of patients developed serum triglyceride elevations over 400 mg/dL [232]. Six of these patients had serum triglycerides over 1000 mg/dL and three developed pancreatitis. The median time from start of propofol therapy to identification of hypertriglyceridemia was 54 h. A recent prospective observational study also identified propofol administration as the strongest risk factor for hypertriglyceridemia in 1300 patients admitted consecutively to the intensive care unit [233]. It is recommended that triglyceride levels be checked at least twice a week when patients are on propofol. It is not clear if the triglyceride increase is due to the lipid emulsion or a direct effect of propofol on lipid metabolism.

Protease Inhibitors

The role of human immnuodeficiency virus-1 protease inhibitors in the genesis of hyperlipidemia in association with lipodystrophy has been discussed in another section.

Conclusions

A variety of commonly used drugs including antihypertensives, steroids, immunosuppressants, antineoplastic agents, antipsychotics, and others can cause mild to severe alterations in serum lipid levels. It is important to recognize these effects when evaluating patients with dyslipidemia. The optimal treatment of drug-induced dyslipidemia would obviously be discontinuation of the medication if possible. But sometimes, clinical circumstances may dictate continued use of such medicines. The overall cardiovascular risk profile needs to be considered when managing such patients. Mild hypercholesterolemia due to diuretic use may not have adverse long-term effects. If the medication is being used only temporarily, like isotretinoin for treatment of acne, then it may be sufficient to just monitor lipid levels unless there is risk of acute complications like pancreatitis. Hypolipidemic therapy in addition to lifestyle measures may be necessary in many cases, such as in post-transplant patients, and it is important to be aware of potential drug interactions when doing so. Finally, it must be realized that other drugs, new and old, not discussed here can also potentially affect lipid levels, and clinicians should always consider drug-induced dyslipidemia in the differential diagnosis of hyperlipidemic disorders.

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Lipodystrophies and Dyslipidemias

16

Abhimanyu Garg

Introduction

Patients presenting with extreme hypertriglyceridemias can either have type 1 hyperlipoproteinemias (T1HLP, primarily elevations of chylomicrons) or type 5 hyperlipoproteinemias (T5HLP, elevations of both very low-density lipoproteins (VLDL) and chylomicrons). Clinically, based upon measurement of serum total cholesterol and triglyceride levels, it is difficult to distinguish between type 1 and 5 hyperlipoproteinemias as both can cause acute pancreatitis and eruptive or tuberous xanthomas. Most clinicians when presented with T1HLP or T5HLP will consider mutations in genes involved in lipoprotein metabolism such as lipoprotein lipase, apolipoproteins CII, E, and A5, and the newly recognized glycosylphosphatidyl-inositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) and lipase maturation factor 1 (LMF1). However, patients with inherited or acquired lipodystrophies also present with mild to severe hypertriglyceridemia including T5HLP and these disorders must be considered in the differential diagnosis of T1/5HLP patients. This chapter thus reviews the clinical features and underlying etiology of various lipodystrophy syndromes and the mechanisms of dyslipidemias in these patients.

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Overview of Lipodystrophy Syndromes

Lipodystrophies are rare, heterogeneous, inherited, or acquired disorders characterized by selective loss of adipose tissue [1]. The extent of fat loss can be variable with some having loss of sc fat from small areas (localized), to distinct regions of body such as extremities (partial) to near total loss of adipose tissue (generalized). While patients with localized lipodystrophies do not develop any metabolic complications, those with partial or generalized loss of body fat are predisposed to developing insulin resistance and its complications such as premature diabetes mellitus, hypertriglyceridemia, low levels of HDL cholesterol, and nonalcoholic hepatic steatosis. The severity of metabolic complications in general parallels the extent of body fat loss and patients with generalized lipodystrophies have more severe metabolic derangements compared to those with partial lipodystrophies. A classification of the various lipodystrophy syndromes and their etiopathologic basis is shown in Table 16.1.

Genetic Lipodystrophies

The genetic lipodystrophies so far have been reported in about 1000 patients and their estimated prevalence in general population based on the assumption that only one fourth of the patients may be reported in the published literature that could be less than one in a million. In the past

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Table 16.1 Classification, clinical features, and pathogenetic and molecular basis of genetic and acquired lipodystrophies

A. Genetic lipodystrophies				
I. Autosomal recessive				
Type	Subtypes (Gene)	Key clinical features	Dyslipidemia	Molecular basis/other comments
Congenital generalized lipodystrophy (CGL)[a]	CGL1 (*AGPAT2*)	Lack of metabolically active adipose tissue since birth	T5HLP, mild HTG in children	AGPATs are key enzymes required for triglyceride and phospholipids biosynthesis. AGPATs acylate lysophosphatidic acid to form phosphatidic acid. AGPAT2 is highly expressed in adipose tissue
	CGL2 (*BSCL2*)	Lack of both metabolically active and mechanical adipose tissue since birth, mild mental retardation, cardiomyopathy	T5HLP, mild HTG in children	*BSCL2* encodes seipin which may play a role in fusion of small lipid droplets and in adipocyte differentiation
	CGL3 (*CAV1*)	Single patient with extreme lack of body fat, short stature, and vitamin D resistance	T5HLP	Caveolin 1 is an integral component of caveolae, present in abundance on adipocyte membranes. Caveolin 1 binds fatty acids and translocates them to lipid droplets
	CGL4 (*PTRF*)	Extreme lack of body fat, congenital myopathy, pyloric stenosis, and cardiomyopathy	Mild HTG in children; some have T5HLP	PTRF (also known as cavin) is involved in biogenesis of caveolae and regulates expression of caveolins 1 and 3
Mandibuloacral dysplasia[a]	Type A (*LMNA*)	Skeletal anomalies, loss of sc fat from the extremities and trunk	Mild to moderate HTG	Lamins A and C are nuclear lamina proteins and *LMNA* mutations may disrupt nuclear function resulting in premature cell death in many tissues
	Type B (*ZMPSTE24*)	Skeletal anomalies, more generalized loss of fat, premature renal failure, progeroid features	Mild HTG	ZMPSTE24 is required for posttranslational processing of carboxy-terminal residues of prelamin A to form lamin A. Accumulation of farnesylated prelamin A may disrupt nuclear function in several tissues.
Autoinflammatory syndromes	JMP (*PSMB8*)	Joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy	Low HDL, normal TG	PSMB8 encodes β5i, a catalytic subunit of the immunoproteasomes. Immunoproteasome-mediated proteolysis generates immunogenic epitopes presented by MHC class I molecules
	CANDLE (PSMB8)	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature	Mild HTG	New syndrome reported in six patients

Table 16.1 (continued)

Type	Subtypes (Gene)	Key clinical features	Dyslipidemia	Molecular basis/other comments
Familial partial lipodystrophy (FPL)	(*CIDEC*)	Single patient with loss of sc fat from limbs, multilocular, small lipid droplets in adipocytes	T5HLP	CIDEC is a lipid droplet associated protein that inhibits lipolysis and promotes formation of unilocular lipid droplet in adipocytes
Neonatal progeroid syndrome	Unknown	Generalized loss of body fat and muscle mass, and progeroid appearance at birth	Unknown	Unknown
II. Autosomal dominant				
Familial partial lipodystrophy (FPL)[a]	FPLD1, Kobberling (unknown)	Loss of sc fat from the extremities	HTG	Phenotype not well characterized
	FPLD2, Dunnigan (*LMNA*)	Loss of sc fat from the extremities and trunk (sparing the face and neck) at puberty	Normal to T5HLP	Lamins A and C are nuclear lamina proteins and specific mutations may disrupt nuclear function resulting in premature death of adipocytes
	FPLD3 (*PPARG*)	Loss of sc fat from the extremities, especially from distal regions	Normal to T5HLP	PPAR γ is a critical transcription factor required for adipogenesis. Dominant negative PPAR γ mutations may inhibit adipocyte differentiation
	FPLD4 (*AKT2*)	Single family reported with loss of sc fat from the extremities	Unknown	AKT2, also known as protein kinase B, is involved in adipocyte differentiation and downstream insulin receptor signaling
	FPLD5 (*PLIN1*)	Loss of sc fat from the extremities with small adipocytes and increased fibrosis of adipose tissue	Mild to T5HLP	Perilipin 1 is an integral component of lipid droplet membranes and is essential for lipid storage and hormone regulated lipolysis
Atypical progeroid syndrome	(*LMNA*)	Variable loss of sc fat, progeroid features	Mild to T5HLP	Different heterozygous, mostly de novo mutations in *LMNA*, cause nuclear dysfunction
Hutchinson–Gilford progeria	(*LMNA*)	Generalized loss of sc fat, progeroid features	Normal to mild HTG	Specific de novo *LMNA* mutations induce abnormal splicing and accumulation of truncated farnesylated prelamin A.
SHORT syndrome	(*PIK3R1*)	Short stature, Hyperextensibility or inguinal hernia, Ocular depression, Rieger anomaly, and Teething delay	Normal or borderline HTG	PIK3R1 encodes multiple regulatory subunits of phosphatidylinositol 3 kinase (PI3K), an intracellular enzyme with a central role in insulin signaling
MDP syndrome	(*POLD1*)	Mandibular hypoplasia, deafness, progeroid features, undescended testes, and male hypogonadism	Mild HTG, Low HDL-C	Patients usually present with generalized loss of sc fat POLDI plays key role in DNA replication.
Neonatal progeroid syndrome	NPS1 (*FBN1*)	Generalized loss of body fat and muscle mass, and progeroid appearance at birth	TG normal, low HDL-C	Five patients reported with de novo heterozygous mutations in the penultimate exon of fibrillin 1

Table 16.1 (continued)

B. Acquired lipodystrophies				
Type	Subtypes (Gene)	Key clinical features	Dyslipidemia	Molecular basis/other comments
Lipodystrophy in HIV-infected patients	a. PI-induced b. NRTI-induced	Loss of sc fat from the face and extremities, and excess fat deposition in the neck and abdomen	Mild to T5HLP	Protease inhibitors may inhibit ZMPSTE24 and/or cause dysregulation of transcription factors involved in adipogenesis. NRTIs may inhibit mitochondrial polymerase-γ and cause mitochondrial toxicity
Acquired partial lipodystrophy	a. Autoimmune b. MPGN-associated c. Idiopathic	Loss of sc fat from the face, neck, upper limbs, and trunk, sparing the lower abdomen and lower limbs	Mild HTG in one third	Low serum complement 3 levels and presence of an autoantibody, complement 3 nephritic factor, in most of the patients suggest autoimmune-mediated loss of adipose tissue
Acquired generalized lipodystrophy	a. Autoimmune b. Panniculitis-associated c. Idiopathic	Generalized loss of fat associated with tender sc nodules, autoimmune or other diseases	Mild to T5HLP	Panniculitis preceding the loss of sc fat and association of autoimmune diseases suggest immune-mediated loss of adipose tissue. Other mechanisms may also be involved.
Localized lipodystrophy	a. Drug-induced b. Panniculitis-induced c. Pressure-induced d. Centrifugal e. Idiopathic	Loss of sc fat from small areas of the body	None	Multiple mechanisms including local drug-induced immune-mediated or pressure-induced atrophy of adipose tissue. Other unknown mechanisms may also be involved

[a]Additional rare types for which the genetic basis is not known are not included.

HIV human immunodeficiency virus, *AGPAT* 1-acylglycerol-3-phosphate *O*-acyltransferase, *BSCL2* Berardinelli–Seip congenital lipodystrophy 2, *CAV1* caveolin 1, *PTRF* polymerase I and transcript release factor, *PPAR* peroxisome proliferator-activated receptor, *LMNA* lamin A/C, AKT2, v-AKT murine thymoma oncogene homolog 2, *CIDEC* cell death-inducing DNA fragmentation factor a-like effector c, *PLIN1* perilipin 1, *PSMB*8 proteasome subunit, beta-type 8, *MHC* major histocompatibility complex, *MPGN* membranoproliferative glomerulonephritis, *PIK3R1* phosphoinositide-3- kinase regulatory subunit 1, *POLD1* polymerase (DNA-directed) delta 1 catalytic subunit, *PI HIV-1* protease inhibitors, *NRTI* nucleoside reverse transcriptase inhibitors, *SC* subcutaneous, *SHORT* short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Reiger anomaly and teething delay, *MDP* mandibular hypoplasia, deafness, progeroid features, *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome, *JMP* joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy, *T5HLP* type 5 hyperlipoproteinemias, *HTG* hypertriglyceridemia, *ZMPSTE24* zinc metalloprotease enzyme

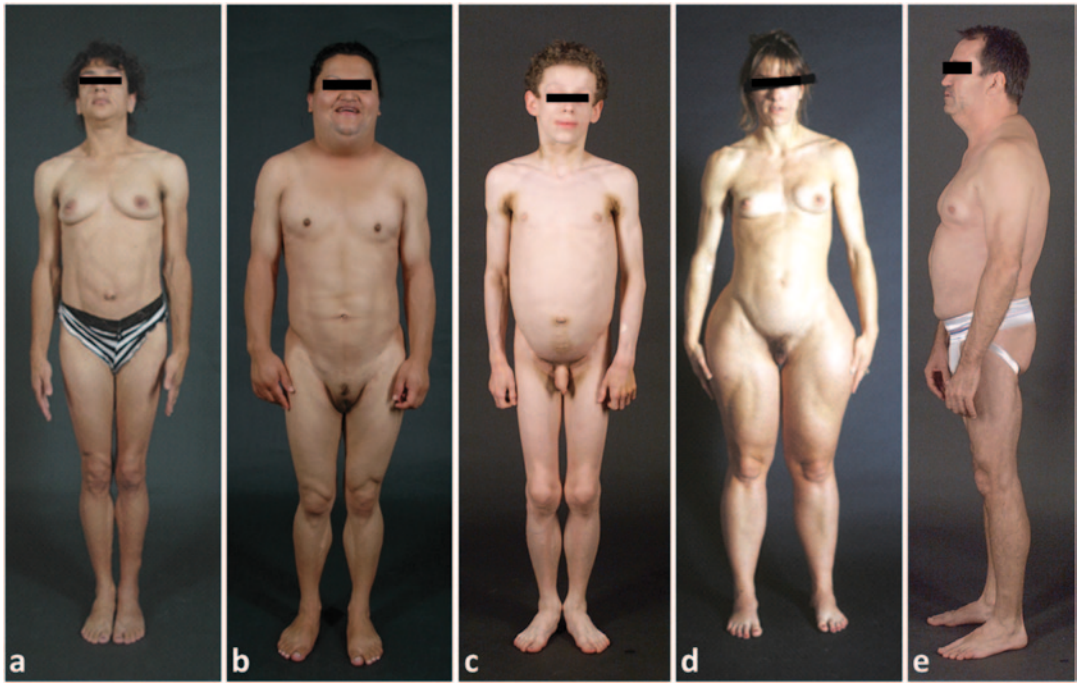

Fig. 16.1 Clinical features of patients with various types of lipodystrophies. **a** Anterior view of a 33-year-old Hispanic female with congenital generalized lipodystrophy (also known as Berardinelli–Seip congenital lipodystrophy), type 1 due to homozygous c.IVS4–2A > G mutation in *AGPAT2* gene. The patient had generalized loss of sc fat with acanthosis nigricans in the axillae and neck. She has umbilical prominence and acromegaloid features (enlarged mandible, hands, and feet). **b** Anterior view of a 27-year-old Native American Hispanic female with familial partial lipodystrophy of the Dunnigan variety due to heterozygous p.Arg482Trp mutation in *LMNA* gene. She had marked loss of sc fat from the limbs and anterior truncal region. The breasts were atrophic. She had increased sc fat deposits in the face, anterior neck, and vulvar regions. **c** Anterior view of an 8-year-old German boy with acquired generalized lipodystrophy. He had severe generalized loss of sc fat with marked acanthosis nigricans in the neck, axillae, and groin. **d** Anterior view of a 39-year-old Caucasian female with acquired partial lipodystrophy (Barraquer–Simons syndrome). She had marked loss of sc fat from the face, neck, upper extremities, chest and had lipodystrophy on localized regions on anterior thighs. She had increased sc fat deposition in the lower extremities. **e** Lateral view of a 53-year-old Caucasian male infected with human immunodeficiency (HIV) virus with highly active antiretroviral therapy induced lipodystrophy. He had marked loss of sc fat from the face and limbs but had increased sc fat deposition in the neck region anteriorly and posteriorly showing buffalo hump. Abdomen was protuberant due to excess intra-abdominal fat. He had been on protease inhibitor containing antiretroviral therapy for more than 8 years. (Reproduced with permission from Garg A. Lipodystrophy. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K (eds). *Fitzpatrick's Dermatology in General Medicine*, 8th Edition. McGraw Hill, New York, NY, pp 755–764, 2012)

two decades, progress in characterization of the phenotypes and elucidation of their molecular defects has led to increased recognition of these syndromes. The most common types of genetic lipodystrophies are congenital generalized lipodystrophy (CGL) and familial partial lipodystrophy (FPL). Other syndromes are quite rare and each have been reported in approximately 30 patients or less. Affected females are recognized easily and thus are reported more often than males.

Congenital Generalized Lipodystrophy

CGL is a rare autosomal recessive disorder in which near-total absence of subcutaneous adipose tissue is evident from birth [2]. Affected subjects have a marked muscular appearance with prominent veins, acromegaloid features, acanthosis nigricans, hepatomegaly, and umbilical prominence (Fig. 16.1a). As children, they are often noted to

have a voracious appetite, and accelerated linear growth. Female subjects with CGL usually have hirsutism, clitoromegaly, oligo-amenorrhea, and polycystic ovaries. Other clinical features which may be noted in some patients include focal lytic lesions in the long bones, hypertrophic cardiomyopathy, and mild mental retardation [3, 4]. Metabolic abnormalities related to insulin resistance, such as diabetes mellitus, hyperlipidemia, and hepatic steatosis, are evident at a young age and often difficult to control. Many of these patients suffer from recurrent attacks of acute pancreatitis due to severe T5HLP.

Positional cloning and candidate gene approach have led to the identification of four genetic loci for CGL: the 1-acylglycerol-3-phosphate-O-acyltransferase 2 (*AGPAT2*) gene on chromosome 9q34 [5, 6], the Berardinelli–Seip congenital lipodystrophy 2 (*BSCL2*) gene on chromosome 11q13 [7], caveolin 1 (*CAV1*) gene on chromosome 7q31 [8], and polymerase I and transcript release factor (PTRF) on chromosome 17q21 [9]. Additional loci remain to be identified as some patients with CGL do not harbor mutations in any of the above genes [3, 4, 10]. The clinical features are by and large similar among patients with CGL due to any of these genetic defects, but some phenotypic differences do exist. CGL patients with *BSCL2* mutation have more severe lipodystrophy in that there is loss of both mechanical adipose tissue (found in retro-orbital, palm, sole, and other areas) and metabolically active adipose tissue (found in subcutaneous, intra-abdominal, intrathoracic, and other areas) when compared to other CGL patients where mechanical fat is preserved [8, 11]. A high prevalence of cardiomyopathy and mental retardation is also seen in those with *BSCL2* mutations [4]. The only reported patient with *CAV1* mutation also had short stature and presumed Vitamin D resistance [8]. Those with *PTRF* mutations have congenital myopathy, pyloric stenosis, skeletal anomalies, and prolonged QT interval and predisposition to catecholaminergic polymorphic ventricular tachycardia that can result in sudden death [12, 13].

Familial Partial Lipodystrophy

FPL is a rare autosomal dominant disorder in which fat loss mostly involves the extremities with variable fat loss from the trunk. It results from heterozygous missense mutations in lamin A/C (*LMNA*) [14–16], peroxisome proliferator-activated receptor γ (*PPARG*) [17–19], v-AKT murine thymoma oncogene homolog 2 (*AKT2*) [20] and perilipin 1 (*PLIN1*). A single patient has been reported to have the autosomal recessive variety of FPL phenotype due to homozygous mutation in *CIDEC*. The most common subtype of FPL is the Dunnigan variety (FPLD), which is due to *LMNA* mutations. Affected subjects have normal body fat distribution at birth and during childhood, but progressive loss of fat occurs from the extremities and trunk during late childhood and puberty. Subcutaneous fat over the face, chin, supraclavicular, and dorsocervical regions, and intra-abdominal fat are spared, and often excess fat accumulates there [21, 22] (Fig. 16.1d). Severity of fat loss may also depend on the site of *LMNA* mutation [23]. Similar to patients with CGL, acanthosis nigricans, and hepatomegaly can be prominent, and about one fourth of the female subjects show features of polycystic ovarian syndrome. Other metabolic abnormalities such as diabetes and hyperlipidemia are also commonly seen, particularly in affected women [24], but usually at a later age than in CGL patients. Affected women with FPLD also tend to have low levels of HDL-cholesterol. Diabetes and associated dyslipidemia can predispose these patients to develop premature coronary heart disease and other atherosclerotic vascular manifestations including peripheral vascular disease and cerebrovascular accidents. Occasionally, patients also develop cardiomyopathy and conduction system abnormalities indicative of a multisystem dystrophy [25, 26].

FPL due to *PPARG* mutations results in a milder phenotype with fat loss being noted usually after the second decade, and usually confined to the distal extremities [17, 19]. It is also less common with about only 30 reported cases, and it is likely that the mild phenotype may hamper recognition. FPL due to *AKT2* mutation has been

described in a single pedigree so far [20], and the phenotype is not well characterized. FPL due to *PLIN1* mutations has been reported in only three pedigrees. It is likely that other genetic loci exist for FPL, as some patients do not show mutations in any of the above FPL genes.

Lipodystrophy in Association with Other Syndromes

Partial to generalized lipodystrophy has been reported to be a part of other rare genetic syndromes, many of which are also characterized by progeroid features. Mandibuloacral dysplasia is an autosomal recessive disorder characterized by postnatal development of osteolysis involving the mandibles, clavicles, and terminal digits. Other associated features include short stature, delayed closure of cranial sutures, mottled skin pigmentation, hypogonadism, and sensorineural hearing loss. Lipodystrophy is another feature of this syndrome, and based on the pattern of fat loss, two varieties of MAD have been described [27]. MAD type A is associated with partial lipodystrophy similar to FPLD, and results from homozygous or compound heterozygous missense mutations in *LMNA* [28]. MAD type B is associated with a more generalized pattern of fat loss, and results from mutations in zinc metalloprotease (*ZMPSTE24*) enzyme, which is critical for posttranslational proteolytic processing of prelamin A to mature lamin A [29]. Some patients with MAD do not have mutations in either of these two loci suggesting the presence of additional loci. Both partial and generalized lipodystrophy has also been reported in patients with Hutchinson–Gilford progeria [30], and atypical progeroid syndrome due to heterozygous *LMNA* mutations [31]. In patients with neonatal progeroid syndrome, generalized diminution of subcutaneous fat has been reported, but it is often accompanied by concomitant reduction in skeletal muscle and lean body mass unlike other lipodystrophy syndromes [32]. Some patients with neonatal progeroid syndrome have been reported to have de novo heterozygous null mutations in penultimate exon of fibrillin 1 (*FBN1*) gene [33–36]. Patients with SHORT syndrome (acronym for short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Reiger anomaly and teething delay) have also been reported to have partial lipodystrophy involving fat loss from the face and upper trunk, or localized fat loss from the hip region and elbows [37, 38]. Recently, heterozygous de novo mutations in phosphatidyl inositol 3 kinase receptor 1 (*PIK3R1*) were reported in many patients with SHORT syndrome including a recurrent mutation [39, 40]. We recently reported a distinct autosomal recessive, autoinflammatory syndrome characterized by *joint* contractures, *muscle* atrophy, *microcytic* anemia and *panniculitis*-induced (JMP) lipodystrophy [41] and reported a homozygous, missense, loss of function, mutation in proteasome subunit, beta-type, 8 (*PSMB8*) gene [42]. *PSMB8* encodes the β5i subunit of the immunoproteasome [43]. Immunoproteasome-mediated proteolysis generates immunogenic epitopes presented by major histocompatibility complex (MHC) class I molecules. Mutation in *PSMB8* may trigger autoinflammatory response resulting in infiltration of adipose tissue with lymphocytes and other immune cells and loss of nearby adipocytes. Mutations in *PSMB8* have since also been reported in *c*hronic *a*typical *n*eutrophilic *d*ermatosis with *l*ipodystrophy and *e*levated temperature (CANDLE) syndrome [44, 45]. Another novel syndrome reported by us is characterized by *m*andibular hypoplasia, *d*eafness, *p*rogeroid features (MDP)-associated lipodystrophy [46]. All males with MDP had undescended testes and hypogonadism. MDP syndrome was recently reported to be due to de novo heterozygous recurrent mutation in polymerase delta 1 (*POLD1*) gene [47].

Peterfy et al. [48] have recently reported a homozygous truncation mutation in lipase maturation factor 1 (*LMF1*), responsible for the maturation of both lipoprotein lipase (LPL) and hepatic lipase (HL) in a woman with marked hypertriglyceridemia since early adulthood and lipodystrophy affecting extremities but sparing her face and abdomen. However, precise characterization of the pattern of lipodystrophy was not reported. Interestingly, patients with LPL deficiency have

normal body fat distribution through enhanced adipocyte lipogenesis [49].

Acquired Lipodystrophies

Acquired lipodystrophy in HIV-infected patients (LD-HIV) is the commonest type of lipodystrophy, estimated to be affecting more than 100,000 patients in the USA and many more in other countries [50–52]. However, acquired generalized lipodystrophy (AGL) and acquired partial lipodystrophy (APL), both of autoimmune origin, have been reported in ~100 and 250 cases, respectively affecting women three to four times more often than men [53, 54].

Acquired Generalized Lipodystrophy (Lawrence Syndrome)

The onset of loss of sc fat in patients with AGL usually occurs during childhood [54] and is quite variable (Fig. 16.1c). While most of them have generalized loss of fat, in some patients, however, certain areas of the body such as intra-abdominal and bone marrow fat are spared. AGL patients are highly likely to develop severe hepatic steatosis, diabetes, and hypertriglyceridemia. Some of them have developed painful eruptive xanthomas requiring plasmapheresis. The mechanisms of fat loss in patients with AGL seem to be variable, including panniculitis, associated autoimmune diseases especially dermatomyositis, and unknown mechanisms [54]. Some patients have low serum complement 4 levels suggesting involvement of the classical complement pathway in the pathogenesis of fat loss [55].

Acquired Partial Lipodystrophy (Barraquer–Simons syndrome)

The onset of APL usually occurs before the age of 15 years and loss of sc fat loss occurs gradually in a symmetric fashion first affecting the face and then spreading downwards. Mostly, sc fat is lost from the face, neck, upper extremities, and trunk, and the lower abdomen and legs are spared (Fig. 16.1d). Metabolic complications are usually not seen. However, approximately one fifth of the patients develop membranoproliferative glomerulonephritis and later on some of them develop drusen [53]. More than 80% of the patients have low serum levels of complement 3 and a circulating autoantibody called complement 3-nephritic factor that blocks degradation of the enzyme C3 convertase [53].

Highly Active Antiretroviral Therapy-Induced Lipodystrophy in HIV-Infected Patients

LD-HIV is usually clinically apparent after patients have been treated with HIV1- protease inhibitors (PIs)-containing HAART for 2 years or more (Fig. 16.1e). The most severely affected regions are the arms, legs, and face and some patients accumulate excess fat in nonlipodystrophic regions presenting as buffalo hump, double chin, and increased waist circumference [50]. The fat loss worsens with ongoing HAART therapy and does not reverse on discontinuation of PIs. Many patients develop hypertriglyceridemia but only a few develop diabetes mellitus.

Both, PIs and nucleoside reverse transcriptase inhibitors (NRTIs) are implicated. PIs may cause lipodystrophy by inhibiting ZMPSTE24 and resulting in the accumulation of toxic farnesylated prelamin A [56] but other mechanisms may also be involved [57]. NRTIs, especially zidovudine and stavudine, have been proposed to induce fat loss by inhibiting mitochondrial polymerase-γ and causing mitochondrial toxicity [58, 59]. Since PIs or NRTIs are usually given together as part of the HAART, the individual effects of these drugs on the phenotype remain unclear.

Hyperlipidemia in Lipodystrophy Syndromes

Dyslipidemia can be recognized as early as in infancy but mostly mild to moderate elevations of TG are seen in childhood. During puberty and

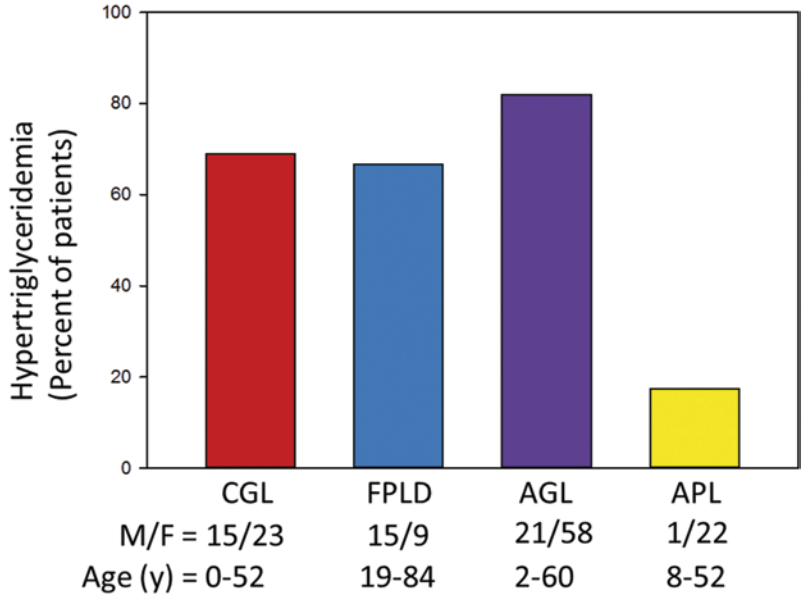

Fig. 16.2 Prevalence of hypertriglyceridemia in various types of genetic and acquired lipodystrophies. Hypertriglyceridemia is defined as fasting serum triglyceride concentrations ≥200 mg/dL. The number of males (*M*) and female (*F*) patients is provided under the *x*-axis as well as the age range

after the onset of diabetes mellitus, patients are predisposed to marked HTG or T5HLP. Females with CGL generally develop more severe metabolic derangements than males.

Despite marked phenotypic and genotypic heterogeneity among different lipodystrophy syndromes, there is a high prevalence of dyslipidemia characterized by marked hypertriglyceridemia and reduced HDL-cholesterol levels (Fig. 16.2). The severity of these metabolic abnormalities, however, varies, and is closely related to the extent of fat loss [60]. Severe hypertriglyceridemia, often associated with eruptive xanthoma and recurrent pancreatitis, is seen in patients with all varieties of CGL. The prevalence of hypertriglyceridemia in case series of CGL patients is over 70% [3], but it may actually be even more common as some of the patients were very young. Usually, serum triglycerides are normal or slightly increased during early childhood and severe hypertriglyceridemia manifests after puberty along with onset of diabetes mellitus. FPLD patients may also present with eruptive xanthomas and pancreatitis (Fig. 16.1c, d). Interestingly, serum triglyceride elevation in female subjects with FPLD is about 2–3 times higher than in male subjects [24]. Modest elevation in serum lipids are noted in FPL subjects with *PPARG* and *AKT2* mutations, and in subjects with MAD. However, dyslipidemia is not a typical feature of lipodystrophic subjects with progeria syndromes such as Hutchinson–Gilford progeria [61] or neonatal progeroid syndrome [32], where fat loss appears to be proportionate to loss of total and lean body mass. On the other hand, elevated serum triglyceride levels are reported in patients with atypical progeroid syndrome due to *LMNA* mutations [31, 62]. Lipid abnormalities are also mild in patients with SHORT syndrome who only have mild fat loss from the upper body. Patients with JMP syndrome interestingly have markedly low levels of HDL cholesterol but only have mild hypertriglyceridemia. Those with MDP syndrome do not manifest dyslipidemias. All these observations suggest that hyperlipidemia is a direct consequence of fat loss, and more severe the fat loss, greater is the severity of lipid abnormalities. As far as acquired lipodystrophies are concerned, most patients with AGL develop extreme hypertriglyceridemia. Hypertriglyceri-

demia in AGL is more prevalent in those with associated autoimmune diseases or idiopathic varieties than in those with panniculitis variety [54]. In contrast, only about one third of the patients with APL have hypertriglyceridemia or low levels of HDL-cholesterol [53]. Patients with HIV-LD also manifest mild to severe hypertriglyceridemia [50].

Mechanisms of Fat Loss and Dyslipidemia in Generalized Lipodystrophy

Despite much progress in understanding the genetic and autoimmune basis of lipodystrophy syndromes, the exact molecular mechanisms which lead to fat loss and dyslipidemia are not entirely clear. The adipocytes are specialized cells designed for synthesis and storage of neutral lipids. In the absence of adipocytes, dietary lipids accumulate in aberrant sites such as the liver and muscle leading to cellular toxicity and metabolic abnormalities. Accumulation of liver triglycerides leads to fatty liver and increased hepatic VLDL secretion. Excessive VLDL production resulting in saturation of the catalytic sites of LPL further causes accumulation of chylomicrons and T5HLP [63]. There is also a role of deficiency of adipocytokines such as leptin and adiponectin in inducing metabolic abnormalities [64]. Leptin can influence both energy intake and peripheral lipid deposition in nonadipose tissues, and its deficiency may contribute to hyperphagia and hepatic steatosis.

Lipid droplet formation in adipocytes involves accumulation of newly synthesized triglycerides in the endoplasmic reticulum and coating by amphipathic proteins belonging to the PAT family, such as perilipin, and by glycerophospholipids (Fig. 16.3). The small lipid droplets coalesce to form a single large lipid vacuole in the mature adipocyte [65]. Deficiency of AGPAT2, a critical enzyme involved in the biosynthesis of triglyceride and glycerophospholipid, could therefore restrict lipid droplet synthesis at an early stage. The *BSCL2* encoded protein, seipin, is involved in fusion of lipid droplets, in adipocyte differentiation and can inducibly bind lipin 1, a phosphatidic acid phosphatase, involved in biosynthesis of di- and tri-acylglycerol [66, 67]. Caveolin-1 and PTRF through their role in caveolae formation may also contribute to lipid droplet formation [68, 69].

The Agpat2-deficient mice develop hepatic steatosis through increased TG biosynthesis involving an alternate monoacylglycerol pathway using monoacylglycerol acyltransferase 1 (MGAT1) [70]. Further, dietary fat restriction ameliorated hepatic steatosis and hyperlipidemia in the Agpat2-deficient mice, which may have implications for the treatment of CGL patients. Interestingly, the Agpat2-deficient and Bscl2-null mice have normal or low serum free fatty acid levels despite diabetes and insulin resistance which raises doubts on the previously proposed role of increased free fatty acid turnover in causing metabolic complications in patients with generalized lipodystrophy [71].

Mechanisms of Fat Loss and Dyslipidemia in Partial Lipodystrophy

Pathogenesis of fat loss and dyslipidemia in partial lipodystrophies is not well understood. Mutations in *LMNA* may result in premature death or apoptosis of adipocytes resulting in lipodystrophy (Fig. 16.4). PPARγ is a key regulator of adipocyte differentiation and AKT2 is also necessary for adipocyte differentiation besides its involvement in post receptor insulin signaling. Perilipin 1 is the most abundant lipid droplet protein. Thus, mutations in these genes could cause lipodystrophy by affecting adipocyte differentiation or lipid droplet formation. However, why certain fat depots undergo atrophy while others are spared in partial lipodystrophies remains a mystery.

The pathogenesis of hyperlipidemia in partial lipodystrophies also likely involves increased hepatic VLDL secretion. Semple and colleagues [72] showed elevated liver fat and secretion of triglyceride-enriched VLDL in patients with *LMNA* and *AKT2* mutations but normal levels of fasting free fatty acids. Since de novo lipogenesis

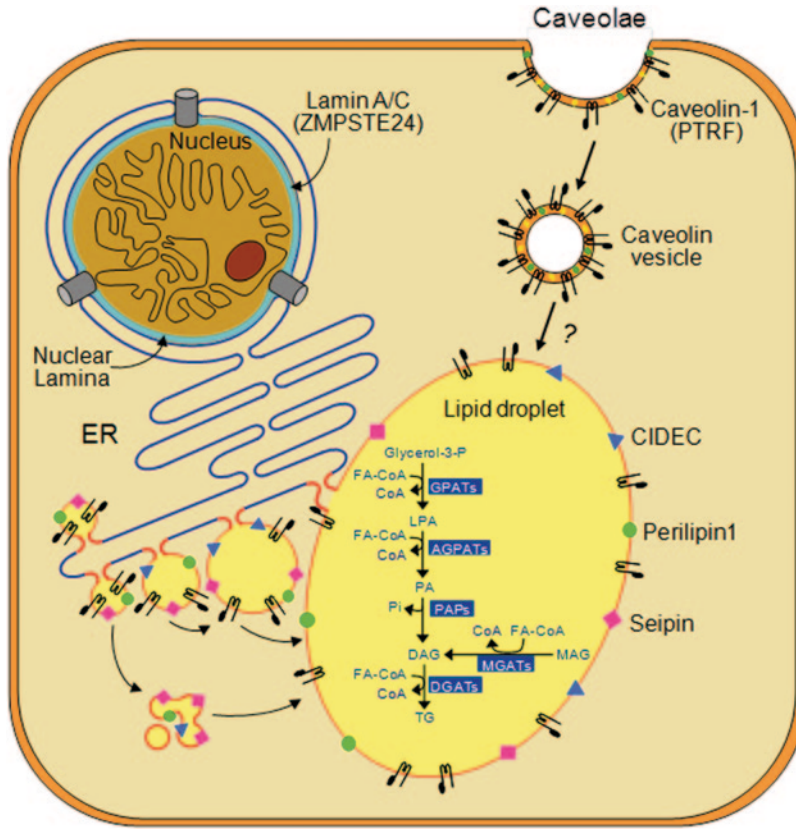

Fig. 16.3 Lipid droplet formation in adipocytes. Lipid droplets (LD) are organelles that store triglycerides (TG) intracellularly. They form as budding vesicles at the endoplasmic reticulum (ER) which fuse in adipocytes to form one large LD. Many proteins, such as cell death-inducing DNA fragmentation factor a-like effector c (CIDEC, shown in *blue triangles*), seipin (*pink squares*) and perilipin 1 (*green circles*) are present on the LD membrane. CIDEC and seipin may be involved in fusion of LDs to form a larger LD while perilipin 1 is essential for lipid storage and hormone mediated lipolysis. Caveolae are formed from lipid rafts on the cell surface which include cholesterol (*yellow symbols*), glycosphingolipids (*green symbols*) and caveolin-1 (*black hair pin like symbols*). Endocytosis of caveolae forms caveolin vesicles which may directly merge with lipid droplets and thus translocating fatty acids to LDs. Polymerase I and transcript release factor (PTRF) controls expression of caveolin 1 and 3 (not shown). The classical and alternative pathways involved in the biosynthesis of TG are shown inside the lipid droplet. In the adipose tissue, TG synthesis requires glycerol-3-phosphate as the initial substrate (classical pathway), whereas in the small intestine, synthesis of TG can occur via an alternative pathway using monoacylglycerol (MAG) as the initial substrate. Acylation of glycerol-3-phosphate using fatty acyl coenzyme A (FA-CoA) at the sn-1 position is catalyzed by glycerol-3-phosphate acyltransferases (GPATs) resulting in the formation of 1-acylglycerol-3-phosphate or lysophosphatidic acid (LPA). LPA is then acylated at the sn-2 position by 1-acylglycerol-3-phosphate acyltransferases (AGPATs) to yield phosphatidic acid (PA). Removal of phosphate group from PA by PA phosphatases (PAPs) produces diacylglycerol (DAG). Further acylation of DAG at the sn-3 position by diacylglycerol acyltransferases (DGATs) finally produces TG. In the alternative pathway, MAG is acylated to DAG by monoacylglycerol acyltransferases (MGATs) which is then further converted to TG. Lamin A/C are integral components of nuclear lamina (shown in blue color) and interact with nuclear membrane proteins as well as chromatin. Zinc metalloproteinase (ZMPSTE24) is critical for post-translational processing of prelamin A to its mature form, lamin A. (Reproduced with permission from: Garg A. Lipodystrophies: genetic and acquired body fat disorders. J Clin Endocrinol Metab 96:3313–25, 2011)

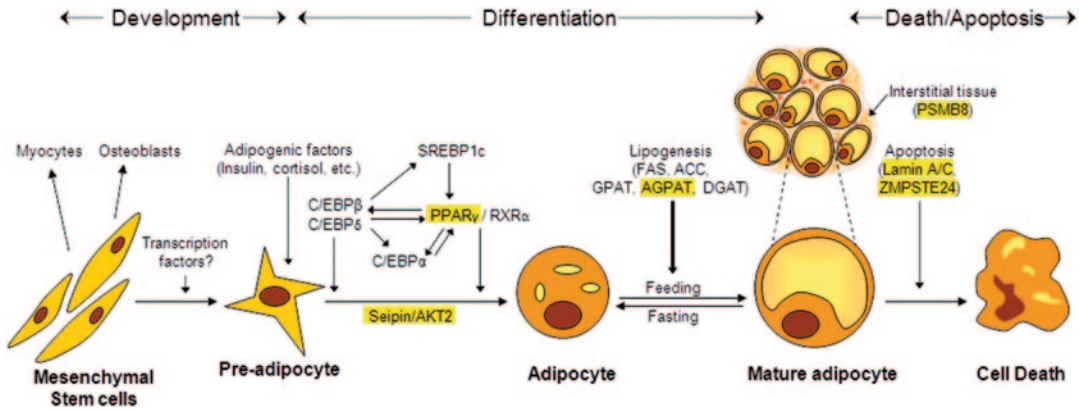

Fig. 16.4 Pathways involved in the development, differentiation, and death of adipocytes. The pluripotent mesenchymal stem cells can form preadipocytes, myocytes, or osteoblasts depending upon the various cues. In response to various signals from hormones such as insulin and steroids and induction of adipogenic transcription factors, a series of changes are initiated in preadipocytes which lead to their differentiation to adipocytes. The transcription factors, CCAAT (cytidine–cytidine–adenosine–adenosine–thymidine)-enhancer-binding proteins (C/EBP) β/δ, are the first to be upregulated and then stimulate other transcription factors such as PPARγ, C/EBPα, and *sterol regulatory element-binding protein (*SREBP) 1c. Some other genes such as preadipocyte factor 1 (Pref1), a known adipogenesis inhibitor are downregulated. Mature adipocytes are activated resulting in the overexpression of lipogenic genes like fatty acid synthase (FAS), acetyl coenzyme A carboxylase (ACC), GPATs, AGPATs, and DGATs for biosynthesis of triglycerides and phospholipids. The size of the lipid droplets is reduced upon fasting and increases with increased substrate availability. Available data suggest that the *BSCL2*-encoded protein, seipin, and v-AKT murine thymoma oncogene homolog 2 (AKT2) may be involved in adipocyte differentiation, whereas AGPAT2 affects triglyceride synthesis. Clinical evidence from lipodystrophy patients harboring *LMNA* or *ZMPSTE24* mutations suggests that nuclear dysfunction may accelerate apoptosis/death of mature adipocytes. Interstitial tissue may also play an important role in adipocyte survival. Mutations in *PSMB8* which encodes β5i, a catalytic subunit of the immunoproteasomes, may induce autoinflammatory syndrome resulting in infiltration of lymphocytes in adipose tissue (panniculitis) and death of nearby adipocytes. (Reproduced with permission from: Garg A. Lipodystrophies: genetic and acquired body fat disorders. J Clin Endocrinol Metab 96:3313–25, 2011)

was significantly increased, the authors speculate that partial post-receptor insulin resistance (resistance to glucose uptake, but not to lipogenesis) contributes to hyperlipidemia [72].

Therapeutic Options for Dyslipidemias in Lipodystrophies

While mild to modest hypertriglyceridemia can predispose patients with lipodystrophy to premature atherosclerosis, extreme HTG can lead to acute pancreatitis and death. T5HLP in lipodystrophy patients is often resistant to conventional therapy [73] but various approaches can be suggested. In general, there are no well-controlled trials of diet or lipid-lowering drugs to guide decisions. Recently, a growth hormone releasing factor analogue, tesamorelin, was approved for reducing excess visceral fat in LD-HIV patients [74]. However, it does not improve fat loss and may not improve hyperlipidemia. Switching of PI-containing HAART to other HIV-treatment regimen may improve dyslipidemia.

Low-Fat Diet Since dietary fat directly contributes to chylomicronemia, it appears prudent to give patients with acute pancreatitis and chylomicronemia, fat-free or extremely low-fat diets. During the acute episode of pancreatitis, the patients are not given any energy orally which drastically reduces chylomicron formation and gradually the chylomicronemia abates.

Thiazolidinediones Arioglu et al. [75] showed lowering of fasting triglyceride and free fatty acid levels in patients with both generalized and partial lipodystrophy upon treatment with troglitazone for 6 months in an open-labelled trial. Similar improvement in serum lipids have been

reported anecdotally in a few FPLD patients with both rosiglitazone [76] and pioglitazone [77, 78], though some case reports suggest worsening of dyslipidemia [79]. Interestingly, we recently noted that thiazolidinediones do not reverse fat loss in patients with FPLD, and in fact may worsen excess deposition in nonlipodystrophic regions [80]. Although thiazolidinediones can be useful in FPL patients with *PPARG* mutations, the data on their efficacy are equivocal [81].

Fibrates Gemfibrozil or fenofibrate increase fatty acid oxidation by virtue of their PPAR-α agonist action, increase lipoprotein lipase activity and reduce apo C3 levels and thus can reduce serum TG by about 50% and should be used in lipodystrophy patients with severe hypertriglyceridemia as the first line therapy. However, there are no efficacy trials in patients with inherited lipodystrophies.

Fish Oil Concentrated fish oil preparations containing the n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can reduce hepatic TG synthesis by competitive inhibition and thus can lower serum triglycerides. Administration of 3–9 g of EPA and DHA can reduce serum TG by up to 50%. Recently, two prescription brand preparations, Lovaza and Vascepa, have also been available. However, controlled trials supporting their efficacy in lipodystrophic patients are lacking.

Leptin Marked improvement in diabetes control, dyslipidemia, and other metabolic abnormalities has been reported with leptin replacement therapy in patients with both generalized and partial lipodystrophy, though the former show a more robust response [73, 82–84]. Serum triglycerides decreased by over 60% in nine patients with lipodystrophy (of whom eight had generalized lipodystrophy) upon treatment with leptin for 4 months [73], and the benefits have been shown to persist with long-term treatment in patients with generalized lipodystrophy [82, 83]. Leptin was given twice daily in low dose to patients with lipodystrophy. Leptin therapy has been shown to improve satiety [85] and decrease energy intake [73], and also reduce ectopic lipid deposition in the liver and muscle [86–88]. Thus, both the central and peripheral effects of leptin may be contributing to improved metabolic functions. Leptin therapy has been associated with the development of neutralizing antibodies to leptin, the significance of which is not clear at this time. The other reported side effects include rare development of lymphomas in patients with autoimmune acquired lipodystrophies and increase in proteinuria in some patients. Recently, the US Food and Drug Administration approved metreleptin for managing metabolic complications in patients with generalized lipodystrophies. Further studies are required to identify patients with partial lipodystrophies who will benefit from leptin replacement therapy.

Conclusions

Marked hypertriglyceridemia is a common feature in patients with genetic or acquired lipodystrophies, and is particularly severe in patients with generalized lipodystrophy. Mutations in 14 different genes have been reported to cause various lipodystrophy syndromes, and some of these should be considered as candidate genes in patients with monogenic hypertriglyceridemia. The molecular mechanisms by which many of these genetic defects cause fat loss and dyslipidemia is not entirely clear yet, but data show impaired formation and maturation of lipid droplets in the adipocyte, and interruption of signals necessary for adipocyte differentiation and survival, may cause lipodystrophies. Reduced triglyceride storage capacity in the adipose tissue leads to ectopic fat deposition in other organs such as the liver. Hepatic steatosis may increase VLDL synthesis and can cause hypertriglyceridemia. Besides, conventional therapies including low-fat diets, fish oils, fibrates, and improvement of diabetes control, patients may benefit from metreleptin replacement therapy, especially those with generalized lipodystrophy.

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Novel Genes for Dyslipidemias: Genome-Wide Association Studies

17

Kiran Musunuru

Introduction

As outlined in some of the preceding chapters of this book, a number of genes responsible for dyslipidemias have been discovered because they harbor rare mutations that cause a very small number of individuals—typically in one or a few families—to have profoundly dysregulated blood lipid concentrations. In other words, these few individuals have monogenic or "Mendelian" dyslipidemias caused by the aberrant action of single genes. Although researchers have learned much about cholesterol metabolism by studying these genes over the past few decades, this work has left unanswered the question of what causes a significant proportion of the general population to have what might be thought of as garden variety dyslipidemias—high but not exceptionally high blood low-density lipoprotein cholesterol (LDL-C) concentrations, low but not exceptionally low blood high-density lipoprotein cholesterol (HDL-C) concentrations, and/or high but not exceptionally high blood triglyceride (TG) concentrations. It has been a popular belief that in these patients, in whom there do not seem to be single aberrant genes driving the abnormal lipid levels, the dyslipidemias are polygenic in nature, i.e., caused by the actions of multiple genes in tandem. By this reasoning, if the functions of 5, 10, 20, or 50 genes were slightly dysregulated, the combined effect would result in abnormal lipid levels.

Although the notion of polygenic dyslipidemias is attractive to many researchers, it had not been possible to test this model by detecting and measuring the slight dysregulation of each of the numerous genes that would be involved—indeed, it was not even clear *which* genes out of the roughly 20,000 genes in the human genome might be involved. In the past few years, the completion of the Human Genome Project and advances in genotyping and sequencing technologies have made it possible for the first time to do unbiased searches for genes that make small contributions to blood lipid levels in dyslipidemia patients. This chapter focuses on the methodology known as the genome-wide association study (GWAS) and summarizes the advances in knowledge regarding cholesterol metabolism that have emerged from the application of this methodology to tens of thousands of people and subsequent work to identify and characterize novel genes involved in dyslipidemias.

A Primer on Genome-Wide Association Studies

The human genome is roughly three billion DNA bases in size, spanning 23 chromosome pairs; the vast majority of the sequence is identical across

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the human species. What makes each individual unique is a large number of DNA variants distributed throughout the genome. Some of these DNA variants are extremely rare and have large effects on gene function; as described above, these variants can be responsible for monogenic disorders. Other DNA variants are quite common, occurring in >1% of the general population and, in some cases, the majority of the people in a population. Most of these common DNA variants are of no functional consequence, but some lie either within or near genes and have small effects on gene function. These variants do not alter gene activity enough to cause disease by themselves, but instead need to be combined with other gene variants or with environmental factors in order for disease to occur.

All of these common DNA variants are termed polymorphisms, of which there are several varieties. The most relevant to the use of GWAS to study polygenic disorders is the single nucleotide polymorphism (SNP), in which a single base pair in the DNA differs from the usual base pair at that position. There are an estimated 11 million SNPs across the human genome, occurring on an average every few hundred base pairs. A local area on a chromosome around an SNP is termed a locus. Each person has two copies of each locus because of the pairing of chromosomes (the exceptions are loci on the X or Y chromosome in men, who have only one of each). A person's genotype at an SNP is the identity of the base pair position for each of the two copies—also termed alleles—of the SNP on paired chromosomes; thus, a genotype is typically a combination of two alleles. These two alleles may be identical (termed homozygosity) or different (termed heterozygosity).

Groups of SNP alleles near genes tend to stay together with the genes as they are passed along from parents to children for generation after generation, over thousands of years. Thus, even if it is not known which gene contributes to a disease, one can use an SNP that is not in the gene—but is near to and therefore linked to the gene—as a "tag" for the gene. In the past decade, the technology has become available to determine the genotypes at hundreds of thousands of "tag" SNPs in a person's DNA in a single experiment using a "gene chip." By applying the gene chips to thousands of individuals, some with a disease and some without a disease, researchers are able to identify tag SNPs that are associated with disease. This is the principle underlying GWAS.

As an example of how a GWAS might be performed, imagine a study in which DNA samples are collected from several thousand individuals with high blood LDL-C levels (e.g., >200 mg/dL) and several thousand individuals with low blood LDL-C levels (e.g., <60 mg/dL in the absence of lipid-lowering medications). For each study participant, a gene chip would be used to determine the genotypes of more than 1 million SNPs across the genome. Although the use of gene chips on thousands of people would yield billions of pieces of data, the statistical methods to analyze these data are conceptually straightforward. Computer software is used to analyze each of the 1 million SNPs separately; for each SNP, the question is asked whether allele "A" and allele "B" of the SNP occur in equal proportions in the high LDL-C cohort and in the low LDL-C cohort. For the vast majority of the 1 million SNPs, no difference in the allele proportions would be observed. For a particular SNP, however, there might be a statistically significant difference in the allele proportions such that allele "A" occurs more commonly in the high LDL-C cohort than in the low LDL-C cohort. Because the SNP tags any nearby genes, the conclusion would be that there is a DNA variant in one of the local genes that influences that gene's function in such a way as to influence blood LDL-C levels. From a researcher's perspective, the SNP acts as a "signpost" indicating that somewhere in the locus lies the key to a biological mechanism that contributes to dyslipidemia in the general population. (In fact, some of the million tested SNPs are very close to one another and effectively tag the same locus, so it is the locus rather than any individual SNP that is considered to be a GWAS discovery.)

In practice, it would be difficult to recruit thousands of study participants with either very high or very low LDL-C levels due to their relative scarcity in the population. In an alternative study design, thousands of people from the gen-

eral population at large would be recruited, and computer analysis would be performed using the blood LDL-C levels as a continuous rather than a categorical variable. The question would be framed in a different way: For a given SNP, what are the average LDL-C levels for individuals who are homozygous for allele "A" versus individuals who are heterozygous for alleles "A" and "B" versus individuals who are homozygous for allele "B"? If there are statistically significant differences among the three genotype groups, the SNP/locus would be considered to be associated with blood LDL-C levels.

It is worth noting that in either of these GWAS study designs, there are effectively a million experiments being performed, one for each individual SNP. As such, the traditional statistical significance threshold of $P < 0.05$ is inappropriate, since by that threshold 50,000 SNPs (5% of 1,000,000) would be associated with LDL-C levels by chance alone, with most if not all being false positives. Accordingly, GWAS researchers insist on the statistical significance threshold being much more stringent, e.g., by adjusting for the number of experiments (known as the Bonferroni correction) such that P should be less than $0.05 \div 1{,}000{,}000$, or $P < 5 \times 10^{-8}$, for the SNP/locus to be regarded as having a true association.

GWAS on Blood Lipid Traits

One consequence of the GWAS study design is that it becomes increasingly powered to detect associations as the number of study participants grows. Accordingly, the past several years have seen successive reports of increasingly larger GWAS studies of blood lipid concentrations, beginning with a few thousand individuals and culminating in more than 100,000 individuals. As such, the list of reported lipid-associated GWAS loci has substantially grown over that time period.

The first published high-density GWAS on blood lipid concentrations was performed with data from about 3000 individuals of European descent in the Diabetes Genetics Initiative. This study identified one statistically significant locus each for three lipid traits—LDL-C, HDL-C, and TG [1]. The LDL-C locus contained the apolipoprotein E (*APOE*) gene, and the HDL-C locus contained the cholesteryl ester transfer protein (*CETP*) gene—both well-established regulators of lipoprotein metabolism. The TG locus contained no previously identified lipid regulators; the single gene in the locus is glucokinase regulatory protein (*GCKR*). Subsequent functional experiments pointed to a coding missense variant in the *GCKR* gene as being responsible for the TG association [2, 3]. The fact that this first GWAS identified two known lipid genes provided strong validation of the study design as well as giving confidence that the *GCKR* locus was a true positive (and novel) finding.

A second set of published GWAS studies for blood lipid concentrations added the Finland–US investigation of NIDDM (noninsulin-dependent diabetes mellitus) genetics study (FUSION) and SardiNIA cohorts to the Diabetes Genetics Initiative for a cohort of about 9000 individuals of European descent [4, 5]. As a method of increasing the studies' power to detect statistically significant associations, the researchers used a staged approach: They selected the SNPs with the best P values from the analysis of data from the initial 9000 individuals and genotyped just those SNPs in an additional 18,000 individuals of European descent from several other cohorts. This approach yielded a total of 19 statistically significant lipid-associated loci. In addition to the three loci identified by the first GWAS (*APOE, CETP, GCKR*), the list now included many more well-established lipid regulators, including apolipoprotein A-I (*APOA1*), apolipoprotein B (*APOB*), LDL receptor (*LDLR*), lipoprotein lipase (*LPL*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*). The last is noteworthy because it encodes the enzyme that is targeted by the statin class of LDL-C-lowering drugs. These GWAS studies also identified six novel loci, two of which were also discovered in simultaneously published GWAS studies that focused solely on LDL-C (a locus on chromosome 1p13) or TG (a locus on chromosome 7q11) [6–8].

A third set of GWAS studies on blood lipid concentrations, analyzing data from up to 40,000 individuals of European descent, identified more than 30 lipid-associated loci, half of them harboring well-established lipid regulators, the other half novel, continuing the trend observed from the first two sets of studies [9–11].

Finally, a definitive GWAS combining data from all of the cohorts with which the previous three sets of GWAS studies had been performed—collectively termed the Global Lipids Genetics Consortium (GLGC)—used more than 100,000 individuals of European descent to identify a total of 95 loci associated with one or more blood lipid concentrations—LDL-C, HDL-C, TG, and/or total cholesterol (Table 17.1) [12]. Many of these loci were also shown to be associated with lipids in other ethnic groups, including East Asians, South Asians, and African Americans. About two thirds of these loci are novel. Among the remaining one third of the loci are 16 genes that have been implicated in familial lipid disorders (Table 17.2), highlighting that the same gene can contribute to both monogenic and polygenic dyslipidemias, with a rare variant in the gene greatly perturbing its function and causing disease, and with a common variant in the gene mildly perturbing its function and combining with common variants in other genes to collectively produce disease.

In support of the polygenic model of disease, the GLGC study calculated SNP "risk scores" that summarized the number of LDL-C-raising, HDL-C-raising, or TG-raising SNP alleles in each individual who had high or low LDL-C levels (mean 219 mg/dL vs. mean 110 mg/dL), high or low HDL-C levels (mean 90 mg/dL vs. mean 36.2 mg/dL), or high or low TG levels (mean 1,079 mg/dL vs. mean 106 mg/dL). Individuals with LDL-C risk scores in the top quartile of the combined GLGC cohort were 13 times as likely to have a high-LDL-C level than individuals with scores in the bottom quartile; individuals with HDL-C risk scores in the top quartile of the combined GLGC cohort were four times as likely to have a high-HDL-C level than individuals with scores in the bottom quartile; and individuals with TG risk scores in the top quartile of the combined GLGC cohort were 44 times as likely to have a high-TG level than individuals with scores in the bottom quartile [12]. These results confirm that the additive effects of multiple common variants do indeed contribute to dyslipidemias in many individuals.

In spite of these data, there have continued to be criticisms that the common variants discovered by GWAS have little clinical relevance, since the effects on gene function are small. Besides disregarding the polygenic model of disease, such arguments ignore the possibility that a GWAS gene can turn out to be clinically important if its activity is modulated by a large degree with pharmacological intervention, with *HMGCR* being the prototypic example. If the discovery of statins had not predated the GWAS era, the finding that *HMGCR* is in a locus associated with LDL-C levels would have suggested to researchers that pharmacological inhibition of *HMGCR* might be a viable therapeutic strategy. By this reasoning, some of the novel lipid GWAS genes may emerge as clinically useful drug targets, as described below.

Novel Genes That Have Emerged from GWAS

Although the 95 lipid-associated loci identified by the GLGC study potentially point to dozens of novel genes, to date only a few such candidates have been studied with functional experimentation. We focus on three genes from which novel insights into lipoprotein metabolism have started to be gleaned.

SORT1. Perhaps the most compelling of the novel lipid GWAS loci lies on chromosome 1p13. SNPs in the 1p13 locus have among the strongest associations with LDL-C of any loci in the genome. Individuals who are homozygous for the more common allele of one of these SNPs have an average 16 mg/dL higher blood LDL-C concentration than individuals who are homozygous for the less common allele [4]. The former also have about a 40% increase in risk of myocardial infarction compared to the latter [13]. Thus, the 1p13 locus is strongly associated with both blood

Table 17.1 GWAS loci associated with blood lipid traits

Locus/gene	Chr	Best SNP	Best trait	Other traits	*P*	Locus/gene	Chr	Best SNP	Best trait	Other traits	*P*
LDLRAP1	1	rs12027135	TC	LDL	4E-11	*ABCA1*	9	rs1883025	HDL	TC	2E-33
PABPC4	1	rs4660293	HDL		4E-10	*ABO*	9	rs9411489	LDL	TC	6E-13
PCSK9	1	rs2479409	LDL	TC	2E-28	*JMJD1C*	10	rs10761731	TG		3E-12
ANGPTL3	1	rs2131925	TG	TC, LDL	9E-43	*CYP26A1*	10	rs2068888	TG		2E-8
EVI5	1	rs7515577	TC		3E-8	*GPAM*	10	rs2255141	TC	LDL	2E-10
SORT1	1	rs629301	LDL	TC	1E-170	*AMPD3*	11	rs2923084	HDL		5E-8
ZNF648	1	rs1689800	HDL		3E-10	*SPTY2D1*	11	rs10128711	TC		3E-8
MOSC1	1	rs2642442	TC	LDL	6E-13	*LRP4*	11	rs3136441	HDL		3E-18
GALNT2	1	rs4846914	HDL	TG	4E-21	*FADS1-2-3*	11	rs174546	TG	HDL, TC, LDL	5E-24
IRF2BP2	1	rs514230	TC	LDL	5E-14	*APOA1*	11	rs964184	TG	TC, HDL, LDL	7E-240
APOB	2	rs1367117	LDL	TC	4E-114	*UBASH3B*	11	rs7941030	TC	HDL	2E-10
		rs1042034	TG	HDL	1E-45	*ST3GAL4*	11	rs11220462	LDL	TC	1E-15
GCKR	2	rs1260326	TG	TC	6E-133	*PDE3A*	12	rs7134375	HDL		4E-8
ABCG5/8	2	rs4299376	LDL	TC	2E-47	*LRP1*	12	rs11613352	TG	HDL	4E-10
RAB3GAP1	2	rs7570971	TC		2E-8	*MVK*	12	rs7134594	HDL		7E-15
COBLL1	2	rs10195252	TG		2E-10	*BRAP*	12	rs11065987	TC	LDL	7E-12
		rs12328675	HDL		3E-10	*HNF1A*	12	rs1169288	TC	LDL	1E-14
IRS1	2	rs2972146	HDL	TG	3E-9	*SBNO1*	12	rs4759375	HDL		7E-9
RAF1	3	rs2290159	TC		4E-9	*ZNF664*	12	rs4765127	HDL	TG	3E-10
MSL2L1	3	rs645040	TG		3E-8	*SCARB1*	12	rs838880	HDL		3E-14
KLHL8	4	rs442177	TG		9E-12	*NYNRIN*	14	rs8017377	LDL		5E-11
SLC39A8	4	rs13107325	HDL		7E-11	*CAPN3*	15	rs2412710	TG		2E-8
ARL15	5	rs6450176	HDL		5E-8	*FRMD5*	15	rs2929282	TG		2E-11
MAP3K1	5	rs9686661	TG		1E-10	*LIPC*	15	rs1532085	HDL	TC, TG	3E-96
HMGCR	5	rs12916	TC	LDL	9E-47	*LACTB*	15	rs2652834	HDL		9E-9
TIMD4	5	rs6882076	TC	LDL, TG	7E-28	*CTF1*	16	rs11649653	TG		3E-8
MYLIP	6	rs3757354	LDL	TC	1E-11	*CETP*	16	rs3764261	HDL	TC, LDL, TG	7E-380
HFE	6	rs1800562	LDL	TC	6E-10	*LCAT*	16	rs16942887	HDL		8E-33
HLA	6	rs3177928	TC	LDL	4E-19	*HPR*	16	rs2000999	TC	LDL	3E-24
		rs2247056	TG		2E-15	*CMIP*	16	rs2925979	HDL		2E-11
C6orf106	6	rs2814944	HDL		4E-9	*STARD3*	17	rs11869286	HDL		1E-13
		rs2814982	TC		5E-11	*OSBPL7*	17	rs7206971	LDL	TC	2E-8

Table 17.1 (continued)

Locus/gene	Chr	Best SNP	Best trait	Other traits	*P*	Locus/gene	Chr	Best SNP	Best trait	Other traits	*P*
FRK	6	rs9488822	TC	LDL	2E-10	*ABCA8*	17	rs4148008	HDL		2E-10
CITED2	6	rs605066	HDL		3E-8	*PGS1*	17	rs4129767	HDL		8E-9
LPA	6	rs1564348	LDL	TC	2E-17	*LIPG*	18	rs7241918	HDL	TC	3E-49
		rs1084651	HDL		3E-8	*MC4R*	18	rs12967135	HDL		7E-9
DNAH11	7	rs12670798	TC	LDL	9E-10	*ANGPTL4*	19	rs7255436	HDL		3E-8
NPC1L1	7	rs2072183	TC	LDL	3E-11	*LDLR*	19	rs6511720	LDL	TC	4E-117
TYW1B	7	rs13238203	TG		1E-9	*LOC55908*	19	rs737337	HDL		3E-9
MLXIPL	7	rs17145738	TG	HDL	6E-58	*CILP2*	19	rs10401969	TC	TG, LDL	3E-38
KLF14	7	rs4731702	HDL		1E-15	*APOE*	19	rs4420638	LDL	TC, HDL	9E-147
PPP1R3B	8	rs9987289	HDL	TC, LDL	6E-25			rs439401	TG		1E-30
PINX1	8	rs11776767	TG		1E-8	*FLJ36070*	19	rs492602	TC		2E-10
NAT2	8	rs1495741	TG	TC	5E-14	*LILRA3*	19	rs386000	HDL		4E-16
LPL	8	rs12678919	TG	HDL	2E-115	*ERGIC3*	20	rs2277862	TC		4E-10
CYP7A1	8	rs2081687	TC	LDL	2E-12	*MAFB*	20	rs2902940	TC	LDL	6E-11
TRPS1	8	rs2293889	HDL		6E-11	*TOP1*	20	rs6029526	LDL	TC	4E-19
		rs2737229	TC		2E-8	*HNF4A*	20	rs1800961	HDL	TC	1E-15
TRIB1	8	rs2954029	TG	TC, LDL, HDL	3E-55	*PLTP*	20	rs6065906	HDL	TG	2E-22
PLEC1	8	rs11136341	LDL	TC	4E-13	*UBE2L3*	22	rs181362	HDL		1E-8
TTC39B	9	rs581080	HDL	TC	3E-12	*PLA2G6*	22	rs5756931	TG		4E-8

The listed gene is either an established lipid-related gene in the locus or the gene closest to the best SNP. *P* values are given for the associations of each best SNP with each best lipid trait for each locus

HDL high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *SNP* single-nucleotide polymorphism, *TG* triglycerides, *TC* total cholesterol

Table 17.2 Monogenic dyslipidemias caused by genes with nearby common DNA variants identified in lipid GWAS

Gene	Chr	GWAS SNP	Monogenic dyslipidemia	Associated traits
ABCA1	9	rs1883025	Tangier disease	Low HDL
ABCG5	2	rs4299376	Sitosterolemia	High LDL
ABCG8	2	rs4299376	Sitosterolemia	High LDL
ANGPTL3	1	rs2131925	Familial combined hypolipidemia	Low LDL, low HDL, low TG
APOA1	11	rs964184	ApoA-I deficiency	Low HDL
APOA5	11	rs964184	ApoA-V deficiency	High VLDL, high chylomicrons
APOB	2	rs1367117	Familial hypobetalipoproteinemia	Low LDL
			Familial defective ApoB-100	High LDL
APOC2	19	rs4420638	Familial ApoC-II deficiency	High chylomicrons
APOE	19	rs4420638	Familial dysbetalipoproteinemia	High VLDL, high chylomicrons
CETP	16	rs3764261	Cholesteryl ester transfer protein deficiency	High HDL
LCAT	16	rs16942887	LCAT deficiency (fish-eye disease)	Low HDL
LDLR	19	rs6511720	Familial hypercholesterolemia	High LDL
LDLRAP1	1	rs12027135	Autosomal recessive hypercholesterolemia	High LDL
LIPC	15	rs1532085	Familial hepatic lipase deficiency	High VLDL remnants
LPL	8	rs12678919	Lipoprotein lipase deficiency	High chylomicrons
PCSK9	1	rs2479409	PCSK9 deficiency	Low LDL
			Autosomal dominant hypercholesterolemia	High LDL

GWAS genome-wide association study, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SNP* single-nucleotide polymorphism, *TG* triglycerides, *VLDL* very low-density lipoprotein

lipids and the most serious clinical phenotype resulting from dyslipidemias.

The 1p13 locus harbors several genes including *CELSR2, PSRC1,* and *SORT1*, none of which had previously been linked to lipid metabolism in the pre-GWAS era. Functional experimentation with these genes in mouse models revealed that *SORT1* (which encodes the sortilin protein) modulated blood lipid levels when its expression was either increased or decreased in mouse liver, or when it was deleted in mice altogether [14, 15]. Cell-based experiments have discovered two roles for sortilin in lipoprotein metabolism. First, it regulates blood LDL-C levels by reducing the secretion of VLDL particles from the liver into the bloodstream, where the VLDL particles are ultimately converted to LDL particles [14]. It appears to do this in hepatocytes by directly binding apolipoprotein B (apoB)—the core protein of VLDL/LDL particles—in the endoplasmic reticulum/Golgi apparatus and trafficking apoB to the endolysosomal compartment for degradation, thereby reducing the number of VLDL particles produced and, ultimately, secreted [14, 16]. Second, sortilin appears to be able to function as an alternative LDL receptor by binding to apoB-carrying LDL particles in the bloodstream and facilitating the endocytosis of the particles into the cell, followed by their degradation in the endolysosomal compartment [16]. Both mechanisms should have the effect of lowering blood LDL-C concentrations (Fig. 17.1), consistent with the association of the 1p13 locus with LDL-C in human populations.

The strong association of the 1p13 locus with myocardial infarction suggests *SORT1* as a plausible clinical drug target, as modulation of the gene would be expected to not only reduce blood LDL-C levels but also the risk of myocardial infarction. However, the biological evidence indicates that increasing sortilin activity in liver would be a therapeutically useful intervention, which may be difficult to achieve with traditional therapies (in contrast to inhibiting an enzyme's activity, as is the case with statin drugs and *HMGCR*).

TRIB1. Another compelling novel lipid GWAS locus lies on chromosome 8q24. SNPs in

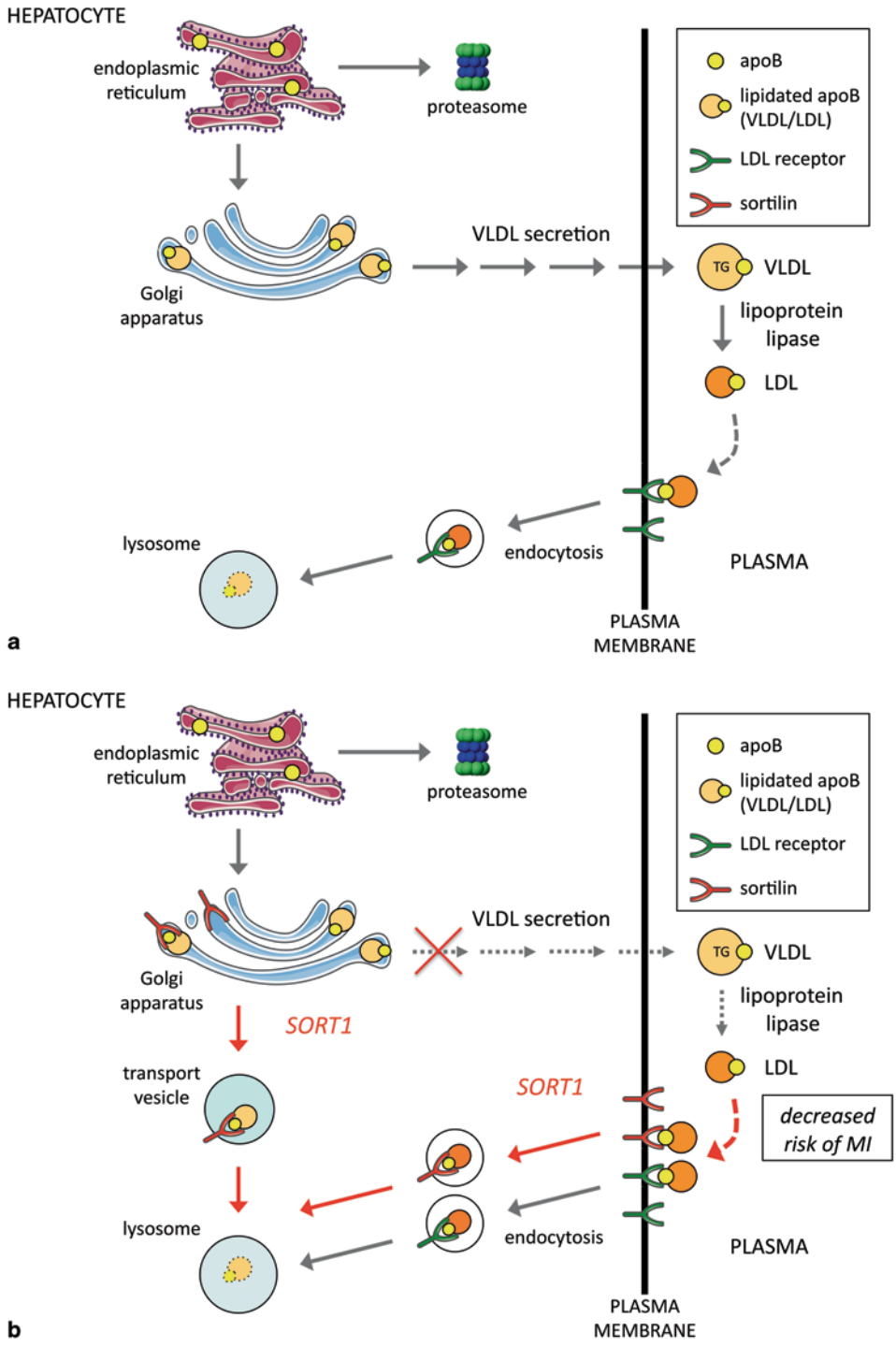

Fig. 17.1 Model of sortilin actions in hepatocytes **a** Lipoprotein production, secretion, uptake, and degradation in hepatocytes. **b** Sortilin decreases very low-density lipoprotein (VLDL) particle secretion by trafficking nascent particles to the endolysosomal compartment. It also acts as an alternative LDL receptor to facilitate endocytosis of LDL particles into the cell and degradation in the endolysosomal compartment. The consequence of both actions is to reduce LDL cholesterol levels in the blood and thereby reduce the risk of myocardial infarction (MI)

this locus have a distinctive pattern of association with multiple lipid traits, with the minor allele conferring decreased LDL-C levels, increased HDL-C levels, and decreased TG levels—all changes that are epidemiologically associated with lower risk of coronary artery disease (CAD) [4, 5]. Perhaps not surprisingly, then, the same SNPs are themselves associated with CAD risk [12].

There is a single gene in the locus, *TRIB1* (tribbles homolog 1), which had not previously been linked to lipid metabolism in the pre-GWAS era. Two types of functional experimentation have been undertaken in mice [17]. First, *Trib1* knockout mice (in which the gene has been deleted) were observed to have increased blood levels of cholesterol and TG. Second, mice in which *Trib1* was overexpressed in liver showed the opposite effect—decreased blood levels of cholesterol and TG. The mechanism appears in part to be related to hepatic production of VLDL particles; *Trib1* overexpression resulted in decreased particle production and secretion, whereas the knockout mice displayed the opposite effect [17]. This was reproduced in cell-based experiments in which *TRIB1* was overexpressed in cultured human hepatoma cells, resulting in decreased apoB particle secretion. Furthermore, *Trib1* appears to reduce the hepatic expression of genes involved in lipogenesis, including *Acc1, Fasn*, and *Scd1* [17]. The mechanism(s) through which the protein produces these effects remains to be defined.

As with *SORT1*, it would appear that increasing *TRIB1* expression in the liver would be a therapeutically useful intervention. Indeed, a *TRIB1*-targeting strategy might be of even greater clinical benefit than a *SORT1*-targeting strategy since GWAS data suggest that *TRIB1* is linked to decreased LDL-C, increased HDL-C, and decreased TG in the blood, whereas *SORT1* appears to be linked solely to decreased LDL-C. Moreover, the genetic association of SNPs in the *TRIB1* locus with CAD risk offers reassurance that targeting *TRIB1* would have the desired clinical outcome—a reduction in CAD rather than just modulation of blood lipid levels.

GALNT2. A novel lipid GWAS locus on chromosome 1q42 that is associated with both HDL-C and TG levels in the blood harbors a single gene, *GALNT2,* which encodes UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-2, a member of a family of proteins that are involved in the initiation of mucin-type O-linked glycosylation on various proteins. As with *SORT1* and *TRIB1*, no prior role of GALNT2 in lipoprotein metabolism was known. Unlike *SORT1* and *TRIB1,* SNPs in the locus do not have any significant association with CAD.

Functional experimentation with *GALNT2* in mice in which the gene was either overexpressed or knocked down in the liver indicated that the gene negatively regulates blood HDL-C levels [12]. The connection of *GALNT2* with blood HDL-C and TG concentrations in humans was confirmed by the identification of two families with individuals with dyslipidemia—very high HDL-C and low TG levels—who were heterozygous for a loss-of-function missense mutation in *GALNT2* [18]. Physiological studies of these individuals suggested that they had improved postprandial TG clearance due to impaired glycosylation of apolipoprotein C-III (apoC-III), which normally inhibits LPL, which itself hydrolyzes and thus decreases TG in the blood. Thus, *GALNT2* represents another example of a gene for which common DNA variants produce a small effect on blood lipid levels and rare DNA variants can single-handedly produce dyslipidemias. However, to date there is no evidence that *GALNT2* is associated with a change in CAD risk, and thus its relevance as a therapeutic target remains in question.

Conclusion

The past few years have witnessed remarkable progress in human genetics towards the understanding of polygenic disorders. Blood lipid concentrations represent some of the most successfully studied clinical traits with the use of GWAS. Whereas our prior knowledge of the genetics of

dyslipidemias was limited to rare variants causing monogenic disorders, GWAS studies have now identified dozens of novel loci that appear to contribute to polygenetic dyslipidemias. A key challenge will be to determine the molecular mechanisms by which these loci influence blood lipid concentrations. Although functional studies of the novel loci are starting to yield new insights into lipoprotein metabolism, as demonstrated by the examples of the genes *SORT1, TRIB1,* and *GALNT2,* there are undoubtedly as many new discoveries to be made with respect to lipoprotein metabolism as have been made in the past few decades of investigation.

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Perspectives on Cholesterol Guidelines

18

Scott M. Grundy

For over 50 years, scientific evidence has grown that low-density lipoproteins (LDL) are a strong risk factor for atherosclerotic coronary heart disease (CHD). The relation between LDL levels and CHD risk is bidirectional and log-linear [1]. Bidirectional means that increasing LDL raises risk for CHD, whereas decreasing levels reduces risk. Log-linear means that progressively lower levels are accompanied by diminishing absolute risk reduction. That higher LDL levels increase atherosclerosis or CHD has been shown in laboratory animals, in patients with familial hypercholesterolemia, and in populations with higher LDL levels [1]. Over the past 30 years, randomized controlled trials (RCTs) with pre-statin and statin drugs have demonstrated that lowering of LDL levels reduces CHD [1, 2]. More recently, genetic epidemiology has shown and confirmed that a lifetime of low LDL levels, secondary to pro-protein convertase subtilisin kexin 9 (PCSK-9) null or loss-of-function mutations, essentially eliminates CHD [3].

Without doubt, the strongest evidence for the relation between LDL and CHD comes from statin RCTs. A substantial number of major statin trials have shown a progressively lower risk for CHD events as LDL levels fall [1]. These trials reveal two things. First, the lower the LDL level, the lower is the risk for CHD; and second, statins are highly efficacious for reducing LDL levels and CHD risk. These RCTs have generated some debate. For example, one view holds that prevention guidelines should be constructed around LDL goals [1]; another view contends that guidelines should be defined exclusively in terms of statin therapy [4]. These two views can be considered and contrasted in this chapter. Guidelines previously divided preventive strategies into secondary prevention and primary prevention. This still appears to be a good strategy. This will be the approach taken in this chapter.

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Secondary Prevention

Secondary prevention targets patients with existing or manifest atherosclerotic cardiovascular disease (ASCVD). Included in this category are individuals with CHD, previous thrombotic stroke, peripheral arterial disease (PAD), aortic aneurysm, and other atherosclerotic diseases. Such persons are at high risk for recurrent vascular events. Once a person manifests vascular disease in one arterial bed, there is a high likelihood for events in other beds. For example, patients with PAD are at high risk for developing CHD.

Clinical Trial Evidence in Secondary Prevention

A large number of clinical trials have documented that cholesterol-lowering therapy will reduce

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Table 18.1 Hazard ratios (HR) for major cardiovascular events and major coronary events in meta-analysis of statin trials

On-treatment LDL-cholesterol	LDL-cholesterol category	Adjusted hazard ratios for major cardiovascular events	Adjusted hazard ratios for major coronary events
<50 mg/dL	Extremely low	0.44	0.47
50–74 mg/dL	Very low	0.51	0.53
75–99 mg/dL	Low	0.56	0.58
100–124 mg/dL	Borderline low	0.58	0.62
125–149 mg/dL	Borderline high	0.64	0.67
150–174 mg/dL	High	0.87	0.78
>175 mg/dL	Very high	1.00	1.00

LDL-C low-density lipoprotein cholesterol

risk for ASCVD in patients with established atherosclerotic disease [1, 2]. In the pre-statin era, meta-analysis of trials with cholesterol-lowering drugs showed a significant reduction in CHD events [5]. After the introduction of statins, a host of studies documented significant risk reduction [6–21]. These trials have convinced the medical community that cholesterol lowering is beneficial for patients with established ASCVD. They further show that statin therapy can reduce risk for future atherosclerotic events by 30–50%. Today, use of statins in secondary prevention has become routine.

LDL-C Goals

The US National Cholesterol Education Program (NCEP) has championed the LDL-centered approach to ASCVD reduction. The NCEP Adult Treatment Panel III [1] set an LDL-C goal of <100 mg/dL for patients with ASCVD; later, ATP III recommended an LDL-C of <70 mg/dL for ASCVD patients at very high risk for future cardiovascular events [2]. The latter included those ASCVD patients with diabetes, metabolic syndrome, or multiple risk factors. Recently, the American Heart Association (AHA) and the American College of Cardiology (ACC) made a similar recommendation for secondary prevention [22]. Other organizations, i.e., European [23] and Canadian [24] guidelines, recommend LDL-C goals of <1.8 mmol/L (<70 mg/dL) or <2.0 mmol/L (<77 mg/dL), respectively, for those with established CHD.

Justification for these lower goals comes from several statin trials (or subgroup analyses of these trials). In these trials, very low levels of LDL-C were achieved and showed incremental benefit at these levels compared to higher on-treatment levels [15, 19–25]. Perhaps the best example to support the notion that "the lower, the better" for LDL-C comes from a recent meta-analysis that includes 38,153 patients allocated to statin therapy; in this analysis, a total of 6286 major cardiovascular events occurred in 5387 study participants during follow-up [26]. Key results of this study are shown in Table 18.1. In this analysis, compared to subjects who achieved an LDL-C >175 mg/dL, those who reached an LDL-C 75–100 mg/dL, 50–75 mg/dL, and <50 mg/dL had adjusted hazard ratios for major cardiovascular events of 0.56, 0.51, and 0.44, respectively.

Thus, regarding goals for LDL therapy, it is reasonable to achieve as low an LDL as possible within the bounds of realistic clinical practice. In the meta-analysis described above, 40% of subjects given high-dose statins did not reach an LDL-C target <70 mg/dL. For those who do not achieve very low LDL-C levels, clinical judgment is required whether to add a second LDL-lowering drug.

Other Lipid Targets

Although LDL-C is generally recognized as the primary target of lipid-lowering therapy, other lipid measures have been identified as contributing to ASCVD risk. For example, there is a growing recognition that very low-density lipoproteins

(VLDL) are atherogenic like LDL. This has led to the suggestion that the cholesterol contained in LDL + VLDL, i.e., non-high-density lipoprotein cholesterol (non-HDL-C), may be a better target of lipid-lowering therapy than LDL-C alone [1, 25, 27]. At the least, non-HDL-C is equivalent to low-density lipoprotein cholesterol (LDL-C) as a target of treatment; and in the view of many, it is the preferred target. If non-HDL-C is made the primary target, the goal for patients with ASCVD would be a level of < 100 mg/dL [1].

Another view favors apolipoprotein B (apo B) as the primary target of treatment in secondary prevention [28, 29]. All atherogenic lipoproteins contain one apo B molecule per lipoprotein particle. Some authors contend that serum apo B levels are a better indicator of atherogenicity than non-HDL-C [29–32]. The usual method for measuring apo B is immunological; this method has limitations and universal standardization has not been achieved [33, 34]. Measurement of apo B moreover costs more than does estimation of non-HDL-C. Finally, there is the question of what is the appropriate goal of therapy for apo B in secondary prevention. This question has been discussed thoroughly by Harper and Jacobson [35] and by Brunzell et al. [31]. A consensus goal for total apo B has not been reached. Until agreement can be reached on what is the appropriate apo B goal for secondary prevention, it is difficult to produce a solid clinical recommendation. Standardization and costs are added limitations. Of course, all of these limitations potentially could be overcome. But even so, whether apo B is more desirable than non-HDL-C as a target in secondary prevention is doubtful [36].

Secondary Prevention Without Specific Lipid-Lowering Goals

Most RCTs in secondary prevention have been carried out with statin therapy. Recently, the ACC/AHA [4] released a new set of treatment guidelines in which no LDL-C goals were identified. Instead, these guidelines recommended that high-intensity statins be used in all patients with established ASCVD. High-intensity statins include atorvastatin 80 mg and rosuvastatin 20–40 mg. They claim that RCT evidence for other cholesterol-lowering drugs is too weak to justify any recommendations. They declined to support any particular goal for LDL-C.

ACC/AHA guidelines [4] discount the value of other lipid-lowering drugs because their efficacy in large-scale RCT have not been adequately demonstrated. Thus, the new guidelines are essentially statin-treatment guidelines. They consider LDL to be only a risk marker but without RCT-proven atherogenic potential. Therefore, these guidelines negate any value to other cholesterol-lowering agents based on their ability to lower LDL alone. This view of course runs counter to the basic premise of 25 years of the NCEP, which holds that any form of LDL reduction will reduce risk.

Combination Drug Therapy in Secondary Prevention

According to ATP III [1], but contrary to ACC/AHA guidelines [4], two drugs are available to add to high-intensity statin to increase the proportion of patients who can achieve an LDL-C < 70 mg/dL. These are bile-acid-binding resins and ezetimibe. Bile-acid-binding resins have been shown to reduce ASCVD in patients with hypercholesterolemia [37]. They reduce the absorption of bile acids by the intestine. This decreases return of bile acids to the liver, which releases feedback inhibition of bile acids on conversion of cholesterol into bile acids. The result is a reduction of hepatic cholesterol, which increases the activity of LDL receptors. By this mechanism, bile acid resins lower LDL-C levels by 15–25%. Ezetimibe partially blocks the absorption of cholesterol, reduces the return of cholesterol to the liver, lowers hepatic cholesterol, and increases LDL receptors. This action also lowers LDL-C levels by 15–25% [38]. The addition of bile-acid-binding resins or ezetimibe to statins enhances LDL-C reduction. For example, adding colesevelam, a bile acid resin, to atorvastatin 10 mg lowers LDL-C similarly to atorvastatin 80 mg [39]. Likewise, combining ezetimibe

plus atorvastatin 40 mg lowered LDL-C more than atorvastatin 80 mg [40]. In the latter study, only 32% of patients treated with atorvastatin 80 mg attained an LDL-C level of <70 mg/dL, whereas 74% of those treated with atorvastatin 40 mg + ezetimibe 10 mg achieved an LDL-C <70 mg/dL.

Although adding colesevelam or ezetimibe to atorvastatin 80 mg undoubtedly would increase the proportion of subjects who would attain an LDL-C <70 mg/dL, whether such a combination will reduce risk for ASCVD events has not been tested in RCTs. A very large clinical trial would be necessary to test the efficacy of these add-on drugs because: (a) the addition in LDL lowering is relatively small, and (b) the risk would already be reduced substantially by high-dose statin. To address these issues, one RCT is currently underway to test the efficacy of ezetimibe as add-on to statin therapy. The IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) is a multicenter RCT designed to test whether the addition of ezetimibe to statin therapy, using ezetimibe/simvastatin, will produce increased clinical benefit on cardiovascular outcomes relative to simvastatin monotherapy in patients with acute coronary syndrome [41]. The trial was designed to recruit up to 18,000 patients, stabilized after an acute coronary syndrome. They were randomized in a 1:1 ratio to once-daily doses of either ezetimibe/simvastatin 10/40 mg or simvastatin monotherapy 40 mg or 80 mg. The primary end point is the first occurrence of an ASCVD event. The IMPROVE-IT investigators estimate a requirement for 5250 events to maintain a desired 90% power to detect the expected reduction in cardiovascular events, the expected decrease in LDL-C levels, and the anticipated rate of loss of subjects to follow-up.

The IMPROVE-IT trial has now been completed. The results have not been published but were presented at the 2014 American Heart Association Scientific Sessions. Detailed findings of IMPROVE-IT are available on-line (clinicaltrialresults.org/Slides/AHA2014/Cannon). The results of this study can be briefly summarized. It is the first large RCT to show incremental clinical benefit when adding a non-statin agent (ezetimibe) to statin therapy. It demonstrated that "even lower is better", that is, incremental reduction in ASCVD when mean LDL-C on statins (70 mg/dL) was reduced to 53 mg/dL. There were no adverse effects when ezetimibe was added to a statin. The authors claim that this study reaffirms the LDL hypothesis justifying combined lipid lowering drugs in secondary prevention.

Another lipid-lowering drug, nicotinic acid, has been shown to reduce ASCVD events in monotherapy [42, 43]. Nicotinic acid, when combined with statins, apparently retards progression of subclinical atherosclerosis [44, 45]. Whether nicotinic acid as an add-on to maximal statin therapy reduces risk for ASCVD events beyond statin therapy alone had not been adequately tested until recently. In 2011, nicotinic acid combined with maximal statin therapy in a smaller RCT failed to give added risk reduction in secondary prevention [46]. A larger trial of combination therapy therefore was needed to test whether nicotinic acid provides incremental benefit. Such a trial (http://clinicaltrials.gov/ct2/show/NCT00461630) has recently been completed; this trial assessed the effects extended-release niacin/laropiprant versus matching placebo on ASCVD events in 25,000 men and women with existing ASCVD and who were taking high-dose statin. A recent statement from the investigators indicates that this study failed to document benefit from niacin add-on to statin therapy. However, results of the trial have not been published.

The demand that only RCTs can be used to make lipid-lowering guidelines limits the potential for add-on drugs to give additional risk reduction beyond the use of high-dose statin in secondary prevention. However, if the IMPROVE-IT trial is positive, this would open the door to use of non-statin drugs. If not, it is doubtful whether new RCTs will be done with either bile acid resins or ezetimibe. The costs are too high. Trials with more potent lipid-lowering drugs nonetheless may be tested as add-on therapy. Several of these latter drugs can be considered.

LDL-Lowering Therapies Under Development

Microsomal triglyceride transfer protein (MTP) inhibitors block the incorporation of triglyceride into VLDL and reduce the secretion of these lipoproteins. VLDL are precursors of LDL. Reducing hepatic input of VLDL thereby reduces LDL levels. Available MTP inhibitor, Lomitapide, reduces LDL-C levels by approximately 50% [47]. The combination of an MTP inhibitor with a statin markedly reduces LDL levels. Unfortunately, blockage of transfer of triglyceride into VLDL causes triglyceride retention in liver. The resulting fatty liver stands in the way of routine use of MTP inhibitors. But in patients with severe hypercholesterolemia, who are resistant to other lipid-lowering drugs, MTP inhibitors may be acceptable. Even so, they must be monitored carefully for liver dysfunction.

Another class of drugs that will substantially lower LDL-C levels are antisense oligonucleotides. These agents target apo B synthesis. The currently available agent is named Mipomersen. One recent study tested Mipomersen as an add-on drug to maximally tolerated statins in patients with heterozygous familial hypercholesterolemia. This product reduced LDL-C levels by an additional 26% [48]. Like MTP inhibitors, blockage of apo B synthesis potentially causes fatty liver. In this recent study [48], Mipomersen increased liver fat content by 5%. An increase in alanine aminotransferase values $\geq$3 times the upper limit of normal also was observed in 6% of subjects. To date, there are only a few reported studies with the oligonucleotide; more studies will be required to determine whether this approach is safe and practical.

Another potential class of add-on drugs includes cholesterol-ester transfer protein (CETP) inhibitors. Inhibition of CETP retards transfer of cholesterol ester from HDL to VLDL and LDL. This increases HDL-C and lowers LDL-C. The first drug in this class was torcetrapib [49]. In a large RCT of 15,067 patients at high cardiovascular risk, participants received either torcetrapib plus atorvastatin or atorvastatin alone. The primary outcome was a composite of ASCVD events. Compared to atorvastatin alone, there was an increase of 72.1% in HDL-C and a 24.9% decrease in LDL-C. Unfortunately, torcetrapib treatment caused more ASCVD events and total deaths. Because of these side effects, the trial was terminated early. Despite this termination, testing of other CETP inhibitors has continued.

The second CETP inhibitor to be tested was dalcetrapib. This RCT recruited 15,871 with acute coronary syndromes and randomized them to dalcetrapib 600 mg or the best available evidence-based care. The primary end point was composite ASCVD [50]. During the trial, HDL-C levels in the dalcetrapib group increased by 31–40%, whereas LDL-C were essentially unchanged. After a median of 31 months, 1135 primary end points were achieved (71% of the projected total number). At this analysis, an independent data and safety monitoring board recommended ending the study for futility. There may be several explanations for the failure to attain a reduction in events. First, dalcetrapib was a relatively weak CETP inhibitor and failed to reduce LDL-C levels. Alternatively, a rise in HDL-C by CETP inhibition may not be antiatherogenic.

To test whether a more efficacious CETP inhibitor will be effective, a 1623-patient, a phase II trial with anaceptrapib was carried out in patients treated with statins [51]. This study showed that anaceptrapib reduced LDL-C by an additional 40% and raised HDL-C by 138%. To date, anaceptrapib has shown no significant side effects, and a large RCT has been initiated to determine its safety and efficacy as an add-on drug to maximal statin therapy.

Another class of LDL-lowering agents consists of the PCSK9 inhibitors. PCSK9 is a serum protein that blocks the ability of LDL receptors to remove LDL from the circulation. Apparently, PCSK9 reduces LDL receptor levels by binding and targeting the receptor for lysosomal degradation [52, 53]. Persons who have a mutation in PCSK9 that prevents the interaction of the protein with LDL receptors have a high expression of receptors and low serum levels of LDL throughout life. These persons appear to be protected against

CHD [5, 54, 55]. Recently, the pharmaceutical industry has developed antibodies against PCSK9 that block its action on LDL receptors and lower LDL levels [56, 57]. The addition of PCSK9 inhibitors to statin therapy enhances LDL lowering. Although these agents are promising for achieving and exceeding an LDL-C goal of < 70 mg/dl, their efficacy and safety must be demonstrated in clinical trials.

Lifestyle Therapy in Secondary Prevention

Although in the secondary prevention arena emphasis has been on lipid-lowering drugs, nonetheless, the potential benefit of therapeutic lifestyle changes should not be overlooked. Lifestyle therapies have two major goals: (a) to reduce LDL-C levels and (b) to reduce the metabolic syndrome [1]**.** The first can be achieved largely by reducing intakes of saturated fats, *trans* fats, and dietary cholesterol. The second is best approached through both weight reduction and increased physical activity. All patients with established ASCVD should be educated and encouraged to adopt effective lifestyle therapies.

Treatment of Hypertriglyceridemia in Secondary Prevention

For patients who remain hypertriglyceridemic on statin therapy, fibrates can be considered as a second drug [58]. RCTs have reported reductions in ASCVD in primary and secondary prevention with fibrates; a meta-analysis of these trials found that fibrates lower risk by approximately 10% [59]. Moreover, meta-analysis in patients with hypertriglyceridemia has shown even greater reductions in risk [60]. The safest fibrate to be used with statins appears to be fenofibrate, which is largely devoid of myopathy risk [61].

In summary, the strongest evidence of benefit for risk reduction in secondary prevention trials has been obtained with RCTs using high-intensity statins (e.g., atorvastatin 80 mg). RCTs and subgroup analysis support reducing LDL-C to very low levels (60–75 mg/dL). However, the majority of subjects treated with high-intensity statins fail to achieve these levels. For this reason, consideration can be given to use of an add-on drug to maximal statin therapy to achieve this lower goal. Drugs currently available to obtain very low levels of LDL-C are bile-acid-binding resins and ezetimibe. Whether the addition of these drugs to maximal statin therapy will further reduce risk for ASCVD has not been tested, although one RCT in which ezetimibe is added to maximal statin dose is underway. Two recent clinical trials using niacin as add-on therapy to maximal statin therapy failed to document added benefit. Fenofibrate can be considered as a second drug in patients with hypertriglyceridemia. Newer drugs are currently undergoing testing to determine whether they may have some potential as add-on drugs to achieve very low LDL levels. These include CETP inhibitors, PCSK9 inhibitors, apo B synthesis inhibitors, and MTP inhibitors. Only when these trials are complete will it be known whether they are incrementally beneficial.

Primary Prevention

Because of the efficacy of statin therapy in secondary prevention trials, many investigators believe that this same drug-treatment strategy can be extended to primary prevention. Since statins are powerful LDL-lowering drugs and are relatively safe, why not just treat large segments of the population with statins *before* they develop ASCVD? In fact, recent ACC/AHA guidelines [4] have moved in this direction. They have done this in two ways: (a) by lowering the risk threshold for starting statins, and (b) by ignoring baseline LDL-C levels for statin initiation. Through these changes, all people will eventually become candidates for statin therapy. In other words, these guidelines are a step towards making statins a public health measure rather than a clinical therapy.

Risk Assessment: Selection of Patients for Drug Treatment

Ten-year Risk Assessment for CHD In ATP III [1], 10-year risk for CHD is estimated by an algorithm developed by the Framingham Heart Study. These guidelines recommended that intensity of LDL-lowering therapy be adjusted according to 10-year risk for CHD. According to ATP III, risk can be categorized as high, moderately high, moderate, and low [1]. High risk was classified as a 10-year risk for hard CHD of ≥ 20 %; moderately high risk was 10–19 %; moderate risk was approximately 5–9 %; and low risk was < 5 %. This classification of risk has been widely accepted in the USA. In Europe, 10-year risk for cardiovascular mortality is preferred over morbidity for risk assessment [23].

Ten-year Risk for Total ASCVD The ACC/AHA guidelines [4] expanded the Framingham end point to include both CHD and stroke. Here the algorithm to assess ASCVD risk becomes all-important for selection of patients for statin therapy. If it overpredicts risk, more low-risk persons will be selected for drug therapy. Prior to ACC/AHA guidelines, Framingham investigators published a risk-prediction algorithm that includes CHD, stroke, peripheral vascular disease, and heart failure [62]. To date, it has not been tested for its practicality. One study showed that its use will markedly change therapeutic strategies [63]. ACC/AHA instead utilized a different algorithm to predict ASCVD. This algorithm was obtained by combining data from five large epidemiologic studies sponsored by NHLBI [64]. Since the publication of the ACC/AHA algorithm, a question has been raised as to whether it overestimates risk in the current US population. If so, an excess of low-risk patients would be treated with statins. There is some evidence that population risk has declined since the earlier studies contained in this algorithm. For example, Ridker and Cook [65] recently reported that three US populations have approximately half the risk calculated by the ACC/AHA algorithm. Moreover, several studies have indicated that the Framingham algorithm, which is contained in the new ACC/AHA tool, overestimates risk in several European populations [66–72].

Table 18.2 Number needed to treat to prevent one ASCVD event over 10 years with high-intensity statin therapy

Ten-year risk for ASCVD (%)	ACC/AHA algorithm	One-half ACC/AHA algorithm
5	50	100
7.5	33	66
10	25	50
12.5	20	40
15	17	34
17.5	14	28
20	12	24
22.5	11	22
25	10	20

ASCVD atherosclerotic cardiovascular disease, *ACC* American College of Cardiology, *AHA* American Heart Association

This uncertainty must be taken into account when estimating risk in the US population; it requires considerable clinical judgment as to whether patients at low risk are being overtreated with cholesterol-lowering drugs.

The uncertainty over the reliability of the ACC/AHA algorithm requires us to consider another metric for statin therapy namely the number needed to treat (NNT) to prevent one ASCVD event over 10 years. This is illustrated in Table 18.2. If we assume that statin therapy reduces risk for ASCVD by 40 % and if the ACC/AHA algorithm is correct, the NNT for each risk category is shown in the first column. But if the algorithm overestimates risk by twofold, NNT is shown in the second column. As low risk, the NNT is relatively high, but particularly so if risk is overestimated. A more acceptable NNT is obtained as the risk becomes higher. There is no consensus number on NNT for statin drugs, so a decision about initiation of drug therapy depends on agreement between physician and patient.

Assessment of Lifetime Risk The 10-year risk assessment for ASCVD is problematic because the purpose of primary prevention is to reduce lifetime risk. This fact has led to increased interest in estimating lifetime risk [73–77]. Donald Lloyd-Jones and associates [73, 78–85] have

published a series of papers on estimation of lifetime risk. Other investigators have projected lifetime risk based on Framingham data [75]. Another lifetime risk predictor is the QRISK model [77, 86, 87]. This model was derived from a prospective cohort study with data from general practices in the UK between 1994 and 2010. Recent guidelines from the International Atherosclerosis Society [88] have adopted lifetime risk assessment as a basis for recommendations of intervention to treat elevated cholesterol and other lipid abnormalities.

Risk Assessment by Atherosclerosis Imaging Framingham risk scoring is highly dependent on age as a risk factor. Since atherosclerosis increases progressively with age, age essentially becomes a *surrogate* for atherosclerosis burden. The relation between age and plaque burden may hold for populations but not necessarily for individuals. Therefore, some investigators have postulated that a better way to estimate risk would be to replace age with a more direct measure of atherosclerosis.

One method for determining subclinical atherosclerosis burden is by measuring coronary artery calcium (CAC) [89]. CAC measurements are strongly correlated with coronary artery plaque burden [90–94]. Some years ago, Grundy [95] proposed using CAC to replace chronological age as a risk factor when using Framingham risk scoring. This approach has been validated in more recent studies [96]. One utility of this adjustment is to identify persons who are at low risk and who are unlikely to benefit from cholesterol-lowering drugs. Although some persons lacking in CAC can still have coronary plaques, these individuals are relatively rare and do not negate the value of CAC testing in individuals. This method of risk assessment appears to be particularly attractive for older persons. If it is used, many fewer older people will require statin therapy than is selected by the ACC/AHA algorithm.

Since the ACC/AHA algorithm includes stroke as well as CHD, imaging of the carotid arteries may also be helpful in the selection of patients for statin therapy. This is best done by measurement of carotid intimal medial thickness (IMT) with sonography [97]. If the patient is found to have an increased IMT, prevention of stroke through use of statins is reasonable.

Role of Emerging Risk Factors in Risk Assessment A variety of other factors have been found to associate with increased risk for ASCVD [1, 98]. These include various lipid factors (e.g., low HDL, small LDL particles, and lipoprotein [a]), pro-inflammatory factors (e.g., C-reactive protein [CRP]), prothrombotic factors (e.g., PAI-1), insulin resistance, and hyperglycemia. A low HDL strongly correlates with ASCVD risk, but whether it is a cause of atherosclerotic disease has not been determined. Further, a low HDL is confounded by non-HDL-C levels; nonetheless, HDL-C is incorporated into most risk algorithms because of the strong association with ASCVD risk. A high CRP appears to reflect a pro-inflammatory state; it has been included in one CHD risk algorithm [99, 100]. Small LDL particles are confounded by a high non-HDL-C, but whether they are more atherogenic than normal-sized LDL is uncertain. Diabetes is accompanied by increased ASCVD risk; but whether this is due to hyperglycemia per se is uncertain. Diabetes and insulin resistance are components of the metabolic syndrome, which likewise highly correlates cardiovascular risk [101–102]. Finally, a strong family history of premature ASCVD associates with risk. Thus, the presence of all of these emerging risk factors is strongly suggestive of higher risk, although they have not been incorporated into either Framingham or ACC/AHA algorithms. Patients who exhibit one or more of these risk factors can be considered to be at higher risk, but clinical judgment is required whether to modify therapy beyond that advocated using standard algorithms. In the cardiovascular field, opinion is divided on this point.

LDL-C Goals in Primary Prevention

If guidelines are going to employ LDL-C goals, it may be useful to classify LDL-C levels according

to relative risk reduction (Table 18.1). These ranges are potential goals for therapy.

In 2002, ATP III set a goal of < 130 mg/dL for individuals with a moderate-to-moderately high risk [1]. Since that time, there has been growing evidence for further risk reduction by reducing LDL-C levels below 130 mg/dL [103]. The ATP III update [2] indicated that when drug therapy is employed, it is reasonable to set an LDL-C goal of < 100 mg/dL (e.g., 75–99 mg/dL). The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial [103] showed that still lower LDL-C levels reduce ASCVD risk even more. In strictly scientific terms, therefore, it can be said for LDL-C that "the lower, the better" for risk reduction. But to achieve very low LDL-C levels (50–74 mg/dL), high-intensity statins are required for many people. On practical grounds, therefore, clinical judgment is needed to determine whether an effort should be made to achieve a very low LDL-C level. Recent Canadian guidelines [24] favored obtaining a very low LDL-C for primary prevention when statins are employed. Certainly, for high-risk patients, (e.g., those with diabetes and cigarette smokers) attaining very low levels is reasonable. But for those at lower risk, reducing LDL-C to < 100 mg/dL (75–99 mg/dL) should be sufficient. According to current epidemiologic data [55], once LDL-C levels reach 100 mg/dL, additional lowering appears to be accompanied by diminishing returns for risk reduction. From RCTs, it is uncertain whether the decline in risk accompanied a decline in LDL-C is linear or curvilinear (log-linear).

Statin Therapy Without LDL-Cholesterol Goals in Primary Prevention

Since most clinical trials have employed statin therapy, it can be expected that statins will receive highest priority in any guidelines for cholesterol-lowering drugs. As mentioned before, ACC/AHA guidelines hold that statin therapy should be based exclusively on risk estimates regardless of LDL-C levels and without any defined LDL-C goals of therapy. If this approach is to be recommended for primary prevention, a nontrivial question is whether to use moderate-intensity or high-intensity statins. ACC/AHA [4] appears to favor high-intensity statins where possible, although they seemingly accept moderate-intensity statins for primary prevention. The latter include simvastatin 20 mg, pravastatin 40 mg, lovastatin 40 mg, atorvastatin 10 mg, and rosuvastatin 5 mg. Some investigators favor starting with moderate-intensity drugs and triturating the drug upwards as tolerated and according to the degree of LDL lowering.

Since publication of ACC/AHA guidelines, concern has been raised about elimination of LDL-C goals from recommendations [104–107]. Several advantages to use of goals have been claimed: (a) monitoring adherence to drug therapy, (b) monitoring LDL-C response to therapy, (c) allowing adjustment of drug dosage to achieve the goal of therapy, and (d) ensuring maximal LDL-C reduction in higher-risk patients.

When to Start Drug Therapy in Primary Prevention

Most investigators would agree that statin therapy is indicated for persons with 10-year risk for CHD of ≥ 20 % (ASCVD > 27 %). Likewise, in accord with ATP III, cost-effective statin therapy can be recommended for persons whose 10-year risk for CHD is ≥ 10 % (ASCVD > 15 %). Whether to recommend statins for a CHD-risk threshold of 5 % (e.g., ASCVD risk 7–10 %) is more open to question. This question cannot be separated from the age group of the patient. For convenience, it may be useful to separate subjects and at 20-year periods: 60–79 years, 40–59 years, and 20–39 years. Older persons have the highest risk for ASCVD; but reliability for risk assessment is lowest in this age group. Measurement of subclinical atherosclerosis can be particularly useful for deciding when to initiate statin therapy. If the ACC/AHA algorithm is employed, it may be preferable to set a 10-year risk threshold for ASCVD of ≥ 15 %. This corresponds to adding a major risk factor (e.g., hypertension, cigarette smoking, or diabetes) to an optimal or near-optimal baseline

risk. In middle age, the ACC/AHA algorithm appears to be adequate. Likewise, a risk threshold for statin therapy of 7.5% appears appropriate. Finally, for young adults, emphasis should be on lifestyle therapy. Combining a non-atherogenic diet with weight control and exercise and avoidance of cigarette smoking in most cases should be sufficient to retard development of atherosclerosis. However, if major risk factors including high LDL-C have taken hold at a young age, it seems appropriate to intervene with drug therapy if necessary to reverse the risk factor.

Non-Statin Drugs in Primary Prevention

Let us next ask whether statins are the only acceptable drugs for primary prevention. Other agents are approved for LDL lowering: bile acid sequestrants, nicotinic acid, ezetimibe, and fibrates. LDL lowering with bile acid sequestrants have been shown to safely reduce risk in hypercholesterolemic subjects without established ASCVD [108–110]; but it has not been adequately tested for primary prevention. Ezetimibe has not been studied for primary prevention, although theoretically should lower risk if used for a long period. Fibrates have been reported for lower risk in primary prevention [111], but they are not strong LDL-lowering drugs. Thus, statins are obviously first-line therapy for primary prevention; ezetimibe and bile acid sequestrants have potential, but are not adequately tested for efficacy to satisfy most investigators. However, they might be used as add-on drugs to statins in patients with hypercholesterolemia in whom low LDL levels are not attained by statins alone. The potential value of combining another LDL-lowering drug with a statin has been shown by the IMPROVE-IT trial.

If a 15% reduction in LDL-C starting in young adulthood with mildly elevated LDL-C can reduce risk by 50% over a lifetime, it might be worthwhile to consider using a bile acid sequestrant, to age 60; thereafter, a moderate dose of statin could be introduced to achieve further reduce risk for the remainder of life. Such a strategy could be based on currently available information, although it has not been tested with RCTs.

Lifestyle Intervention for Primary Prevention

The goal for lifestyle intervention in primary prevention is to reduce all of the risk factors for ASCVD. Highest on the list is elimination of tobacco use. Cigarette smoking is the most important lifestyle factor responsible for ASCVD. Other lifestyle factors have been discussed in detail previously [1]. To achieve maximal LDL lowering, saturated fatty acids should be reduced $<7\%$ of total calories, or at least to $<10\%$. Intake of *trans* fatty acids should be lowered to $<1\%$ of total calories and dietary cholesterol to <200 mg/day. For greater LDL lowering, plant sterols/stanols (2 g/day) can be used as a dietary adjunct. The preferable total fat intake is about 30% of total calories, with most fatty acids being unsaturated. Total caloric intake should be adjusted to achieve a desirable body weight. Finally, many investigators believe that the diet should be enriched in fruits, vegetables, and fibers. Sodium intake should be <2 gm/day, and foods high in potassium should be encouraged. A common recommendation is for a diet containing fish rich in omega-3 fatty acids.

Population studies show that favorable life habits can greatly reduce the population volume of ASCVD. This combined with judicious use of agents that will moderately reduce LDL-C levels should magnify the reduction of atherosclerosis burden in the population. Finally, use of statins later in life can take advantage of their ability to markedly lower lifetime risk for ASCVD.

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Nutrition and Coronary Heart Disease Prevention

19

Ernst J. Schaefer and Mariko Tani

I. National Guidelines

First we review current national dietary guidelines.

A. US Dietary Guidelines

Every 5 years the dietary guidelines in the USA are updated [1, 2]. In the 2010 version [2], the following initial four recommendations were made to prevent chronic disease and promote health:

1. Prevent or reduce overweight or obesity through improved eating and physical activity behaviors.
2. Control total calorie intake to manage body weight. For people who are overweight or obese, this will mean fewer calories from foods and beverages.
3. Increase physical activity and reduce time spent in sedentary behaviors.
4. Maintain appropriate calorie balance during each stage of life—childhood, adolescence, adulthood, pregnancy and breastfeeding, and older age.

The following recommendations were made for foods to decrease:

1. Reduce daily sodium intake to less than 2300 mg and further reduce intake to 1500 mg in those who are 51 and older and those of any age who are African American or have hypertension, diabetes, or chronic kidney disease. The 1500-mg recommendation applies to about half of the US population, including children, and the majority of adults.
2. Consume less than 10% percent of calories from saturated fat by replacing them with *cis*-monounsaturated and polyunsaturated fatty acids.
3. Consume less than 300 mg per day of dietary cholesterol.
4. Keep *trans*-fatty acid consumption as low as possible by limiting foods that contain synthetic sources of *trans* fats, such as partially hydrogenated oils, and by further limiting solid fats.
5. Reduce the intake of calories from solid fats and sugars.
6. Limit the consumption of foods that contain refined grains, especially grain foods that contain solid fats, added sugars, and sodium.
7. If alcohol is consumed, it should be consumed in moderation—up to one drink per day in women and two drinks per day in men—and only by adults of legal drinking age.

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The following recommendations were made with regard to foods to increase:

1. Increase vegetable and fruit intake.
2. Eat a variety of vegetables, especially dark green and red and orange vegetables, and beans and peas.
3. Increase intake of fat-free or low-fat milk and milk products, such as milk, yogurt, cheese, or fortified soy beverages.
4. Choose a variety of protein foods, which include seafood, lean meat, and poultry, eggs, beans and peas, soy products, and unsalted nuts and seeds.
5. Increase the amount and variety of seafood consumed by choosing seafood in place of some meat and poultry.
6. Replace protein foods that are higher in solid fats with choices that are lower in solid fats and calories and/or are sources of oils.
7. Use oils to replace solid fats where possible.
8. Choose foods that provide more potassium, fiber, calcium, and vitamin D, which nutrients of concern in American diets. These foods include vegetables, fruits, whole grains, and milk and milk products.

The following recommendations were made for special populations.

Women capable of becoming pregnant:

1. Choose foods that supply heme iron, which is more readily absorbed in the body, additional iron sources, and enhancers of iron absorption, such as vitamin C-rich foods.
2. Consume 400 μg per day of synthetic folic acid (from fortified foods and/or supplements) in addition to food forms of folate from a varied diet.

Women who are pregnant or breastfeeding:

1. Consume 8–12 ounces of seafood per week from a variety of seafood types.
2. Due to their high methyl mercury content, limit white (albacore) tuna to six ounces per week, and do not eat the following four types of fish: tilefish, shark, swordfish, and king mackerel.
3. If pregnant, take an iron supplement, as recommended by an obstetrician or other healthcare provider.

Individuals aged 50 and older:

1. Consume foods fortified with vitamin B_{12}, such as fortified cereal, or dietary supplements.

In addition, the following recommendations were made for building healthy eating patterns:

1. Select an eating pattern that meets nutrient needs over time at an appropriate calorie level.
2. Account for all foods and beverages consumed and assess how they fit within a total healthy eating plan.
3. Follow food safety recommendations when preparing and eating foods to reduce the risk of foodborne illnesses.

B. New Guidelines from the American College of Cardiology and the American Heart Association

In 2013, the ACC and the AHA in conjunction with the National Institutes of Health released the American Heart Association (AHA)/American College of Cardiology (ACC) Guideline on Lifestyle Management to Reduce Cardiovascular Risk, as well as guidelines on the assessment of cardiovascular risk, and on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults [3–5]. For the new cardiovascular disease (CVD) risk assessment, the variables utilized included gender, age, race, total cholesterol, high-density lipoprotein cholesterol (HDL-C), systolic blood pressure, treatment for hypertension, diabetes, and smoking, and can be accessed at http://www.myamericanheart.org/cvriskcalculator [4]. Individuals recommended for statin therapy in addition to lifestyle treatment include: those with a 10-year risk of CVD $\geq 7.5\%$, those with CVD, those with diabetes, and those with an low-density lipoprotein cholesterol (LDL-C) value >190 mg/dL [5]. Surprisingly, no LDL-C targets of therapy were recommended. For the set of lifestyle recommendations provided below for lowering LDL-C and blood pressure, the

panel considered that the dietary evidence supporting the recommendations to be in category A or highest category, while for their exercise recommendations they classified the supporting evidence as being in category B [3]:

1. **For adults who would benefit from LDL-C lowering the panel recommended:**
   1A. Consume a dietary pattern that emphasizes intake of vegetables, fruits, and whole grains; includes low-fat dairy products, poultry, fish, legumes, nontropical vegetable oils, and nuts; and limits intake of sweets, sugar-sweetened beverages, and red meats.
   1B. Adapt this dietary pattern to appropriate calorie requirements, personal and cultural food preferences, and nutrition therapy for other medical conditions (including diabetes mellitus).
   1C. Achieve this pattern by following plans such as the Dietary Approaches to Stop Hypertension (DASH) dietary pattern, the United States Department of Agriculture (USDA) food pattern, or the AHA diet.
   1D. Aim for a dietary pattern that achieves 5–6 % of calories from saturated fat.
   1Da. Reduce percent of calories from saturated fat.
   1Db. Reduce percent of calories from *trans* fat.
2. **For adults who would benefit from blood pressure lowering the panel recommended:**
   2A. Consume a dietary pattern that emphasizes intake of vegetables, fruits, and whole grains; includes low-fat dairy products, poultry, fish, legumes, nontropical vegetable oils, and nuts; and limits intake of sweets, sugar-sweetened beverages, and red meats.
   2B. Adapt this dietary pattern to appropriate calorie requirements, personal and cultural food preferences, and nutrition therapy for other medical conditions (including diabetes mellitus).
   2C. Achieve this pattern by following plans such as the DASH dietary pattern, the USDA food pattern, or the AHA diet.
   2D. Lower sodium intake.
   2D1. Consume no more than 2400 mg of sodium/day.
   2D2. Further reduction of sodium intake to 1500 mg/day is desirable since it is associated with even greater reduction in blood pressure.
   2D3. Reduce intake by at least 1000 mg/day since that will lower blood pressure, even if the desired daily sodium intake is not yet achieved.
   2D4. Combine the DASH dietary pattern with lower sodium intake.
3. **Exercise Recommendations:**

The panel recommended that adults engage in aerobic physical activity to reduce LDL-C, non-HDL-C, and blood pressure: three to four sessions a week, lasting on average 40 min per session, and involving moderate-to-vigorous intensity physical activity.

II. Justification for Dietary Recommendations

Now we review the justification for these dietary recommendations.

A. Controlled Metabolic Studies

In our own studies under controlled circumstances, a diet meeting current criteria for LDL-C lowering [3] will lower LDL-C levels by about 12–20 % as compared to an average American diet [6–8]. Moreover, dietary cholesterol as well as saturated and *trans*-fatty acids can both definitely raise LDL-C levels significantly, and therefore should be restricted [9, 10]. Three different sets of investigators have done composite analyses to determine what effects different constituents of the diet have on LDL-C levels under controlled metabolic ward conditions.

Hegsted and colleagues [11] published the following formula:

$$\Delta\text{LDL-C} = 1.74\Delta\text{S} - 0.766\Delta\text{P} + 0.0439\Delta\text{C}.$$

Mensink and Katan [12] published the following formula:

$$\text{Change in LDL-C } (\text{mg/dl}) = 1.28$$
$$\text{Change in S} - 0.24 \text{ Change in M} - 0.55$$
$$\text{Change in P}.$$

Yu et al. [13] published the following formula:

$$\text{Change in LDL-C } (\text{mg/dl}) = 1.46 \text{ Change in S*} - 0.07 \text{ Change in Stearic Acid} - 0.69 \text{ Change in M} - 0.96 \text{ Change in P.}$$

In these equations, S is saturated fat intake as a percentage of caloric intake when exchanged for carbohydrate, S* in the formula by Yu et al. is stearic acid not included in the saturated fat category which includes only lauric (12:0), myristic (14:0), and palmitic acid (16:0), M is monounsaturated fatty acid intake as a percentage of calories (mainly oleic acid or 18:1, n9) when exchanged for carbohydrate, and P is polyunsaturated fatty acid intake as a percentage of calories (mainly as linoleic acid or 18:2, n6, arachidonic acid or 20:4, n6, and alpha linolenic acid or 18:3, n3) when exchanged for carbohydrate. Saturated fatty acids are solid at room temperature, while M fatty acids with one or more double bonds are liquid at this temperature, and confer greater fluidity to the phospholipids that they are attached often in membranes.

As can be seen, changes in S have the largest effects on LDL-C, followed by change in P intake, and then followed by changes in M intake category M [11–13]. Only Hegsted and colleagues included dietary cholesterol in their formula where C = change in dietary cholesterol in milligrams per day. Therefore, using the Mensink and Katan formula, if a subject lowered S from 14 to 7% of calories, and raised P from 5 to 12%, with no change in dietary carbohydrate intake, their predicted LDL-C level would be reduced by about 12 mg/dl or about 10% in a subject with normal cholesterol levels. This equation does not take dietary cholesterol into account. It should be stated that under controlled conditions there is a marked variability in LDL-C lowering response due in part to gender differences as well as to apoE genotype [14–16]. Of course it is important to decrease the intake of *trans*-fatty acids since these in the form of hydrogenated vegetable oils will raise LDL-C as much as saturated fat [10]. It should be noted that almost all soft margarines are now *trans*-fat-free or very low in *trans*-fatty acids, and they are clearly a better option than butter, since their fatty acid content is usually similar to that of soybean oil. We and others have also documented that when one replaces saturated fatty acids with polyunsaturated fatty acids in the diet, both LDL-apoB and HDL apoA-I decrease significantly because of an enhanced fractional catabolic rate [1]. These changes appear to be related to upregulation of the both the LDL receptor and scavenger-receptor B1 in the liver [1].

Four vegetable oils make up 70% of the world's plant oil consumption in a variety of foods including cooking oils, salad dressings, and margarine products. These are: (1) soybean oil at 26% (16% S, 24% M, 60% P, 1% *trans*), (2) palm oil at 18% (52% S, 39% M, 10% P), (3) sunflower oil at 13% (11% S, 20% M, 69% P), and (4) canola oil at 12% (7% S, 64% M, 28% P; (Fig. 19.1). The major vegetable oil produced in the USA is soybean oil, while for Malaysia, Indonesia, and Africa it is palm oil, for Canada, China, and India it is canola or rapeseed oil, and for most of Europe, Russia, and Argentina it is sunflower oil. Other oils that make up the remaining 30% (all below 10% of the total) include safflower oil (10% S, 13% M, 77% P), corn oil (14% S, 29% M, 57% P), peanut oil (18% S, 9% M, 34% P), and cottonseed oil (27% P, 19% M, 54% P). The highly touted olive oil only comprises 2% of the world's oil consumption and contains 14% S, 75% M, and 11% P. Olive oil is expensive to produce and is harvested mainly in Spain, Italy, and Greece.

Fats of animal origin are found in meats with the most consumed worldwide in order being pork, poultry, beef, and mutton, along with dairy products. Meats are also more expensive to produce than plant products because of having to use grain to feed the animals, along with plenty of water and a large need for land. All fats of animal origin contain cholesterol. While butter and dairy fat have the highest S fat content (65% S, 27% M, and 4% P, 4% *trans*), followed by beef fat (52% S, 44% M, 4% P), animal fat shortening (43% S, 48% M, 6% P), lard (41% S, 47% M, 12% P), and chicken fat (31% S, 47% M, 22% P) being somewhat lower (Fig. 19.2). The highest

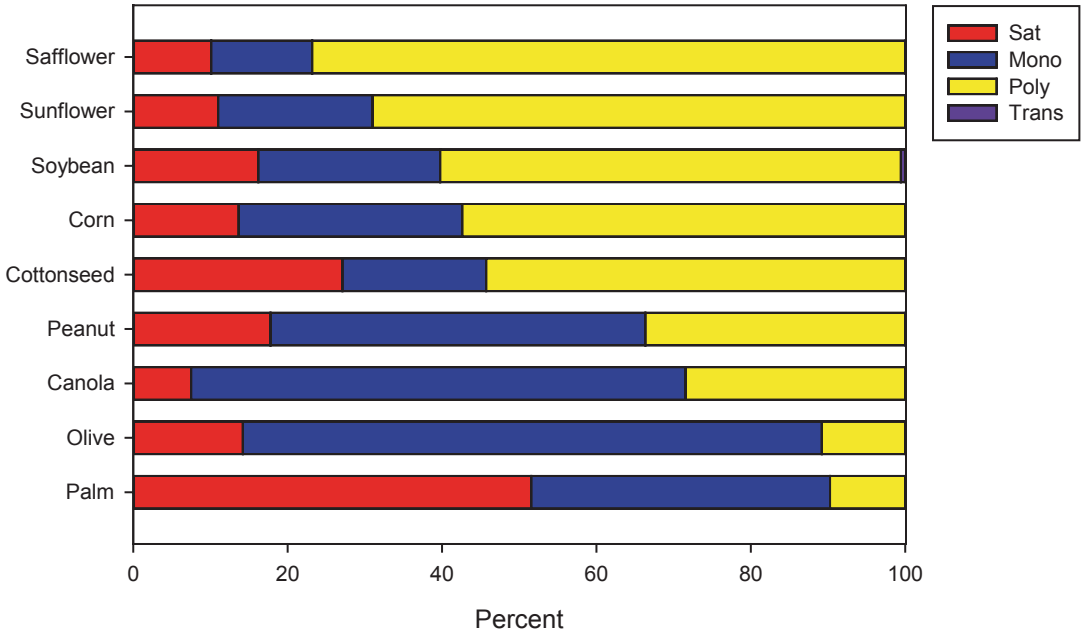

Fig. 19.1 Fatty acid composition of the major plant oils. *Red* indicates the percent of saturated fat; *blue,* monounsaturated fat; *yellow,* polyunsaturated fat and *purple, trans* fats. Figure courtesy of Abhimanyu Garg, M.D. Data from USDA National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov)

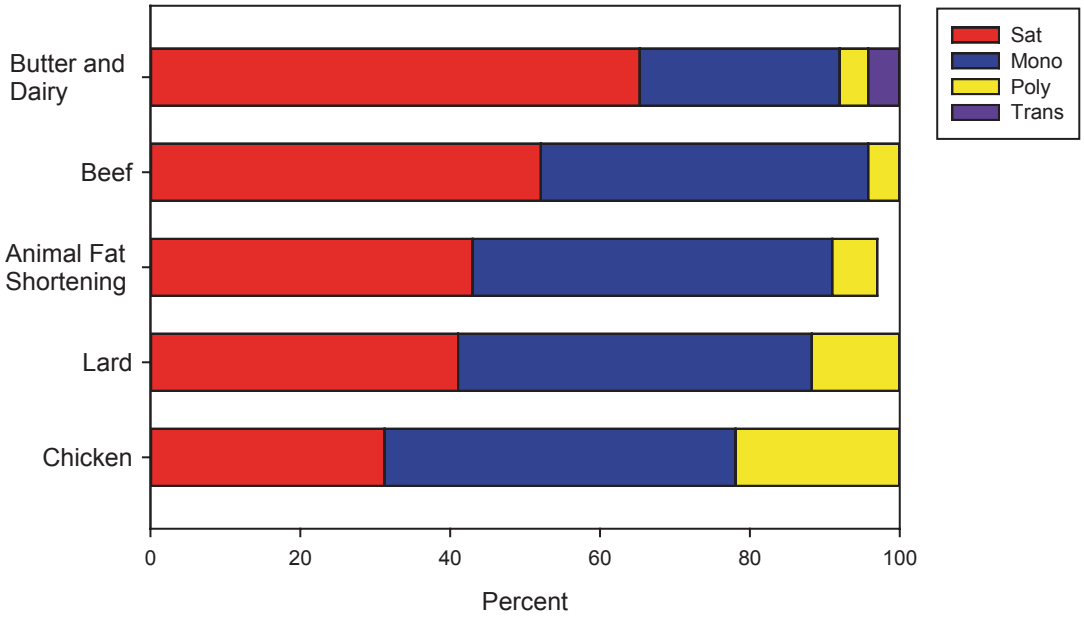

Fig. 19.2 Fatty acid composition of the major animal fats. *Red bar* indicates the percent of saturated fat; *blue,* monounsaturated fat; *yellow,* polyunsaturated fat and *purple, trans* fats. Figure courtesy of Abhimanyu Garg, M.D. Data from USDA National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov)

intake of animal fat in the world is currently in Russia and the Eastern Bloc countries, and these countries according to the World Health Organization's website also have the highest rates of coronary heart disease (CHD) in the world. It should also be mentioned that when one restricts animal fat to lower saturated fat intake, one usually lowers monounsaturated fat intake because most animal fats have almost as much or more monounsaturated fat as saturated fat. Therefore, the only logical way to replace saturated fat is with vegetable oil that is rich in both polyunsaturated and monounsaturated fats.

Additional ways to lower LDL-C with lifestyle modification include adding two serving per day of plant stanol/sterol margarine which will decrease cholesterol absorption [17]. These products will lower LDL-C levels up to 10% [17]. Another way to lower LDL-C 5–10% is to increase dietary fiber including the daily use of psyllium [18]. Another issue that is beyond the scope of this chapter is effects of different types of carbohydrate on lipid levels. It does appear that dietary fructose is more deleterious in terms of effects on visceral fat, triglyceride levels, and HDL-C levels than is glucose [19]. It should be recognized that in the real world most physicians do not refer patients to the dietitian, in part because one or two visits with the dietitian in their experience has virtually no effect on lowering LDL-C levels. It is now becoming clear that for both LDL-C lowering and weight loss, much more intensive group approaches lasting several months are required to achieve any significant type of lifestyle change.

B. Population Studies

There have been many population studies examining the interrelationships between diet and heart disease. The earliest of these was the Seven Countries Study conducted by Ancel Keys and colleagues [20]. This study clearly documented that the key dietary ingredient linked to prospective CHD in seven different countries and 16 populations (including Finland, Greece, Italy, Japan, the USA, and Yugoslavia) was the level of saturated fat intake ($r=0.84$). The Ni-Hon-San Study involving men living in Japan, Hawaii, and California confirmed this relationship [21]. In the Twenty Countries Study, Stamler and colleagues reported significant positive correlations between CHD mortality and intake of butter ($r=0.55$), all dairy products ($r=0.62$), eggs ($r=0.59$), meat and poultry ($r=0.56$), and sugar and syrup ($r=0.68$), and a significant inverse association with intake of grains, fruit, starchy, and nonstarchy vegetables ($r=-0.63$) [22]. The strengths of these earlier studies are that they used 7-day food records which are much better and more reliable and accurate assessments of actual dietary intake than food-frequency questionnaires [23].

More recently in the INTERHEART Study, Yusuf and colleagues collected data on 15,152 men and women with CHD, and 14,820 age- and gender-matched controls in 52 countries from all six inhabited continents [24]. Nine risk factors accounted for 90% of the risk in men and 94% of the risk in women. The significant positive risk factors were: (1) elevated apolipoprotein (apo) apoB/apoA-I ratio (relative risk 3.25), (2) smoking (relative risk 2.87), (3) psychosocial stress (relative risk 2.67), (4) diabetes (relative risk 2.37), (5) hypertension (relative risk 1.91), and (6) obesity (relative risk 1.62), while the three significant negative risk factors were: (1) daily intake of vegetable and fruits (relative risk 0.70), (2) regular physical activity (relative risk 0.86), and alcohol intake (relative risk 0.91) [17]. ApoB is the major protein of LDL, while apoA-I is the major protein of HDL. In INTERHEART, apoB and apoA-I were measured instead of total cholesterol, triglycerides, and HDL-C.

C. Dietary Intervention Studies

The most compelling data justifying any treatment strategy remain results from large-scale randomized trials. Surprisingly, the number of dietary intervention studies to examine CHD risk reduction have been rather limited, in part because such trials are much more labor inten-

sive and difficult to carry out than the placebo-controlled trials with pills.

C1. Oslo Diet Heart Studies

The first significant study was the Oslo Diet Heart Study I in which 412 men were equally randomized to the standard Norwegian diet or a diet low in animal fat (8.5% of calories as saturated fat), but rich in vegetable oil (21% of calories as polyunsaturated fat and 10% of calories as monounsaturated fat) for a total of 5 years [25]. Group dietary counseling was provided over the 5-year period, and the intervention group had a 33% decrease on myocardial infarction ($p<0.05$) at 5 years, and a 44% decrease in mortality from myocardial infarction after 11 years of follow-up as compared to the control group [25, 26]. Oslo Diet Heart Study II was performed by Hjermann and colleagues which enrolled 1232 men with elevated blood cholesterol values (290–380 mg/dl), none of whom had CHD, but 80% of whom were cigarette smokers [27]. Subjects were randomized to usual care or dietary advice and smoking cessation program for 5 years. The dietary advice focused on replacing animal fat with vegetable oil. At an average follow-up of 60 months, the risk of fatal and nonfatal myocardial infarction and sudden death was reduced by 47% ($p=0.028$), and after 102 months of follow-up there was a significant reduction ($p<0.05$) in total mortality [28, 29]. Most of the benefit in the study was related to dietary change and a 10% reduction in total cholesterol levels, because the smoking cessation rates of 25% in the intervention group versus 17% in the control group were only marginally different [28, 29].

C2. Los Angeles Veterans Administration Study

Another important dietary intervention study done at that time was the Los Angeles Veterans Administration (VA) Study, in which 846 men living in the Los Angeles domicile were randomized equally to their usual diet ($n=422$) or an experimental diet ($n=424$) in which saturated fat was replaced by vegetable oil (corn, cottonseed, safflower, and soybean), as part of a diet was contained approximately 40% of calories as total fat in both groups [27]. As compared to the usual diet with 18 % of total calories as saturated fat and 5 % as polyunsaturated fat, the experimental diet contained 11% of total calories as saturated fat, and 16% of total calories as polysaturated fat. Over an average follow-up of 6.5 years, there was a 13% reduction in total cholesterol levels in the treatment group, and a significant ($p<0.01$) 31% decrease in the end points of myocardial infarction, CHD mortality, and other cardiovascular end points (stroke, ruptured aneurysms, and ischemic gangrene) in the treatment group versus the control group [30, 31]. There was a 20% reduction in the primary end point of myocardial infarction and sudden death in favor of the treatment group, but this did not reach statistical significance [27, 30]. However, the authors subsequently reported higher cancer rates in the intervention group [31], as well as a more than twofold increased risk of the presence of gallstones at autopsy (34 vs. 14%, $p<0.01$) [32]. It is known that diets high in polyunsaturated fats will enhance liver biliary cholesterol content, thus promoting cholesterol gallstones. However, the connection with cancer remains unclear.

C3. Finnish Mental Hospital Study

In this landmark study, 5115 men and women in mental hospital N and 5497 men and women in mental hospital K in Helsinki were placed on either an experimental diet (hospital N) or the usual Finnish diet (hospital K) between 1959 and 1965. Between 1965 and 1971, the hospitals were crossed over with hospital N subjects receiving the usual Finnish diet and hospital K receiving the experimental diet. The goal was to replace the dairy and butter fat in the diet in the usual Finnish diet by using skimmed milk "filled" with soybean oil instead of full-fat milk and replacing butter with margarine high in soybean oil [33–35]. Both diets contained about 2800 calories with approximately 110 g of fat (35% of calories). However, the usual Finnish diet contained about 19% of calories as saturated fat, and about 4.5% as polyunsaturated fat, with 480 mg of cholesterol/day. For the experimental diet, these parameters were about 9% S and 14% P, with 280 mg of cholesterol per day, respectively.

In subsets of individuals, the fatty acid content of adipose tissue for linoleic acid and myristic acid were about 10 and 4.3% on the usual diet and about 30 and 1.5% on the experimental diet, respectively. Mean CHD mortality rates were significantly ($p=0.002$) lower by 53% at 6.6/1000 men per year on the experimental diet than on the usual diet at 14.1/1000 men per year. For hospital K these rates were 50.6% lower on the experimental diet versus the usual Finnish diet, while for hospital K these rates were 56.1% lower. Blood cholesterol levels were also significantly lower by 12% for hospital K (236 vs. 268 mg/dl) and by 19% for hospital N (216 vs. 267 mg/dl) on the experimental diet than the usual diet [33–35]. Similar effects were seen in women with a 34% mean reduction in CHD mortality rates in favor of the experimental diet group, but these differences did not reach statistical significance, in part because of substantially lower event rates in women overall as compared to men of similar ages [35].

C4. Minnesota Mental Hospital Study

In this open-label randomized study, 9057 men and women of all ages at six mental hospitals and one nursing home in Minnesota were placed on diets containing about 40% fat, but with different polyunsaturated fat content (5 vs. 15%), saturated fat (18 vs. 9%), and dietary cholesterol (466 vs. 166 mg/day) [36]. The treatment group had 14% lower serum cholesterol levels, but no significant difference in CHD morbidity or mortality was noted between groups [36]. The negative result may have been due to the relatively normal mean serum cholesterol of the study population at 207 mg/dl at baseline, the relatively young age of the study population, with the largest single age group being less than 30 years, and the relatively short duration of subjects actually being at the test diets (mean 384 days) [36]. The reason for this shorter duration was discharges from the mental hospital in part due to the introduction of the medication thorazine.

C5. The Lyon Diet Heart Trial

This trial was a secondary prevention study in 605 men and women who had a prior myocardial infarction and were randomized to a usual French diet or a more "Mediterranean diet" in which all subjects also received two servings per day of a specially prepared high α-linolenic acid margarine [37]. After a 44-month average follow-up, the subjects in the diet group had a 76% decrease in cardiac deaths (with 6 deaths in the treatment group and 19 deaths in the control group, $p<0.01$) [37]. The benefit in this trial was related to increases in levels of plasma α-linolenic acid [37].

C6. The Women's Health Initiative

The largest dietary intervention trial to have ever been run using dietary modification instead of supplements was the Women's Health Initiative. In this trial, 48,835 postmenopausal women aged 50–79 years were randomly allocated (40% of total or 19,541) to a diet low in fat versus the usual diet (60% of total or 29,294). All subjects in the control group received a copy of "Dietary Guidelines for Americans." The dietary intervention was implemented by group classes and individual interview sessions including dietary assessments using food frequency questionnaires [38]. The goals of the intervention were to decrease total fat intake to 20% of calories, and increase the intake of vegetables and fruits to five servings/day and grains to six servings/day [39]. The study subjects in the active group at 6 years of follow-up had a total fat intake of 28.8% of calories (vs. 37.0% in the control group), saturated fat intake of 9.5% (vs. 12.4% in the control group), monounsaturated fat intake of 10.8% (vs. 14.2% in the control group), and polyunsaturated fat intake of 6.1% (vs. 7.5% in the control group) [40]. They had increased their vegetable and fruit servings by 1.1 servings/day and their grain intake by 0.5 servings/day [41]. One of the confounding features of the study was that of those participating in the active dietary arm, 8052 women also participated in the hormone replacement arm of the Women's Health Initiative and 5017 participated in the calcium and vitamin D arm of this study [41].

The primary aim of the study was to ascertain whether a low-fat diet would reduce the risk of breast cancer. Over 8.1 years of follow-up,

0.42% per year developed breast cancer in the diet group versus 0.45% per year in the control group [39]. Therefore, subjects in the active diet group lowered their risk of developing an invasive breast cancer by 9% (hazards ratio 0.91, confidence interval (CI) 0.83–1.01, $p=0.07$) [39]. The investigators also assessed the impact of the diet intervention on CVD[41]. After 8.1 years of follow-up the risk of CHD was reduced by 3% (hazards ratio 0.97, CI 0.90–1.06) and the risk of stroke was increased by 2% (hazards ratio 1.02, CI 0.90–1.15) [41]. The dietary intervention also had no significant impact on risk of colorectal cancer or the development of diabetes [39–42]. The diet group did have statistically significant ($p<0.05$) 3.55 mg/dl lower LDL-C, 0.31 mmHg lower systolic blood pressure, and 4.29% lower factor VIIC values than the control group [41]. Moreover, in a subgroup analysis of those women who achieved <6.1% of calories as saturated fat, their risk of CHD was reduced 19% (hazards ratio 0.81, CI 0.69–0.95) [41]. Such differences were also observed in those subjects in the diet group who had the lowest *trans*-fatty acid intake (hazards ratio 0.81, CI 0.69–0.95) [41]. The major issue with the Women's Health Initiative was the emphasis on total fat reduction, rather than reduced saturated fat, and increasing omega-3 and omega-6 fatty acids.

C7 Diet Atherosclerosis Treatment Trials

Studies in the UK have documented beneficial effects of fish consumption or the use of two fish oil capsules per day in reducing CHD death by 29% in more than 2000 patients with established CHD [43]. However, this was not confirmed in a follow-up study, possibly because of much greater aspirin use in the second study [44].

C8 Gruppo Italiano per lo Studio della Soppravvivenza nell'Infarto miocardico (GISSI)-Prevenzione

In GISSI-Prevenzione, a large Italian study of 11,323 post-myocardial infarction patients, the use of 1 g per day of concentrated fish oil (containing 465 mg of eicosapentaenoic acid or EPA and 375 mg of docasahexaenoic acid or DHA) was associated with a reduction in overall recurrence of CHD, and a very striking 53% reduction in sudden death in the first 4 months after myocardial infarction in those receiving the active supplement versus the control group [45, 46]. This product is now marketed in the USA as a triglyceride-lowering agent known as Lovaza™ given at 4 g per day, and this product will lower triglycerides significantly (up to 50% or more) on top of statin therapy in patients with triglycerides >500 mg/dl [47]. A pure EPA product known as Vascepa™ has also come on the market as a prescription item approved by the Food and Drug Administration for triglyceride lowering at 4 g/day. It should be noted that these products are expensive, and similar efficacy for triglyceride lowering can be achieved with over-the-counter fish oil capsules at a dose of 6 g/day.

C9 Japan Eicosapentaenoic Acid Lipid Intervention Trial

Japan Eicosapentaenoic Acid Lipid Intervention Trial (JELIS) was designed to test the hypothesis that 1800 mg/day of pure EPA would reduce CVD risk in Japanese subjects who had elevated baseline total blood cholesterol of more than 250 mg/dl [48]. In this study, 15,000 subjects without CHD (4204 men and 10,796 women) and 3645 subjects with CHD (1656 men and 1989 women), between 40 and 75 years of age, were all placed on statin and then randomized in an open-label, end point-blinded manner to an EPA 1800 mg/day group or a control group. The primary end point was major cardiovascular events (sudden death, fatal or nonfatal myocardial infarction, unstable angina, angioplasty, or coronary artery bypass surgery). After 4.6 years of follow-up, there were 9326 who received EPA and 9319 in the control group, and 262 events (2.8%) were observed in the EPA group versus 324 events (3.5%) in the control group (relative risk reduction of 19%, $p=0.011$) [48]. No significant differences in sudden death rates between the groups were noted, although the overall rates were low.

In the patients with a history of prior CHD, events were also reduced by 19% (event rates of 8.7 vs. 10.7%) by EPA versus no treatment ($p=0.048$). The number needed to treat to prevent one CVD event was low at 49 subjects [49]. In 1050 subjects

with a history of prior myocardial infarction, risk of subsequent CHD events was reduced by EPA by 27% from 20.0 to 15.0%, $p=0.033$, with the number needed to treat to prevent one event being only 19 [50]. Risk reduction in subjects without CHD was 18% with event rates of 1.4 versus 1.7% ($p=0.132$) [48]. Use of EPA in JELIS was not associated with a significant reduction in stroke (1.3 vs. 1.5%) for the entire cohort [50]. However, for those subjects with a prior stroke, the use of EPA was associated with a 20% relative risk reduction in recurrent stroke (6.8 vs. 10.5%, $p<0.05$) [50].

The most striking effect on CHD risk reduction benefit was noted in those subjects with triglyceride levels >150 mg/dl and HDL-C levels <40 mg/dl [51]. In this group, the risk of developing CHD on trial was increased 1.71 as compared to controls, and the use of EPA in this group reduced CHD events by 53% ($p=0.043$) [51]. A subgroup analysis of JELIS was also carried out in subjects with impaired glucose tolerance (fasting glucose >110 mg/dl) [52]. In this group, the CVD risk was increased 1.63 versus the controls, and EPA reduced their risk by 22% ($p=0.048$), versus 18% in the normal glucose group ($p=0.048$). The use of statin resulted in a 25% mean reduction in LDL-C level as compared to baseline, but the use of EPA was not associated with any significant effects on lipid levels [47–51, 53]. A modest 5% reduction in fasting plasma triglyceride levels was noted. Most recently, the JELIS investigators reported in a subanalysis based on 15,534 patients that plasma EPA, but not DHA levels, were related to overall CVD risk on trial (hazards ratio 0.83, $p=0.049$ for the entire group, and hazards ratio 0.71, $p=0.018$ for the intervention group) [54]. Patients with plasma EPA levels >150 μg/ml had the lowest risk, while those with levels <0.87 μg/ml had the highest risk [54]. These data indicate that EPA at a dose of 1800 mg/day is effective in reducing major CVD events in patients with prior CHD and stroke, as well as those with impaired glucose tolerance, and especially in those with dyslipidemia. Moreover, these effects were independent of LDL lowering or HDL raising, but did relate to changes in plasma EPA levels [48–54].

C10. Alpha Omega Trial

A more recent study of 4837 post-myocardial infarction patients randomized to placebo margarine, margarine containing 2 g of alpha linolenic acid, margarine containing a total of 400 mg of combined EPA and DHA, or a margarine containing the combination of these fatty acids was carried out over 40 months [52]. No significant effects were noted on CVD end points. However, this study may have been underpowered, and the dose of omega 3 fatty acids given may have been too low.

C11. Primary Prevention of CVD with a Mediterranean Diet

In this recent trial, 7447 subjects at high risk for CVD, but without CVD, were enrolled (age range, 55–80 years, 57% were women) in a randomized multicenter trial in Spain [55]. Subjects were placed on one of three diets: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet (advice to reduce dietary fat). Participants received quarterly individual and group educational sessions and, depending on group assignment, free provision of extra-virgin olive oil, mixed nuts, or small nonfood gifts. The primary end point was the rate of major cardiovascular events (myocardial infarction, stroke, or death from cardiovascular causes). On the basis of the results of an interim analysis, the trial was stopped after a median follow-up of 4.8 years. The two Mediterranean-diet groups had good adherence to the intervention, according to self-reported intake and biomarker analyses. The primary end-point event (fatal and nonfatal CVD events) occurred in 288 participants. The multivariable-adjusted hazard ratios were 0.70 (95% CI, 0.54–0.92) and 0.72 (95% CI, 0.54–0.96) for the group assigned to a Mediterranean diet with extra-virgin olive oil (96 events) and the group assigned to a Mediterranean diet with nuts (83 events), respectively, versus the control group (109 events). No diet-related adverse effects were reported. The conclusion of the authors was that among persons at high cardiovascular risk, a Mediterranean diet supplemented with extra-virgin olive oil or nuts reduced the incidence of

major cardiovascular events [55]. This randomized trial clearly supports the daily use of about 30 g/day of either almonds or walnuts, or the daily consumption of 143 mL of olive oil (almost 10 tablespoons per day).

C12. Conclusions from Dietary Interventions Trials

The overall data from the dietary intervention studies support the concept of decreasing saturated fat to <7% of calories, dietary cholesterol to <200 mg/day, and increasing polyunsaturated fatty acids to >10% of calories (ideally about 12%). In the Women's Health Initiative, the women in the control group were consuming 14% of calories as monounsaturated fat, 12.5% as saturated fat, and 7.5% of calories as polyunsaturated fat. Benefit was noted if they got their saturated fat intake to <6.1% of calories [41]. However, in the most successful dietary intervention studies such as the Finnish Mental Hospital Study saturated fat was replaced by polyunsaturated fat and not carbohydrate [33–35]. Therefore, if the women in the Women's Health Initiative had gotten significant increases in their intake of polyunsaturated fatty acids from vegetable oil such as soybean oil, canola oil, or olive oil, then they would probably have gotten a much greater benefit in terms of CHD risk reduction [41].

The ideal diet for CHD risk reduction may well be one containing <7% of calories as saturated fat, <200 mg of cholesterol/day, with about 10–20% of calories from monounsaturated fat such as canola or olive oil, and about 10–15% of calories as polyunsaturated fat from vegetable oils such as soybean or canola oil, along with three or more servings of oily fish per week or two fish oil capsules per day. Under controlled conditions, such diets will lower LDL-C by 15% or more, associated with enhanced LDL-apoB fractional catabolism. With the addition of almost daily servings of fish, triglyceride levels are also lowered, associated with decreased very low-density lipoprotein (VLDL) apoB production [1]. It should be noted that large randomized placebo-controlled trials have not shown any significant benefit in terms of CHD risk reduction associated with the use of vitamin E, vitamin C, a mixture of antioxidant vitamins, the potent antioxidant probucol or analogues, or the combination of folate, and vitamins B6 and B12 [56–60].

The following dietary changes are clearly justified by the intervention trial data to reduce CHD risk:

1. Replace butter with two servings per day of soft, *trans*-fat-free margarine made from soybean oil or canola oil.
2. Use vegetable oils for salad dressing (soybean, canola, or olive oil) and cooking (canola or olive oil).
3. Replace beef and pork with chicken or turkey (white meat, remove skin) or fish.
4. If you do not regularly use fish, consider using two fish oil capsules/day.
5. Minimize *trans* fat intake.
6. Replace eggs with egg whites.
7. Replace whole milk with skimmed or 1% fat milk.

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Phytosterol Therapy

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What Are Phytosterols?

Phytosterols, the general name for plant sterols and plant stanols, are bioactive compounds present in different foods of plant origin. Plant stanols are 5-α-saturated derivatives of plant sterols (Fig. 20.1). This minor structural difference transforms their metabolism in humans so that plant stanols are biologically different substances from plant sterols. Even different plant sterols and different plant stanols have their own metabolic characteristics. Phytosterols have similar function in plants as cholesterol has in humans. Compared with cholesterol, plant sterols and plant stanols have a different side chain structure, and in plant stanols the sterol ring is saturated (Fig. 20.1). These structural changes make the three compound groups completely different functionally and metabolically. Phytosterols are normal components of plants, but they are not synthesized in the human body. They are present in vegetable foods, especially in vegetable oils, and in seeds, nuts, and cereals. The main food sources of phytosterols are vegetable oils, vegetable-fat spreads and margarines, cereals and cereal products (bread), and vegetables. These sources contribute to 50–80% of the total daily phytosterol intake [1, 2]. The role of fruits as the phytosterol source is small, around 12% of the total daily phytosterol intake. The mean daily intake of plant sterols in normal Western diet is about 300 mg [1, 2]. The most abundant plant sterols in human diet are sitosterol and campesterol. Sitosterol contributes to 60–66% of total phytosterol intake and campesterol contributes ~22%. The amount of stanols in plants is much smaller than the plant sterols, so that the amount of plant stanols in diet is only about 13–20 mg/day [1, 2], representing 4–8% of the total phytosterol intake [2]. The most common plant stanols are sitostanol and campestanol.

Plant sterols have a low absorption percentage varying from 0.5 to 2%, and the absorption percentage of plant stanols is even lower, about 0.04–0.2% [3]. Because of the low absorption efficiency, the serum levels of plant sterols are low and vary in general population from 3 to 21 μmol/l, and those of plant stanols are even lower and vary from 0.05 to 0.3 μmol/l, respectively. Phytosterols are also delivered to different tissues and cells. In the carotid artery, e.g., the concentration of plant sterols was ~10 mg/100 g of tissue, and that of plant stanols only ~0.3 mg/100 g of tissue, whereas the concentration of cholesterol was several-fold, ~2000 mg/100 g of tissue [4, 5]. The tissue levels of plant sterols are related to their respective serum levels suggesting that the higher the serum

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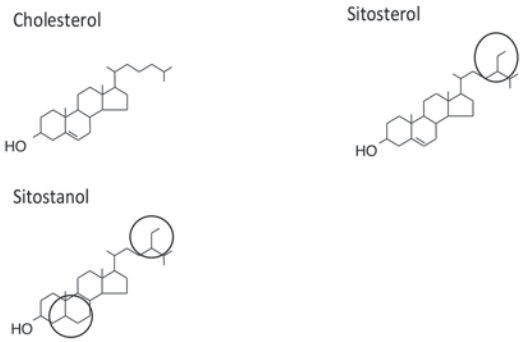

Fig. 20.1 The structure of cholesterol, sitosterol, and sitostanol. The circles in sitosterol and sitostanol demonstrate their difference in structure from cholesterol

plant sterol levels, the higher their tissue levels [4–7].

Phytosterols have been consumed always. In 1000–2000-year-old human coprolites found in dry caves of Nevada, the amount of fecal plant sterols was comparable to that of people today [8]. On the other hand, in a Greenland Eskimo mummy from AD 1475, the amount of fecal plant sterols was only 0.4 % of that of the Americans at present suggesting that the dietary intake of plant sterols varied a lot between ancient populations.

Since the early 1950s, the hypolipidemic effect of large doses of phytosterols has interested scientists as a hypocholesterolemic nonpharmacological means. The cholesterol-lowering effect is based on the partial inhibition of cholesterol absorption [9]. Consuming about 2 g of phytosterols daily, the absorption efficiency of cholesterol is reduced approximately to one half, and cholesterol excretion in feces is increased by 40 %.

In the early 1980s, large doses, up to 15 g/day of crystalline unesterified, poorly soluble plant sterols were used. The concern with large plant sterol doses was the increase of serum and tissue plant sterol levels, even though the clinical relevance of, e.g., the increase of phytosterols in arterial endothelium is still open. Since plant stanols are minimally absorbed, the discovery that free plant stanols lowered cholesterol in experimental animals [10] started a new era of phytosterol research. Fat-soluble plant stanol esters added to food products were introduced to the research field in 1991 as part of a heart healthy diet [11]. The more soluble esterified forms were considered more physiologic in the intestinal milieu than the crystalline ones, and today most of the phytosterols added to food products are in esterified form. The plant sterol and plant stanol dose could also be reduced to 2 g/day from the earlier large unesterified plant sterol doses. However, the efficacy of new formulations of phytosterols needs to undergo specific clinical testing, since the physical dispersion of the phytosterols is important in determining their lipid-lowering efficacy.

The first food product enriched with phytosterols was plant stanol ester margarine launched to market in 1995. Other products followed later on, and at the moment several food products varying from mini drinks to bread and containing esterified or nonesterified plant sterols or plant stanols or their mixture are available worldwide. The amount of naturally occurring phytosterols in regular diet is not large enough to reduce LDL cholesterol level [12], but an added dose of 2 g/day of phytosterols in food items result in a clinically relevant 10 % LDL cholesterol lowering. Consequently, several international recommendations [e.g., ref 13] have included phytosterols as a dietary means to lower serum total and LDL cholesterol concentrations in primary prevention and in secondary prevention combined with hypocholesterolemic drugs.

Introduction to Cholesterol-Lowering Mechanism of Action of Phytosterols

Cholesterol in the small intestinal lumen originates from diet, bile and, to a lesser extent, intestinal epithelial sloughing. The absorption of cholesterol is defined as the transfer of luminal cholesterol into intestinal and thoracic duct lymph. The major intestinal segments involved in cholesterol absorption are the duodenum and the proximal jejunum. Intestinal cholesterol absorption efficiency is influenced by several factors including (1) composition of diet (e.g., amount of phytosterols), (2) secretion and composition of the bile (crucial for the formation of intestinal

mixed micelles), (3) luminal factors in the gastrointestinal tract (e.g., intestinal passage time), (4) cellular factors (e.g., the epithelial sterol transporters), and (5) pharmacological interventions (e.g., ezetimibe). Cholesterol absorption itself is a multistep process, in which the water-insoluble cholesterol molecule has to be emulsified, hydrolyzed (if esterified) by pancreatic esterase, solubilized into intestinal mixed micelles, penetrated through the intestinal diffusion barrier, absorbed by the enterocytes, re-esterified within the enterocytes, and transferred to the lymph. The intestinal diffusion barrier is determined as an unstirred water layer and a surface mucous coat covering the apical membrane of the enterocyte.

The mean cholesterol absorption efficiency among healthy human subjects ranges mainly from 40 to 60% [11, 14]. For comparison, the respective values of absorption of dietary plant sterols campesterol (~1.9%) and sitosterol (~0.5%) are very low in short-term studies [3, 14]. Campestanol and sitostanol have much lower absorption efficiencies (below 0.2%) than the respective plant sterols [3]. Absorption efficiency of sterols and stanols appears to decrease with increasing molecule size from cholesterol to sitosterol and with saturation of the double bond at C-5.

Despite intensive experimental research during recent decades, the exact mechanisms how dietary phytosterols exert their serum LDL cholesterol-lowering influence are not fully solved, and, furthermore, the data concerning the putative metabolic and genetic factors modifying the subject specific LDL cholesterol responses during the phytosterol therapy are mostly suggestive.

Influence of Phytosterols in the Small Intestinal Lumen

In the intestine, several putative mechanisms for the inhibitory effect of phytosterols on cholesterol absorption have been suggested: (i) displacing cholesterol from mixed micelle, i.e., the micelle theory, (ii) competitive blocking of cholesterol absorption from intestinal contents, (iii) co-crystallizing cholesterol and plant sterols to form insoluble crystals, (iv) promoting cholesterol efflux from enterocytes back into the intestinal lumen, (v) decreasing cholesterol-re-esterification rate in the intestinal epithelium, (vi) affecting sterol transport in the enterocytes and hepatocytes, (vii) modifying the expression of the genes encoding sterol transporter proteins, and (viii) increasing cholesterol removal from the body via the transintestinal cholesterol efflux (TICE) pathway [15–18].

The identification of the intestinal and hepatic sterol transporter proteins has helped to understand the metabolism of phytosterols, and their influence on cholesterol metabolism (Fig. 20.2a). The uptake of cholesterol and phytosterols by the enterocyte is a rapid process, and it has been suggested to be mediated by several transporter proteins, of which the Niemann–Pick C1-like 1 (NPC1L1) transporter is the most important. Opposite to the action of the NPC1L1 protein, the adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transporters G5 and G8 are involved in the reverse transport of phytosterols and, to a lesser extent, cholesterol, back from the enterocytes into the gut lumen [14]. This explains the discrimination in the absorption process between cholesterol and phytosterols. Furthermore, ABCG5/G8 transporters are located at the canalicular membranes of the hepatocytes, where they promote efflux of cholesterol, and particularly that of phytosterols into the bile (Fig. 20.2b). The efficiency of the ABCG5/G8 transporters to transfer sterols has a decreasing order: sitosterol—campesterol—cholesterol [14]. Thus, the ABCG5/G8 transporters at the intestinal and hepatic levels seem to be responsible for the differences between serum concentrations of cholesterol and different phytosterols. The evidence above deals with plant sterols, but there is indirect evidence suggesting that plant stanols are using the same transporter mechanisms as the plant sterols. Accordingly, in phytosterolemia serum plant stanols together with plant sterols are increased suggesting that the ABCG5/G8 transporters promote efflux of plant stanols also [19]. Furthermore, ezetimibe not only reduced the serum plant sterol levels but also those of plant stanols suggesting that plant stanols are also transported to the enterocyte using the NPC1L1 transporter.

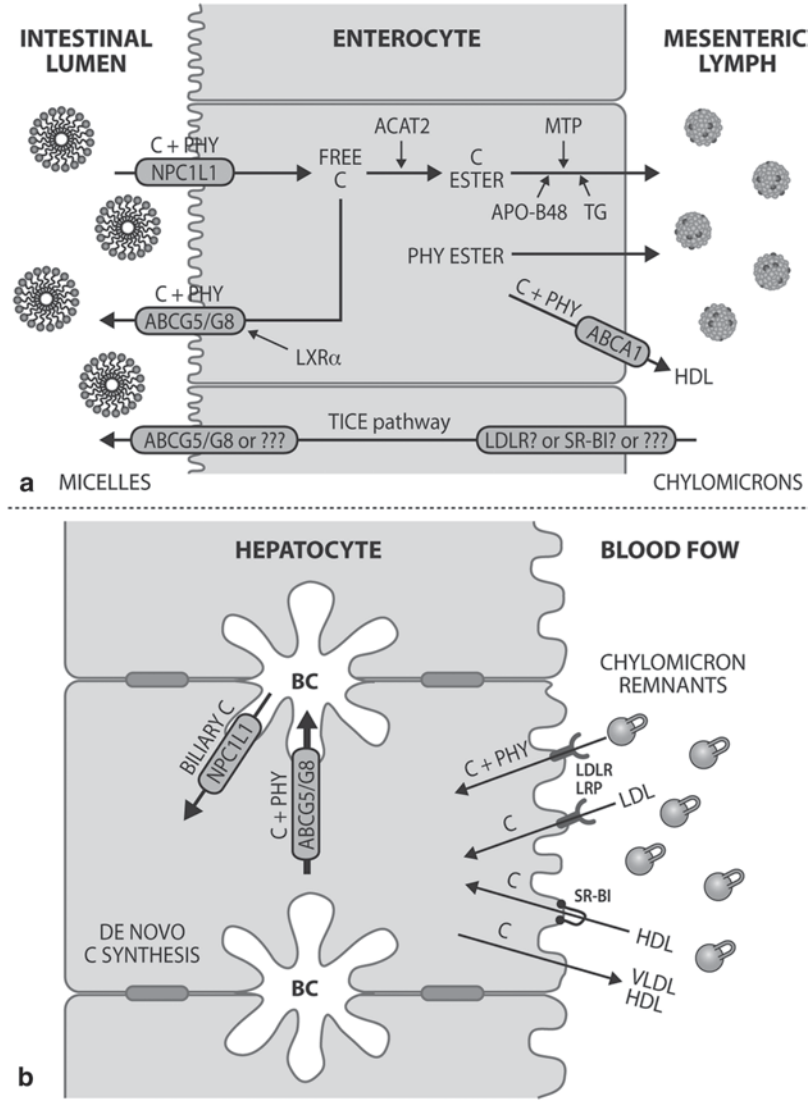

Fig. 20.2 Simplified schematic overview of the major metabolic pathways of cholesterol and phytosterols metabolism in the intestinal epithelium (**a**) and the hepatocyte (**b**). After uptake of cholesterol and phytosterols by the enterocyte, cholesterol is mainly esterified in a reaction catalyzed by acyl CoA: cholesterol acyltransferase 2 (ACAT2) with fatty acids to form cholesteryl esters. Cholesteryl esters can then be secreted into lymph after their packaging into apoB48-containing chylomicrons with the help of microsomal triglyceride transfer proteins (MTP). Small amounts of phytosterols are also esterified and secreted into the lymph. The unesterified sterols including most of the phytosterols are excreted back into the intestinal lumen by the ABCG5/G8 sterol transporter. Activation of LXRα up-regulates expression of ABCG5/G8 sterol transporters. ABCA1 may mediate cholesterol and phytosterol transport to plasma high-density lipoprotein (HDL). The TICE pathway is an important non-biliary route for cholesterol removal from the body. The mediators responsible for cholesterol trafficking in the basolateral and brush border membrane of the enterocytes have been poorly identified.NPC1L1 and ABCG5/G8 sterol transporters are also located in the canalicular membrane of the hepatocytes. The function of these two sterol transporters in the hepatocytes has been suggested to resemble that of enterocytes, so that the former transports sterols into cells and the latter transports sterols out of cells. In the hepatocytes, cholesterol, but not phytosterols, can be synthesized locally. Cholesterol can be taken up by hepatocytes from circulating plasma lipoproteins and chylomicron remnants via respective receptors: LDL receptor (LDLR), HDL receptor scavenger receptor class B type I (SR-BI), and LDLR-related protein (LRP). Of these, the chylomicron remnant route is the most important for

Of the different theories on the mechanism of action of dietary phytosterols to decrease serum LDL cholesterol levels, the micelle theory has gained good experimental support [20–24]. Intestinal mixed micelles are globular aggregates of bile acids, fatty acids, monoacylglycerides, and lysophospholipids. They serve as concentrated reservoir and carrier unit for dietary and biliary cholesterol through the intestinal diffusion barrier onto the surface of the enterocyte. The mixed micelles serve cholesterol and phytosterols to NPC1L1 and other putative sterol transporters of the brush border membrane of the enterocytes in the upper part of the human small intestine. The amount and degree of esterification of dietary phytosterols affect the sterol composition of duodenal mixed micelles. Due to better fat solubility (e.g., in margarines and mayonnaise) of esterified derivatives of plant stanols and sterols as compared with crystalline or poorly soluble ones, they are particularly effective in inhibiting intestinal cholesterol absorption. The results of intestinal plant stanol ester perfusion studies among healthy human subjects support the view that at high intestinal plant stanol concentrations, cholesterol loses its micellar solubility by replacement of its free fraction in the micellar phase by hydrolyzed phytosterols, which consequently leads to a decreased intestinal absorption of cholesterol [21, 22]. Obviously, the micellar replacement occurs, because intestinal hydrolysis of esterified phytosterols occurs rapidly, and phytosterols have higher hydrophobicity than cholesterol. The intestinal perfusion studies also indicate that diacylglycerol oil-based products are not superior to traditional triacylglycerol oil-based ones as intestinal carriers regarding hydrolysis/esterification of administered plant stanol esters and cholesterol and their partition in oil, micellar, and sediment phase in the proximal jejunum. The intestinal perfusion studies were performed with free and esterified plant stanols, but most probably the respective plant-derived Δ^5-sterols might have given similar results. In summary, the present data indicate that the micelle theory comprises the principal mechanisms of action of phytosterols in lowering both intestinal absorption of cholesterol and serum level of LDL cholesterol. At the moment, there is no evidence of a cellular mechanism by which the phytosterols could interfere with cholesterol absorption.

Sterol Transporter Proteins and Dietary Phytosterols

Intervention studies with phytosterols have revealed that study subjects have different subject-specific lowering of their serum LDL cholesterol levels. Some subjects have good LDL cholesterol-lowering response to phytosterol therapy, whereas other subjects, “non-responders,” have no response or a suboptimal response, and, in some subjects, “adverse responders,” serum LDL cholesterol level even increases during the phytosterol therapy [25]. Several subject-specific factors affecting the efficacy of phytosterol therapy have been identified including: (1) metabolic, (2) disease associated, (3) environmental, and (4) genetic factors.

Of the metabolic factors in general, low basal fractional cholesterol synthesis rate, and high cholesterol absorption efficiency, as measured, e.g., by baseline serum ratios of campesterol to cholesterol, are associated with good response to phytosterol therapy. The whole picture is not as

phytosterols. Cholesterol, if not stored in the hepatocyte, can be secreted into blood via the very-low-density lipoprotein (VLDL) or HDL route. Alternatively, cholesterol can be secreted into bile as bile acids via bile salt export pumps or as free cholesterol via ABCG5/G8 sterols transporters. The ABCG5/G8 sterol transporters are much more effective in mediating the hepatobiliary secretion of phytosterols than that of cholesterol. Overall, one of the rationalities in the function of the ABCG5/G8 sterol transporter may be to protect the human body from excessive accumulation of dietary phytosterols. In human subjects, NPC1L1 is expressed in the liver with lower expression than in the small intestine. The obvious role of NPC1L1 in the liver is to counterbalance hepatobiliary cholesterol excretion.ABCA1 intestinal ABC transporter, ACAT2 acyl-CoA: cholesterol acyltransferase 2, BC biliary canaliculus, C free cholesterol, CE cholesteryl ester, HDL high-density lipoprotein, LDLR low-density lipoprotein receptor, LRP LDLR-related protein, LXRα Liver X receptor α, PHY phytosterols, MTP microsomal triglyceride transfer protein, SR-BI HDL receptor scavenger receptor class B type I, TICE transintestinal cholesterol efflux

clear as, contrary to that theory, baseline cholesterol absorption, and synthesis does not predict responsiveness to LDL cholesterol-lowering drugs, e.g., ezetimibe and simvastatin [26]. The putative role of genetic polymorphism in the LDL cholesterol-lowering efficacy of phytosterols has been challenging to resolve, because influx and efflux of sterols at the intestinal and the hepatic level are under polygenic control. Experimental animal studies applying global assessment of gene expression patterns in response to phytosterol treatment suggest that transcriptional changes in ABCA1, ABCG5, ABCG8, and NPC1L1 do not play an essential role in the phytosterol-induced reduction in cholesterol absorption [15].

A recent meta-analysis exploring the associations of *ABCG5* or *ABCG8* polymorphisms to cholesterol metabolism among hypercholesterolemic human subjects indicates that the *ABCG8* p.632V variant is related to a minor, clinically irrelevant LDL cholesterol reduction, and that the 19H allele correlates with decreased cholesterol absorption and increased synthesis without affecting the lipid profile [27]. Overall, Jakulj and coworkers [27] conclude that, in the study cohorts of their meta-analysis, associations between frequently studied missense ABCG5/G8 polymorphisms and markers of cholesterol homeostasis are modest at best [27]. However, results of a genomic approach study show that common variants in ABCG8 and the blood group ABO are associated with serum phytosterol levels, and suggest concordant associations with coronary artery disease [28].

Serum cholesterol lowering with long-term absorption inhibition by plant stanol or sterol ester consumption (2 g/day) was not associated with polymorphic sites of ABCG5 and ABCG8 in a group of 282 mildly to moderately hypercholesterolemic subjects [29]. However, regulation of baseline cholesterol metabolism and vascular function and structure, and the intima media thickness of the carotid artery progression during 1 year were related to some of the common polymorphic sites of these genes, suggesting that low cholesterol absorption and high synthesis as part of the metabolic syndrome is unfavorable regarding the progression of intima media thickness [29]. Plant stanol or sterol esters had no role in this regulation.

TICE Pathway

The enterocytes may mediate the transport of cholesterol from the blood to the intestinal lumen in the TICE pathway [18, 30]. It has been estimated that in human subjects, approximately 20–30% of the endogenous cholesterol in the intestinal lumen could originate from the TICE pathway [18]. Interestingly, the TICE pathway seems to be sensitive to stimulation, e.g., by activation of the liver X receptor (LXR). It is not known which donor particles deliver cholesterol for secretion via TICE. Recent experimental studies in mice suggest that plant sterol feeding results in stimulation of cholesterol excretion via the TICE pathway, and that this cholesterol flux is partly mediated by the ABCG5/G8 transporters [31]. Overall, the TICE pathway seems to be sensitive to manipulation by pharmaceutical drugs and dietary constituents giving intriguing pharmacologic and dietary options to accelerate reverse cholesterol transport from the body.

Effects on Serum Lipids

The pioneer 1-year study of added phytosterols in food products was published in 1995 [32]. The results demonstrated that consuming 1.8–2.6 g of plant stanols daily as esters in margarine lowered LDL cholesterol by 14% compared with controls, and the effect was sustained throughout the 1-year intervention. No side effects were reported. After this, an enormous scientific interest in phytosterols arose. In 2003, a meta-analysis of 41 published randomized controlled studies demonstrated that the daily intake of 2 g of phytosterols reduced LDL cholesterol by 10% [33]. Also the safety of phytosterols was reviewed carefully. By that time, only a few large-dose studies were performed, so that the report's conclusion that higher intakes added little to the LDL cholesterol reduction turned out later on to be untimely.

Recently, two large meta-analyses were published including randomized controlled phytosterol trials in adults [34, 35]. The most recent one contained 114 clinical trials with 182 trial arms including about 8000 subjects [35]. The mean age of the subjects was 49.8 years with a range from 29 to 66 years. Baseline mean LDL cholesterol level was (145 mg/dL) 3.75 mmol/l with a range from 1.98 to 5.35 mmol/l (77–207 mg/dL). Two thirds of the studies dealt with plant sterols and one third with plant stanols. In over 90% of the plant stanol studies, the plant stanols were administered in esterified form, and the respective figure for plant sterol studies was 70%. In four studies, phytosterol was in tablet or capsule form. In the rest of the studies, the phytosterol was in food products and should preferably be taken with meals. The most frequent food matrix was solid nondairy matrix, i.e., margarine. The phytosterol dose varied from 0.8 g to 9 g/day.

The meta-analysis demonstrated that 2 g/day of phytosterols lowered LDL cholesterol about 9% [35]. The results were similar between esterified and nonesterified phytosterols, and they were not related to the fat content of the food format (e.g., margarine or yoghurt), or to solid or liquid food format. The results were comparable to the previous large meta-analysis [34].

However, when the plant sterol and plant stanol studies were analyzed separately, it turned out that across a continuous dose range, LDL cholesterol lowering was dose dependent for plant stanols, but not for plant sterols [35]. Accordingly, the maximal LDL cholesterol lowering with plant stanols was 16.4%, which was significantly higher than the respective reduction of 8.3% with plant sterols. These results suggest that the plant stanol effect does not level off with increasing dose, and it is possible with dietary means to reach an LDL cholesterol reduction comparable with drugs.

Regarding the other serum lipids, phytosterols have in general no effect on HDL cholesterol level, even though in a few studies there has been a slight increase in HDL cholesterol concentration. Similarly, serum triglycerides are generally unaltered in individual studies. However, in further analysis especially in subjects with elevated serum triglyceride levels at baseline and in metabolic syndrome phytosterols reduced serum triglyceride levels in relation to the baseline values [36]. Accordingly, phytosterols in addition to LDL cholesterol reduction have either no or if anything a favorable effect on HDL cholesterol and serum triglyceride levels.

Phytosterols reduce similarly the apolipoprotein B-100 and cholesterol content in LDL particles so that the particle size is unaltered. Regarding inflammation, there is inconsistent information that phytosterols may reduce highly sensitive C-reactive protein levels. While reducing serum total and LDL cholesterol levels, phytosterol consumption increases their own serum levels. During customary plant sterol-enriched margarine consumption, the serum plant sterol concentrations increased from 19 to 30 μmol/l with no change in serum plant stanol values [37]. When plant stanol-enriched margarine was customarily used, the serum plant stanol concentrations increased from 0.2 to 0.7 μmol/l, and serum plant sterols decreased by 16–23% [37]. The clinical relevance of the increased serum and tissue plant sterol levels during long-term plant sterol therapy is not known at the moment.

Phytosterols have been shown to lower serum total and LDL cholesterol levels irrespective of age, gender, and cause of hypercholesterolemia (Table 20.1). A couple of studies in healthy subjects have demonstrated that even in normocholesterolemia phytosterols are safe and without side effects [e.g., 7, 38–40]. Phytosterols have been administered with different food vehicles (Table 20.1). The type of the habitual diet of the subjects, i.e., whether high-fat Western type or low-fat diet, does not affect the hypocholesterolemic effect. The youngest child studied was of the age of 2 years (a child with familial hypercholesterolemia (FH)). Phytosterols have been studied in postmenopausal women, in old age, and most of the studies have included subjects with mild to moderate primary hypercholesterolemia.

The efficacy of phytosterols has been studied in 124 subjects with FH including children and adults, and the latter also in combination with statin treatment. With an average dose of 2.3 g/day, LDL cholesterol was reduced by

Table 20.1 Human studies with phytosterols

Subjects	Age group	Phytosterol vehicles used in different studies in general
Healthy subjects	Children	Margarine
	Adults	Mayonnaise
Primary hypercholesterolemia	Children	Salad dressing
	Adults	Butter, cheese
Familial hypercholesterolemia	Children	Milk
	Adults	Yogurt, yogurt drink
Combined hyperlipidemia	Adults	Orange or vegetable juice
Type 1 diabetes	Adults	Bread, cereal, muesli
Type 2 diabetes	Adults	Tablet, capsule
Metabolic syndrome	Adults	
Patients with renal transplant	Adults	

10–15 % [36]. Phytosterols are an important therapeutic means especially for young FH children, in whom statins are not advised.

In addition to primary hypercholesterolemia and FH, phytosterols are effective also in subjects with type 2 diabetes. A meta-analysis of phytosterols in type 2 diabetes included five randomized controlled studies in 148 subjects [41]. The phytosterol dose varied from 1.6 g/day to 3 g/day. Phytosterols significantly reduced LDL cholesterol by 9 % with a trend towards raising HDL cholesterol level, but with no effect on serum triglycerides. In type 1 diabetes, the efficacy and safety of plant stanol ester were studied in subjects without and with statin treatment. In both studies, plant stanol ester consumption significantly reduced LDL cholesterol up to 16 % compared with controls [38, 39]. An important group of hypercholesterolemic patients is the transplant recipients. The feasibility of phytosterols in this patient group has been evaluated in one randomized controlled study of 84 renal transplant recipients [42]. Thirty to 40 % of the patients had stable statin therapy. Plant stanol ester (2 g of plant stanols/day) significantly reduced serum cholesterol compared with controls, and the reduction was about 9 %. The combination therapy with statins enables to keep the statin dose as low as possible. Accordingly, phytosterols can be administered to subjects with elevated LDL cholesterol level irrespective of the cause of its elevation.

Combination Therapy

The combination of phytosterols with statins inhibits both cholesterol absorption and cholesterol synthesis and should lead to a better LDL cholesterol response than with either of the agents alone. In fact, the combination therapy is working exactly as assumed. A systematic review and meta-analysis of eight randomized controlled studies with 306 patients demonstrated that adding phytosterols to statin-treated subjects significantly lowered LDL cholesterol by 10 % from controls [43]. This reduction is larger than the average decrease of about 6 % achieved by doubling the statin dose. The phytosterol dose varied from 1.8 to 6 g/day, and the baseline LDL cholesterol level varied from 2.9 to 5.9 mmol/l (112–228 mg/dL). HDL cholesterol or serum triglyceride levels were unchanged. The combination therapy is safe and without side effects. The additional efficacy of the combination therapy has been exploited in the hypocholesterolemic treatment schedules.

In addition to statins, phytosterols can be combined with ezetimibe. Even though they both inhibit cholesterol absorption, their mechanism of action is different as discussed in Sections “Introduction to Cholesterol-Lowering Mechanism of Action of Phytosterols” and “Influence of Phytosterols in the Small Intestinal Lumen”. In two studies evaluating the combination therapy of phytosterols with ezetimibe, the results were mixed. One study was negative [44], but in the other with controlled background diet ezetimibe alone reduced LDL cholesterol by 16 %, the addition of phytosterols 2.5 g/day caused a further decrease in LDL cholesterol of 7 %, so that the net reduction was 22 % [45].

Bile acid sequestrants cause bile acid malabsorption followed by increased bile acid synthesis, increased cholesterol synthesis, and upregulated LDL receptor activity. Ultimately, LDL cholesterol is reduced. Because the mechanism of action is different from phytosterols (and from statins), the combination of these agents could

have an additive effect. In fact, when adding statin, plant stanol ester margarine and finally cholestyramine to patients with coronary heart disease whose mean baseline LDL cholesterol level was 4.5 mmol/L (174 mg/dL), each step significantly lowered LDL cholesterol, so that its ultimate concentration was 1.4 mmol/L (54 mg/dL) [46]. On the contrary, in subjects on stable statin therapy and with mean baseline LDL cholesterol concentration of 3.2 mmol/l (124 mg/dL), colesevelam hydrochloride decreased LDL cholesterol by 22%, but the addition of 2 g/day plant sterol-fortified orange juice had no additional effect on LDL cholesterol level [47]. Accordingly, the combination of phytosterols and statins has an additive hypocholesterolemic effect. There is less consistent information on the efficacy of combining phytosterols with ezetimibe or with bile acid sequestrants.

Phytosterol Therapy and Atherosclerotic Cardiovascular Diseases

Daily consumption of at least 2 g of phytosterols reduces the most important risk factor of atherosclerotic cardiovascular diseases, LDL cholesterol level, about 10%, an extent which can be assumed to lower the risk of coronary artery disease [48]. Recently, the Scientific Panel on Dietetic Products, Nutrition and Allergies to the European Commission has stated in its evaluation report, that clinically significant LDL cholesterol lowering of about 10% can be achieved by a daily intake of phytosterols 2 g/day in an appropriate food [49]. The panel considers that such a reduction is of biological significance in terms of reduced risk of coronary artery disease. In addition, in some study populations, phytosterols lower the markers of inflammation and serum triglyceride levels, and increase HDL cholesterol levels, all additional beneficial effects regarding atherosclerosis.

There are, however, no studies of phytosterol consumption and future coronary events. Nevertheless, the effect of phytosterols on subclinical atherosclerosis (intima-media thickness, flow-mediated dilatation, and arterial stiffness) has been evaluated in eight short- and long-term studies including 843 subjects who did not have coronary heart disease. In short-term studies in children with FH, in short-term and in a 1-year study in adults with primary hypercholesterolemia, and in a short-term study in type 1 diabetes, phytosterols had no effect on vascular properties despite serum total and LDL cholesterol lowering [29, 38, 50–52]. On the other hand, customary plant stanol ester consumption at least for 2 years was associated with beneficial changes in carotid artery compliance [53]. In addition, plant stanol esters improved carotid artery compliance and flow-mediated dilatation in subjects with initially reduced respective values [54]. In a third study, arterial stiffness in small arteries and in large arteries in men were improved during a 6-month consumption of plant stanol ester margarine [55]. In addition, endothelial function was improved in relation to LDL cholesterol reduction. In all these studies except the population study of customary users, the phytosterol dose was 2 or 3 g/day, and LDL cholesterol reduction was at least 10% suggesting about a 10% risk reduction in coronary artery disease [48]. There is no explanation at the moment why the beneficial effects were not observed in all of the previous studies. Animal models do not help in this respect, because in animal studies the results are mixed [36]. The previous clinical studies, however, demonstrated that in any case plant sterol and plant stanol consumption did not have any harmful effect on arterial endothelial function, but if anything, a positive effect [36].

To this end, it still remains to be demonstrated, whether phytosterol consumption reduces the atherosclerotic burden and future coronary events.

Safety

The safety of phytosterols has been studied carefully [33, 56]. There is a convincing body of scientific evidence that phytosterol therapy is safe and there are no side effects even with large doses. Phytosterol consumption does not affect blood pressure, weight, or general well-being,

and it does not cause gastrointestinal symptoms. Conventional safety laboratory tests including indicators of liver, kidney, and thyroid function, hemoglobin and blood cells count, or blood coagulation are unchanged. Phytosterols do not interfere with the metabolism or balance of steroid hormones e.g., estrogens, testosterone, or corticosteroids. Plant sterol (but not plant stanol) consumption increases serum plant sterol levels by 50–100 %, but there is no evidence that this increase has any effect on arterial function assessed as arterial stiffness or endothelial function. There are several studies convincing that erythrocyte osmotic fragility, markers of oxidative stress, and the serum levels of fat-soluble vitamins A, D, E, and K remain unchanged. In some, but not in all, studies the serum levels of beta-carotenoids are reduced, but the concentration of the end product, serum vitamin A level, is not reduced. However, dietary intakes of fruits and vegetables in accordance with the recommended reference values have been demonstrated to sustain the serum carotenoid levels regardless of phytosterol consumption [57].

Addendum

After the preparation of this article, European Atherosclerosis Society (EAS) convened an international Consensus Panel to critically evaluate the state-of-the-art knowledge of plant sterols and plant stanols as a dietary means to reduce LDL cholesterol concentration and thereby lower cardiovascular risk. The results are published recently in the following reference [Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård E, Jessup W, Jones PJ, Lütjohann D, Maerz W, Masana L, Silbernagel G, Staels B, Borén J, Catapano AL, De Backer G, Deanfield J, Olivier S. Descamps OS, Kovanen PT, Riccardi G, Tokgözoglu L, Chapman MJ, for the European Atherosclerosis Society Consensus Panel on Phytosterols. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis. 2014;232:346–60].

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N-3 Fatty Acids: Role in Treating Dyslipidemias and Preventing Cardiovascular Disease

21

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Introduction

Much evidence has accumulated over the past 40 years that the long-chain *n*-3 fatty acids (FAs; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA; and, potentially, docosapentaenoic acid, DPA) have beneficial effects in preventing cardiovascular disease (CVD). This evidence comes from various types of research i.e., epidemiological studies, laboratory experiments, animal studies, human trials, which have evaluated many different end points, such as effects on atherosclerosis, blood lipids/lipoproteins, heart rate/rhythm, blood pressure, thrombogenic variables, inflammatory markers, genetic regulation, and disease outcomes. This review focuses specifically on the lipid/lipoprotein effects, and provides a brief overview of the clinical trials which have investigated the effect of *n*-3 FAs on CVD outcomes.

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Overview of *n*-3 FAs

Polyunsaturated fatty acids (PUFAs) are named according to the number of carbon atoms and double bonds in their aliphatic chain, and the numeric position of the first double bond from the methyl end of the chain (the "*n*th" or "omega" carbon atom). Hence, EPA, for example, is designated 20:5, *n*-3, meaning it contains 20 carbon atoms, 5 double bonds, and the first double bond is between the third and fourth carbon atoms when numbering from the CH_3 group. The most biologically important PUFAs are the *n*-6 and *n*-3 FAs. Because normal mammalian metabolism is incapable of inserting double bonds in the *n*-3 and *n*-6 positions, certain *n*-3 and *n*-6FAs are "essential," in that humans must ingest them in order to avoid deficiency syndromes. One of the essential *n*-3 FA is alpha-linolenic acid (ALA; 18:3, *n*-3), which is found in certain seeds, nuts, and their oils. ALA itself has little to no effect on serum lipid levels [1, 2]. ALA can also serve as a precursor to the longer-chain (20–22 carbon) *n*-3 FAs, albeit in a limited and inefficient capacity. For example, only 0.2–8% of ALA is converted to EPA, and 0–4% is converted to DHA (22:6, *n*-3) [3−5] (Fig. 21.1). Although the conversion rate is low, there is interesting evidence that it is

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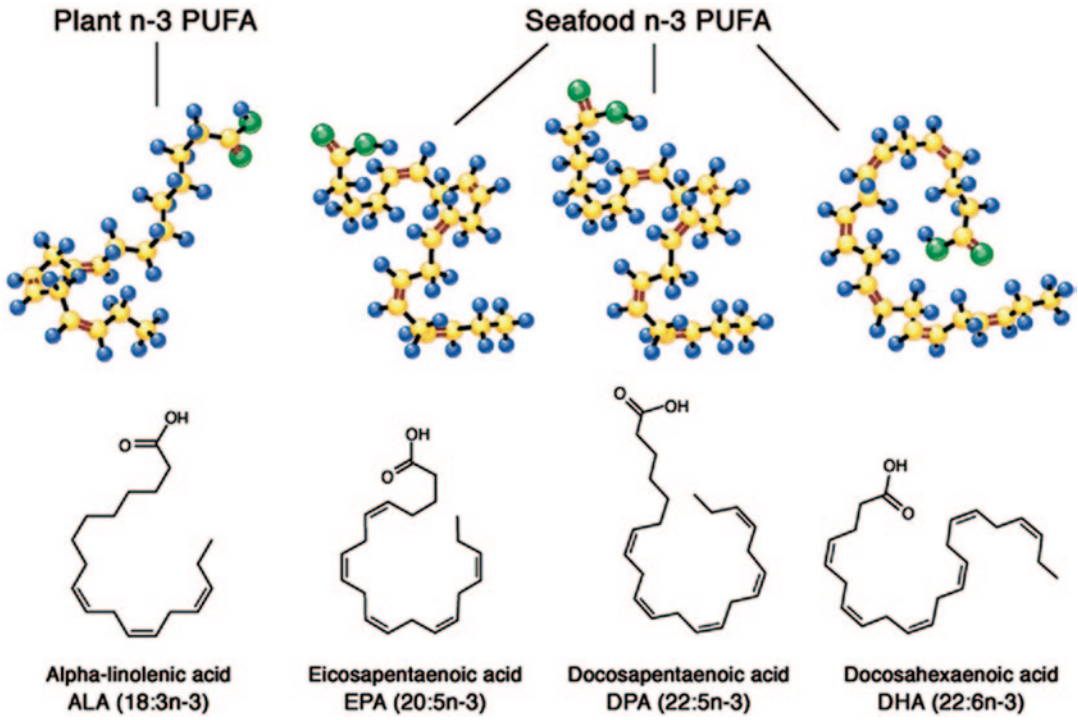


Fig. 21.1 Structure of *n*-3 fatty acids. *PUFA* polyunsaturated fatty acid, *ALA* alpha-linolenic acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid. (Reproduced with permission from Mozaffarian et al. JACC 2011;58:2047–67)

higher in women [6] (possibly because of the effects of estrogen on Δ^5 and Δ^6 desaturase genes), in nonfish consumers [7], and in the setting of low *n*-6 PUFA intake [8]. Nonetheless, conversion to longer-chain *n*-3 FAs is low, and tissue levels of EPA and DHA are much greater when these FAs are consumed directly in food or supplements.

Seafood is the major food source for EPA and DHA, hereafter referred to as long-chain *n*-3 FAs. DPA (22:5, *n*-3) is another long-chain *n*-3 FA somewhat less abundant in fish, but DPA levels correlate stronger with EPA levels than fish consumption, suggesting that endogenous conversion from EPA is primarily responsible for its tissue levels. As shown in Fig. 21.2, there is considerable variation in the amount of EPA, DPA, and DHA in different species of seafood. In addition to nonfish sources of *n*-3 FA, there are new food and beverage products with added *n*-3 FAs in the marketplace. However, many new products labeled high in *n*-3 FA use ALA, derived from plants, such as flax and soy, which may not have effects on lipids and lipoproteins.

History and Development of *n*-3 FAs for Pharmacologic Use

The connection between *n*-3 FAs and CVD was first discovered by the seminal studies of Dyerberg and Bang in the 1970s in Greenland Inuits [9, 10]. Tying their observations of high-plasma *n*-3 FA levels (derived from their *n*-3-rich diet) with the epidemiological studies of Kromann [11], a link between these unique marine FAs, thrombosis, and atherosclerosis emerged [12]. Subsequent randomized trials with fish oils themselves (instead of the complex of seal, whale and fish consumed by the Inuits) confirmed both the antiplatelet [13] and anti-lipidemic [14] effects of the *n*-3 FAs.

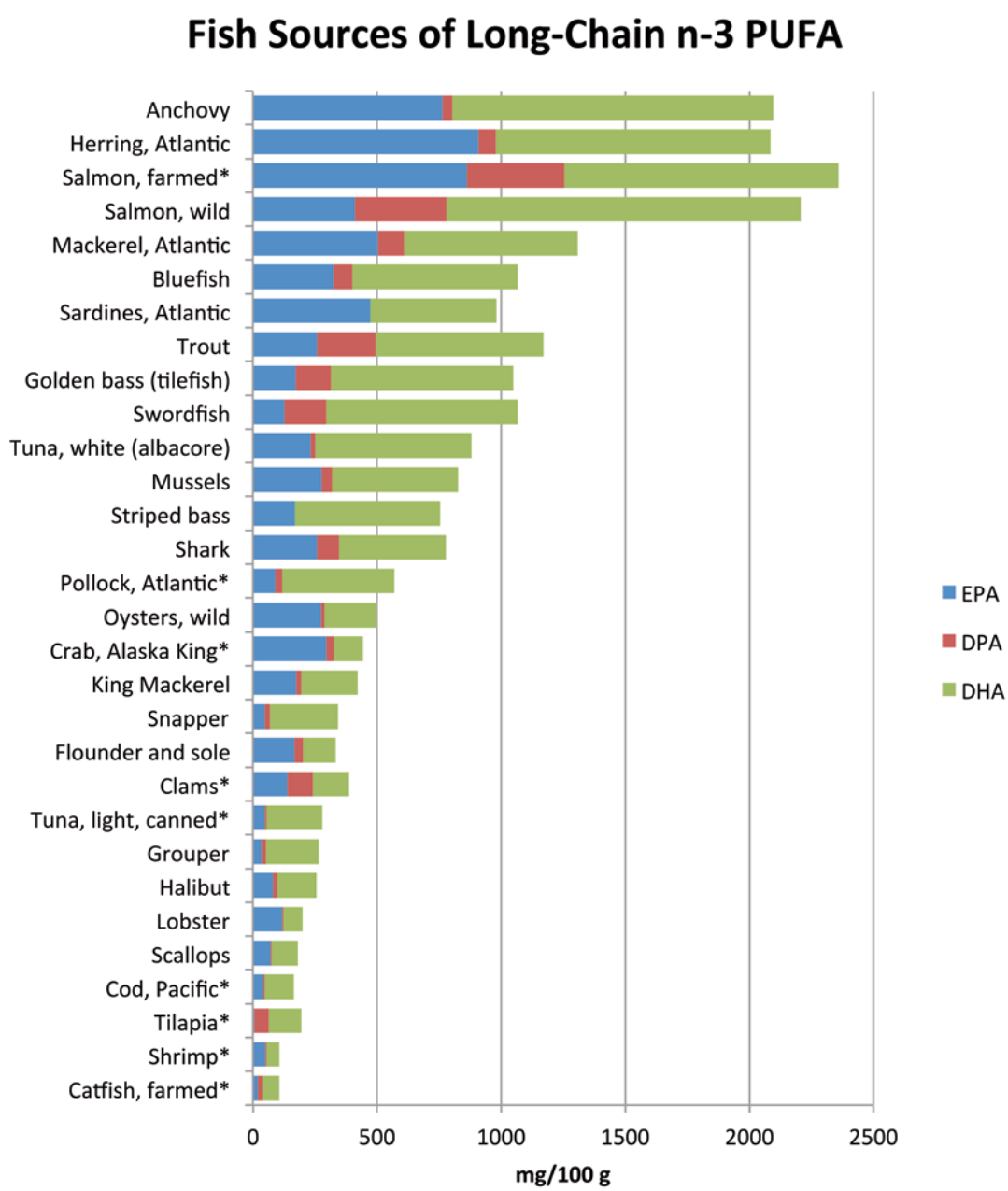


Fig. 21.2 Data derived from USDA National Nutrition Database for Standard Reference Release 23, 2010 (274). *USDA* US Department of Agriculture, *PUFA* polyunsaturated fatty acid, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid. (* Represents seafood included in the National Fisheries Institute "Top Ten" list of most consumed seafood in the U.S.)

N-3 FAs from fish consumption alone, while inversely correlated with CVD mortality in various studies, are not provided in sufficient quantity to significantly affect lipid and lipoprotein levels. Furthermore, concerns have been raised over potential detrimental effects from contaminants (methylmercury, dioxins, and polychlorinated biphenyls, PCBs) present in some fish

species. This has led to the development of a plethora of fish oil supplements, in which the *n*-3 FAs can be concentrated and the fish oil purified, in order to make it relatively easy to consume several grams of long-chain *n*-3 FAs daily. Over 25 years of research with a wide variety of nonprescription (i.e., supplemental) *n*-3 FA products has firmly documented a triglyceride (TG)-lowering effect of these agents [15, 16]. The first prescription product approved by the US Food and Drug Administration (FDA) was renamed Lovaza (also called Omacor, Pronova BioPharma, Oslo) in 2007. Each capsule contains 465 mg of EPA, 375 mg DHA, and 60–90 mg of DPA, all in ethyl ester form, to provide at least 900 mg long-chain *n*-3 FAs in 1000 mg of total fish oil [17]. The second *n*-3 FA pharmaceutical product approved by the FDA for treatment of hypertriglyceridemia contains 96 % EPA ethyl esters and no DHA (Vascepa, or AMR101, Amarin Corp, Mystic, CT) [18, 19]. A third product recently approved by the FDA, called Epanova, contains about 75 % EPA + DHA in free FA form. Unlike the TG and ethyl ester forms of *n*-3 FAs, the free FA form does not require pancreatic lipase hydrolysis, and thus can have significantly greater bioavailability, especially when consumed with a fat-free meal [20, 21]. In addition, in 2014, the first two generic omega-3 products (similar to Lovaza) were approved by the FDA. Plant-derived DHA and EPA are available as dietary supplements derived from specific strains of microalgae.

Mechanism of Action

Although it is well established that long-chain *n*-3 FAs lower serum TGs, the various mechanisms of action responsible are still being elucidated [22, 23]. *N*-3 FAs decrease TG synthesis in the liver via the inhibition of acyl coenzyme A:1,2-diacylglycerol acyltransferase, increased peroxisomal or mitochondrial β-oxidation, and a reduced supply of free FAs. TG clearance from the blood is enhanced through increases in lipoprotein lipase gene expression in adipose tissue [24], as well as decreases in apo CIII content in plasma lipoproteins [25]. The relative importance of each of these mechanisms (reduced synthesis vs. increased clearance) in humans remains unclear.

General Pharmacodynamic Effects on Lipids

The impact of long-chain *n*-3 FAs on lipids has been studied in numerous double-blind, placebo-adjusted randomized clinical trials (RCTs). The earliest studies were first reviewed in the Evidence Report commissioned by the National Institutes of Health [26]. The most consistent and only major effect observed is lowering of TGs. Like most other lipid-lowering treatments, maximum efficacy is seen within 2–4 weeks. The percentage of TG lowering depends upon both the dose of the *n*-3 FAs and the baseline TG level. Higher doses and higher the baseline TG levels result in a greater percentage lowering (Fig. 21.3).

Indications and Dosing Regimen

For patients with fasting TG levels ≥500 mg/dL, the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommends lowering TGs as the primary lipid treatment target. This is because fasting TG levels >500 mg/dL are associated with an increased incidence of pancreatitis. The two prescription *n*-3 FA products currently available (Lovaza and Vascepa) are approved by the FDA, as an adjunct to diet, for treatment of TGs ≥500 mg/dL. The approved daily dose is 4 g, taken either as a single dose (four 1-g capsules) or divided as two 2-g doses. The data supporting this indication for Lovaza are derived from two RCTs that evaluated a combined total of 82 patients with TG levels 500–1999 mg/dL (Fig. 21.4, *top panel*). Using a dose of 4 g EPA + DHA daily for 6–16 weeks, TG levels decreased by 45 % from a baseline mean of 919 mg/dL in the first trial, and by 39 % from a baseline mean of 801 mg/dL in the other trial [27, 28]. High-

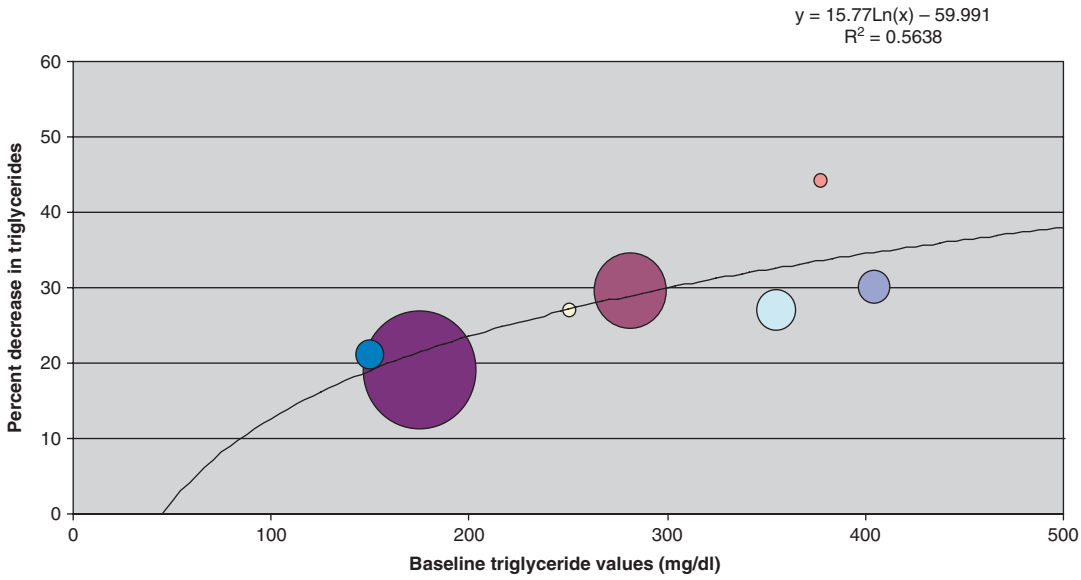


Fig. 21.3 Effectiveness of triglyceride lowering as a function of baseline triglycerides. A *line* of best fit was generated for the results of studies that administered 3.4 g/day EPA + DHA. Bubble size represents population size in each study. (Skulas-Ray et al. Expert Opin Pharmacother 2008;9:1237–1248)

density lipoprotein-cholesterol (HDL-C) levels increased by 13 and 5.9%, very low-density lipoprotein-cholesterol (VLDL-C) levels decreased by 32 and 29%, and low-density lipoprotein-cholesterol (LDL-C) levels increased by 32 and 17%, respectively. Combining the data from both the trials, there was a 14% reduction in non-HDL-C levels [29]. There have been at least three other very small RCTs which enrolled patients with TG levels 500–2000 mg/dL, that had nearly identical results (TG reductions 26–40%) using the same 4 g/day dose [30–32].

The largest study in a very high TG population was the MARINE trial [18], which provides the data supporting the recent FDA-approved indication for Vascepa (AMR101 in the trial). This was a 12-week double-blind study that randomized 229 patients with TGs ≥500 mg/dL and ≤2000 mg/dL (and with or without background statin therapy) to placebo (mineral oil) or Vascepa, an ethyl ester of EPA without DHA, which was given at a dose of 4 g/day or 2 g/day (Fig. 21.4, *bottom panel*). Baseline TG levels in the three treatment arms were 657–703 mg/dL. Vascepa 4 g/day reduced the placebo-adjusted TG level 33.1%, and 2 g/day, 19.7%. In the subgroup of patients with a baseline TG level >750 mg/dL, the reductions were 45.4% for 4 g/day, and 32.9% for 2 g/day. Vascepa did not significantly increase the placebo-adjusted median LDL-C levels at 4 g/day (−2.3%) or 2 g/day (+5.2%; both p = NS). Vascepa significantly lowered non-HDL-C by 17.7% and 8.1%, with 4 g/day and 2 g/day, respectively. The 4 g/day dose lowered apo B by 8.5%, but the 2 g/day dose was ineffective in lowering apo B. HDL-C changes were not statistically significant for either dosing regimens.

Although no head-to-head studies have been conducted with Lovaza and Vascepa in the same population, a comparison of these two studies is instructive. In general, it appears that Lovaza was more effective in reducing TGs and raising HDL-C than Vascepa (at 4 g/day), whereas the latter did not increase LDL-C that was not placebo adjusted (Fig. 21.4). This difference between the effects of products with EPA + DHA versus EPA alone is discussed more fully below.

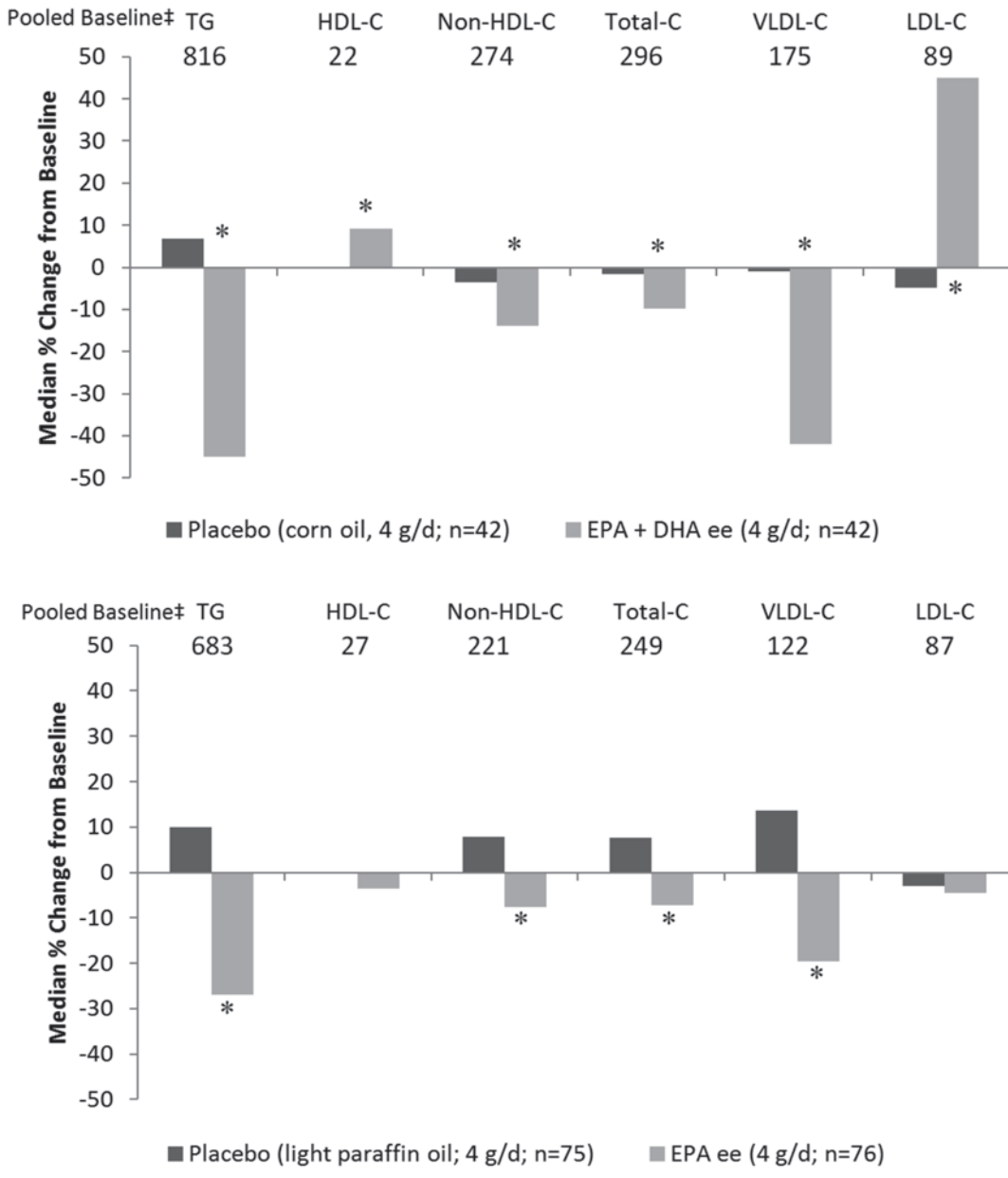


Fig. 21.4 Comparison of the effects of Lovaza after 12 weeks (*top panel*) and Vascepa after 12 weeks (*bottom panel*) on lipid and lipoprotein levels in patients with triglycerides between 500 and 2000 mg/dL. *$p<0.006$; ‡ Values in mg/dL. *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *VLDL-C* very-low-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

Risks and Precautions

Overall, the pharmaceutical *n*-3 FA products have been shown to be safe and well tolerated. The most common treatment-emergent adverse events, compared to placebo, were eructation (4.9 vs. 2.2% in placebo), infection (4.4 vs. 2.2%), flulike syndrome (3.5 vs. 1.3%), dyspepsia (3.1 vs. 2.6%), and taste perversion (2.7 vs. 0%). Alanine transferase levels sometimes increased

transiently to two times the upper limit of normal, without concomitant increases in aspartate transferase. It is therefore recommended to periodically monitor liver enzymes in patients undergoing lipid-lowering therapy.

There are no known drug interactions per se of *n*-3 FAs. In vitro, free forms of EPA and DHA have been shown to inhibit cytochrome P450 enzymes (primarily CYP2E1); however, since free forms of EPA and DHA are not detected in the circulation, clinically significant drug–drug interactions due to inhibition of this system are not expected [29]. Pharmacologically recommended doses of *n*-3 FAs can prolong bleeding times, although always remaining within the normal range, which has raised concern about potentiating the effects of concomitantly administered antiplatelet or antithrombotic drugs. No clinically significant bleeding episodes have been reported in clinical trials [33].

Nevertheless, close monitoring of international normalized ratio (INR) values in patients on warfarin is recommended whenever prescription *n*-3 FAs are added to therapy.

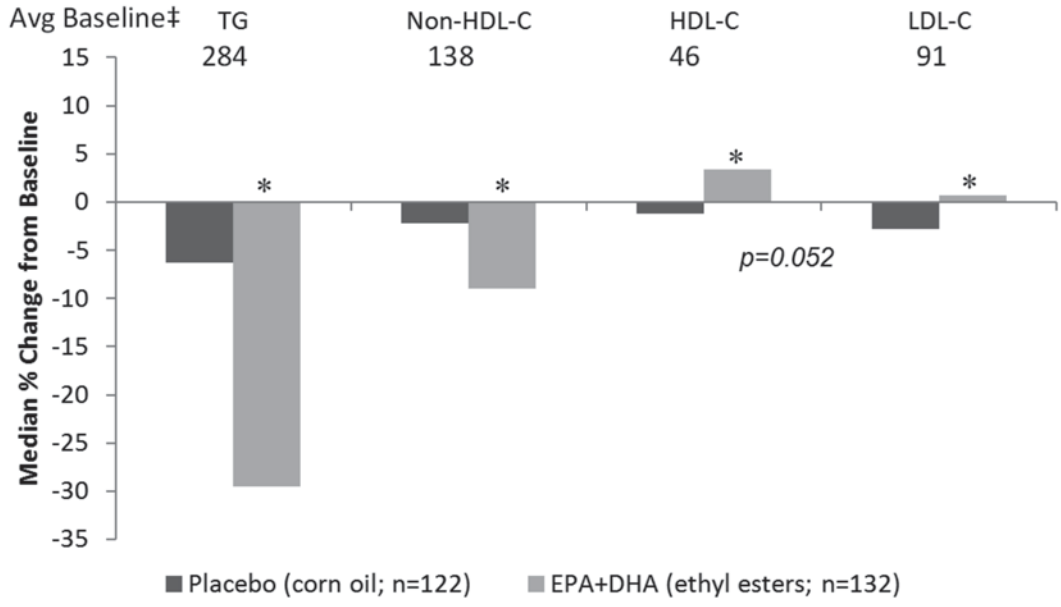
Effects of *n*-3 FAs on TGs < 500 mg/dL

Numerous RCTs have been performed in patients with TGs 150–500 mg/dL (a nonapproved indication at the time of this writing). Skulas-Ray et al. reviewed 19 of these trials, which were quite diverse in study design and population characteristics. Doses ranged from 0.85 to 5.1 g EPA + DHA. The average TG lowering with 4 g/day in subjects with baseline TGs >250 mg/dL was ~30% [34]. Using a 4 g/day dose in subjects with lower TGs at baseline, one study resulted in a 21% reduction, while a study using a higher dose (6 g/day) decreased TGs by ~30% [35, 36]. Thus, baseline TG level and dose of long-chain *n*-3 FAs have independent and additive effects on the TG-lowering response.

The largest trials in this particular patient population have again been conducted with Lovaza and Vascepa, both added to statin therapy (Fig. 21.5). The trial using EPA + DHA (called COMBOS, Combination of Prescription Omega-3 Plus Simvastatin) included 254 patients randomized to 4-g Lovaza versus corn oil placebo [37]. The trial using EPA only (called ANCHOR) included 453 patients randomized to 2 or 4 g of Vascepa or to placebo (light paraffin, or mineral oil) [19]. As with the two monotherapy trials done in patients with TGs >500 mg/dL (discussed above), the EPA + DHA preparation (Lovaza) appeared to have been more effective in lowering TGs and raising HDL-C than the EPA-only preparation (Vascepa; (Fig. 21.5). Compared to placebo, LDL-C increased by 3.5% in the trial using Lovaza, and decreased by 6.2% in the trial using Vascepa 4 g/day. Compared to baseline, however, there were no differences in LDL-C in either trial. In other words, the difference in LDL-C response between Lovaza and Vascepa is due to the different LDL-C responses to placebo. In both of the Lovaza studies, lipids in the placebo arms were either unaffected by treatment or decreased to a small extent, whereas in the two Vascepa studies, lipids increased more than would be expected (e.g. 9% increase in LDL-C and 6% increase in TGs in the placebo arm of the ANCHOR trial). The reason for the difference in placebo responses is unknown. It is possible that the Vascepa study populations became less adherent to lifestyle and/or pharmacologic therapy over the course of the trial. However, the elevation of atherogenic lipids in the placebo arms of the Vascepa trials raises the question of whether the light paraffin oil could have interfered with effectiveness of the concomitant therapies.

Patients with diabetes usually have TG levels that range between 150 and 500 mg/dL. A meta-analysis performed in 2007 included 23 RCTs of long-chain *n*-3 FA therapy in 1075 patients with type 2 diabetes. Doses of EPA + DHA ranged from 1.7 to 6 g daily, with study durations from 4 to 24 weeks (mean 8.9 weeks). Compared to placebo, TGs were reduced by 25%, VLDL-C reduced by 36%, and LDL-C increased by 5.7% [38].

The LDL-C increases observed with long-chain *n*-3 FA monotherapy (i.e., not on concomitant statin therapy) in patients with elevated TGs are generally proportional to the magnitude of the decrease in TGs, and as noted in the studies



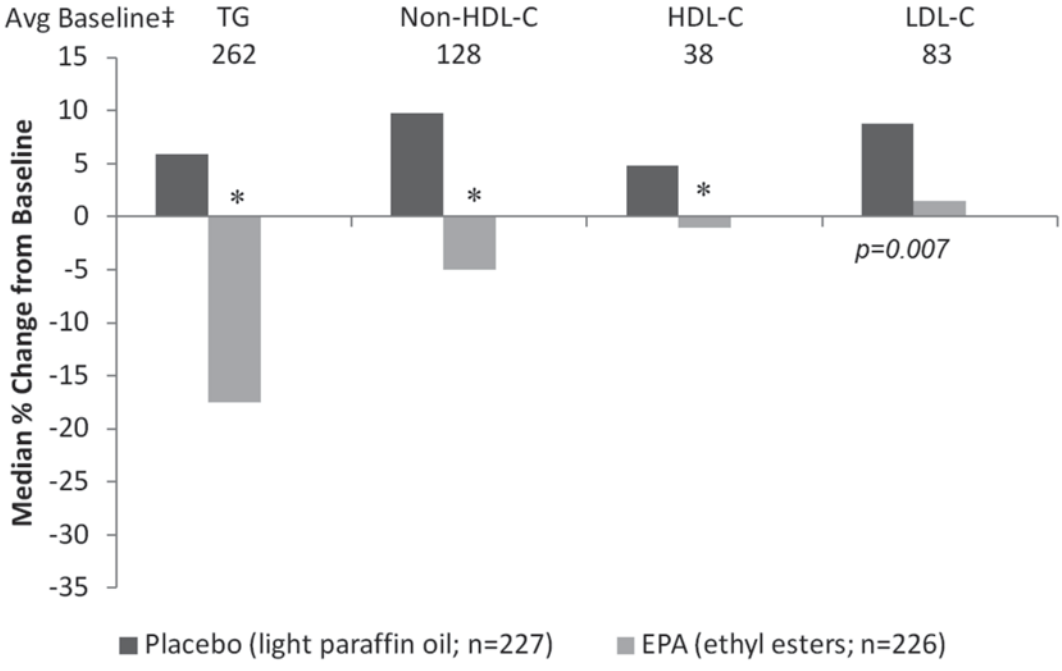


Fig. 21.5 Effects of Lovaza (**a**, *top panel*) and Vascepa (**b**, *bottom panel*) on lipid and lipoprotein levels in patients with triglycerides between 200 and 499 mg/dL while on concomitant statin therapy. *Top panel*: patients were stabilized on simvastatin (40 mg) × 8 weeks, then randomized to placebo or Lovaza (4 g/day) for 8 weeks. B*ottom panel*: patients were stabilized on statins for at least 4–6 weeks, then randomized to placebo or Vascepa (2 or 4 g/day) for 12 weeks. Results for 4 g vs. placebo shown. *$p<0.001$; ‡ Values in mg/dL. *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

above, can be as high as 35–45 % in patients with baseline TGs > 500 mg/dL and low LDL-C levels. Reasons proposed for this increase include an increased rate of conversion of VLDL to LDL, and a reduction in the substrate for cholesteryl ester transfer protein (CETP) [39–41]. CETP is the enzyme that catalyzes the exchange of TG from VLDL for cholesteryl esters from LDL (and HDL) particles. Increased activity of CETP, which occurs when VLDL levels are high, results in more numerous, less cholesterol-rich LDL particles; less activity, such as when VLDL levels are lowered, results in larger, more cholesterol-rich LDL particles [42].

Effect of Long-Chain *n*-3 FAs on Lipoproteins and Apo CIII

The largest long-chain *n*-3 FA trials, which evaluated effects on lipoprotein fractions, were performed in study populations on concomitant statin therapy. The COMBOS trial (protocol summarized above) showed that EPA + DHA (Lovaza) did not change total VLDL particle or LDL particle concentrations relative to placebo, but large VLDL particle and small LDL particle concentrations were lowered, and the large LDL particle concentration was increased. Small LDL particles decreased by an almost identical amount, explaining the unchanged total LDL particles (*p* value 0.07). HDL particle concentration was reduced slightly, owing to a decrease in medium HDL particle that was greater than the increase in large HDL particles [43]. Similarly, in the EPA + DHA trial using atorvastatin in escalating doses, 4 g/day of *n*-3 FA had no significant impact on apo B or total LDL particle concentration compared to placebo, although small LDL particle concentration decreased, large LDL particle concentration increased, and mean LDL particle size increased [44].

In summarizing the effects of high-dose EPA + DHA on the main treatment targets in patients with TGs between 150 and 500 mg/dL, non-HDL-C is reduced (by lowering VLDL-C), but apo B and LDL particles are not significantly lowered. Since approximately 90 % of circulating apo B is associated with LDL particles, the reduction in VLDL particles, which also carry apo B, is apparently not sufficient to produce a significant overall reduction in apo B.

The two trials using EPA only (Vascepa) also evaluated effects on these atherogenic lipoprotein particles. In MARINE (protocol summarized above), the 2 g/day dose had no effect on apo B. The 4 g/day dose lowered apo B by 4 mg/dL from baseline, while the placebo increased apo B by an identical amount, producing a net 8.5 % reduction compared to placebo. Similarly, in ANCHOR, apo B increased 4 mg/dL on the 2 g/day dose, decreased 3 mg/dL on the 4 g/day dose, and increased 7 mg/dL on the placebo. Thus, when compared to placebo, the net effect was a 9.3 % and 3.8 % reduction in apo B with the 4 g/day and 2 g/day doses, respectively. As noted previously, the reason for the consistent and substantial increase in apo B while on placebo is not apparent. Thus, one can conclude that either Vascepa (EPA only) has apo B-lowering effects not seen with EPA + DHA, or more likely, the placebo in these trials (light paraffin oil) was not wholly inert [45].

The effect of EPA + DHA on apo CIII was evaluated in the simvastatin and atorvastatin studies cited above. In both trials, the incremental effect of 4 g/day of *n*-3 FA compared to placebo was an 11–13 % reduction in apo CIII [43, 44].

Differential Effects of EPA Versus DHA on Lipids and Lipoproteins

Compared to the number of studies using combination EPA + DHA, there have been few studies assessing effects on lipids and lipoproteins using EPA or DHA alone. Even fewer were head-to-head comparison studies. Jacobson et al. recently published a review of the most relevant randomized controlled trials to determine if there was a differential effect between EPA and DHA on LDL-C and other lipid parameters [46]. Of the 22 studies which met the selection criteria, 6 compared DHA with EPA directly. A total of 12 trials studied DHA alone (typically from algal sources), and 4 examined

EPA alone (usually ethyl esters derived from fish oils). The mean or median baseline TG level was ≤150 mg/dL in 14 studies, 151–200 mg/dL in 5 studies, and 201–300 mg/dL in 3 studies. In the six head-to-head comparative trials (with doses ranging from 2.2 to 4 g/day), a net increase in LDL-C of 3.3 % was observed with DHA (DHA: +2.6 %; EPA: −0.7 %). In another recent review by Mozaffarian and Wu [47], the increase in LDL-C in response to DHA supplementation was primarily due to an increase in particle size rather than number. DHA was associated with a net decrease in TGs by 6.8 % (DHA: −22.4 %; EPA: −15.6 %), a net increase in non-HDL-C by 1.7 % (DHA: −1.2 %; EPA: −2.9 %), and a net increase in HDL-C by 5.9 % (DHA: +7.3 %; EPA: +1.4 %). In a recent clinical study conducted by Tatsuno et al. [48] with hypertriglyceridemic Japanese subjects (n = 600 subjects) randomized to one of three treatment groups (TAK-085—a mixture of EPA and DHA ethyl esters similar to Omacor/Lovaza; doses were 2 g once daily, 2 g twice daily, or 0.6 g three times daily of an ethyl ester of EPA), TG decreased in subjects in all treatment groups (11, 23, and 11 %, respectively). LDL-C decreased in the EPA ethyl ester group by approximately 4 %, which was greater than the decrease in TAK-085 4 g/day group (−1.1 %); the LDL-C decrease (−2.1 %) for the 2 g/day TAK-085 dose did not differ from EPA ethyl ester group. Interestingly, HDL-C increased only in the TAK-085 4 g/day dose (2.7 %).

It is important to be cautious in our interpretation of the data from these studies. The implementation of "net increase" in analyzing non-HDL-C gives the impression that DHA increases non-HDL-C. This is not true. DHA in fact produced an absolute decrease in non-HDL-C. Statistically, significant increases in LDL-C were also observed in 8 of the 12 DHA-alone trials, but not in any of the 4 EPA-alone trials. As noted in the combination DHA +EPA studies, here too we must exercise caution. DHA as a more effective TG-lowering agent than EPA would be expected to produce a greater increase in LDL-C (see explanation above). The head-to-head and DHA-alone studies were small (mean number of participants per trial 79 and 42, respectively). Nevertheless, in these small studies, DHA was more effective than EPA in lowering TGs, but slightly less effective than EPA in lowering non-HDL-C. Also, DHA usually increased LDL-C, generally in proportion to the baseline TG level, while EPA had no significant effect on this parameter. An important limitation of these studies is the lack of data on apo B or LDL particle-lowering effects, which were end points in the MARINE and ANCHOR trials, as discussed above.

Impact of Genotype on Lipid Effects of *n*-3 FAs

The atherogenic lipoprotein phenotype (ALP) trial was a very small study not designed to examine the impact of genotype on lipid responsiveness to *n*-3 FAs, but a retrospective, hypothesis-generating, analysis has suggested that LDL-C response to EPA + DHA (mean group increase of 7 %) may be modulated by apoE genotype. And 3, 1, and 15 % increases in LDL-C were observed in apoE2 carriers, apoE3/E3 homozygotes, and apoE4 carriers, respectively [49]. Another study, which randomized 38 healthy volunteers according to apoE genotype (apoE3/E3 vs. apoE3/E4) and gave them EPA and DHA separately (3–4 g/day), also showed a 10 % increase in LDL-C with DHA in apoE4 carriers, and no significant impact of treatment in any other apoE genotype **x** EPA or DHA subgroup [50]. However, concomitant with the greater LDL-C increase in apoE4 carriers, *n*-3 FAs also produce a greater reduction in small, dense LDL particles compared to apoE3/E3 genotypes [49]. Yet another study, using EPA + DHA doses of 0.7 g or 1.8 g/day, showed a greater reduction in fasting TGs in apoE4 males only, raising the question of a gender difference [51]. The clinical significance of all of these findings, even if replicated, is completely unknown.

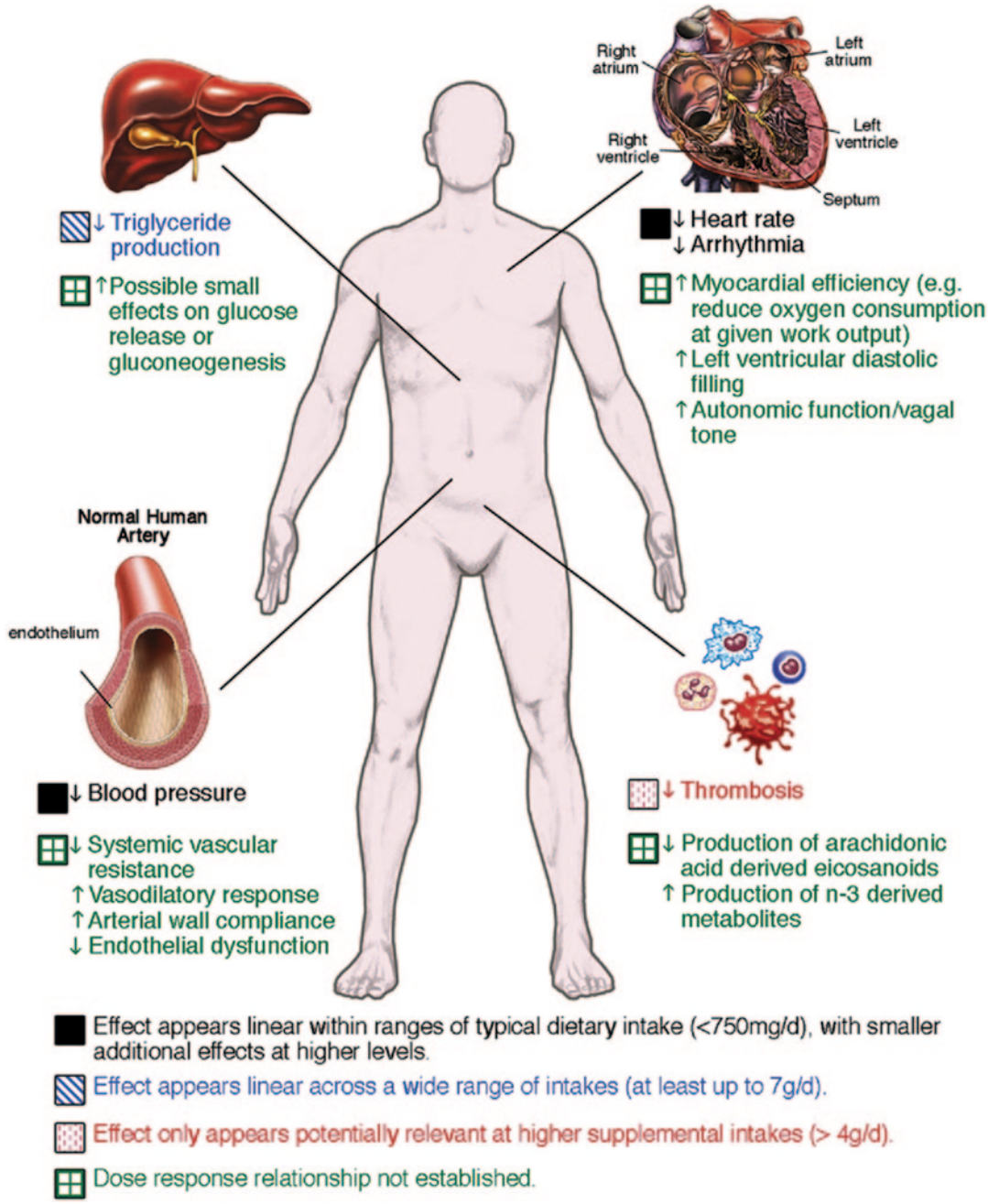


Fig. 21.6 Physiological effects of *n*-3 fatty acids which have potential cardiovascular benefits. (Reproduced with permission from Mozaffarian et al. JACC 2011;58:2047–67)

Table 21.1 Summary of the ten large *n*-3 fatty acid randomized controlled trials assessing cardiovascular outcomes

RCTs of long-chain *n*-3 fatty acids and cardiovascular events						
Trial, Year	Population	Interventions compared	Duration years	Lipid effects	End points	RR (95% CI)
DART 1989	2033 men with recent MI (mean 41 days)	2 servings/wk fatty fish (or fish oil capsules) vs. other dietary advice	2	No change in cholesterol	IHD events Total deaths	0.84 (0.66–1.07) 0.71 (0.54–0.93)
GISSI-P 1999	11,324 men with recent MI (≤3 mo)	Usual care plus 882 mg/day EPA + DHA, vitamin E, both or neither	3.5	No change in C, LDL-C, or HDL-C; 5% decrease in TG	Major CV events Nonfatal events Cardiac deaths Sudden deaths	0.90 (0.82–0.99) 0.98 (0.83–1.15) 0.78 (0.65–0.92) 0.74 (0.58–0.93)
DART 2 2003	3114 men with angina	2 servings/wk fatty fish (or fish oil capsules) vs. other dietary advice	3–9	NR	Cardiac deaths Sudden deaths	1.26 (1.00–1.58) 1.54 (1.06–2.23)
JELIS 2007	18,645 patients with total cholesterol ≥6.5 mmol/l (with and without CHD history)	1.8 g/day EPA vs. usual care	4.6	No change in C, LDL-C, or HDL-C; 6% decrease in TG	Coronary events Nonfatal events Coronary deaths Sudden deaths	0.81 (0.69–0.95) 0.81 (0.68–0.96) 0.94 (0.57–1.56) 1.06 (0.55–2.07)
GISSI-HF 2008	6975 patients with heart failure	840 mg/day EPA + DHA vs. placebo (not defined)	3.9	No change in C, LDL-C, or HDL-C; 5% decrease in TG	Total death Death or hospitalization for CVD	0.91 (0·83–0·99) 0.94 (0.89–099)
Omega 2010	3851 patients with recent MI (≤2 wks)	840 mg/day EPA + DHA vs. placebo (olive oil)	1	No change in LDL-C; 4% decrease in TG	Major CV events Sudden deaths	1.21 (0.96–1.52) 0.95 (0.56–1.60)
Alpha-Omega 2010	4837 patients with history of MI (median 3.7 yrs)	376 mg/day EPA + DHA vs. placebo margarine and ALA (1.9 g/day) groups combined	3.4	No change in any lipid class	Major CV events CHD deaths	1.01(0.87–1.17) 0.95 (0.68–1.32)
SU.FOL.OM3 2010	2501 patients with recent coronary or cerebral ischemic event (median 101 days)	600 mg/day EPA + DHA vs. placebo (not defined) and B vitamin groups combined	4.2	NR	Major CV events CHD deaths	1.08 (0.79–1.47) Not reported
ORIGIN 2012	12,536 patients with diabetes, IGT, or IFG, most with CVD	840 mg EPA + DHA vs. olive oil placebo	6.2	No change in C, LDL-C, or HDL-C; 11% decrease in TG	CVD deaths Major CV events	0.98 (0.87–1.10) 1.01 (0.93–1.10)
Risk and Prevention 2013	12,513 patients at increased risk for CHD but without MI	850 mg EPA + DHA vs. olive oil placebo	5.0	NR	Death, nonfatal or stroke CVD death	0.98 (0.88–1.08) 1.02 (0.82–1.30)

RCT randomized control trial, *MI* myocardial infarction, *IHD* ischemic heart disease, *CV* cardiovascular, *CVD* cardiovascular disease, *CHD* coronary heart disease, *IGT* impaired glucose tolerance, *IFG* impaired fasting glucose, *NR* not reported, *C* cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *DART* Diet and Reinfarction Trial, *GISSI-P* Gruppo Italiano per lo Studio della Soppravvivenza nel Infarto Miocardico—Prevenzione, *JELIS* Japan EPA Lipid Intervention Study, *SU.FOL.OM3* Supplementation en Folates et Omega-3, *ORIGIN* Outcome Reduction with Initial Glargine Intervention, *RR* relative risk, *CI* confidence interval, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *ALA* alpha-linolenic acid

Efficacy of Long-Chain *n*-3 FAs in Preventing Coronary Heart Disease

Long-chain *n*-3 FAs have many physiologic effects beyond the changes observed for lipids and lipoproteins, which could explain their potential cardiovascular (CV) benefits (Fig. 21.6). The review by Mozaffarian and Wu [47] describes some of the specific CV physiologic effects of the individual long-chain *n*-3 PUFA. In short, both EPA and DHA decrease TG levels, and EPA decreases HDL3 cholesterol whereas DHA increases LDL particle size and HDL2 cholesterol. EPA has minimal effects on blood pressure whereas DHA decreases it. DHA lowers heart rate; the effects of EPA on heart rate are unclear. Both FAs increase diastolic filling and increase arterial compliance; for both, there are no clear effects on endothelial function. Both FAs favorably affect inflammation, oxidative stress, thrombosis, and coagulation. DHA, but not EPA, is associated with a decreased risk of fatal coronary heart disease (CHD) and sudden death as well as atrial fibrillation. While progress is being made in understanding the underlying mechanisms of action of the long-chain *n*-3 FAs, based on numerous observational studies over the past 40 years, it is clear that there is an inverse relationship between fatty fish or *n*-3 FA consumption and morbidity or mortality from CHD [52−66]. Among studies which measured blood or tissue levels of *n*-3 FAs, the great majority have shown the same inverse correlation with CVD events [67−72]. However, definitive evidence for a cause-and-effect relationship, or lack thereof, must come from RCTs assessing clinical outcomes, such as myocardial infarction, revascularization, and mortality.

There have been ten RCTs of sufficient size (n=2000–18,000) to provide adequate power for detecting statistically meaningful results [73−80] (Table 21.1). Trial designs, *n*-3 FA doses, and study populations were quite different, and results have been inconsistent. Nevertheless, in a recent meta-analysis, fish oil supplementation was associated with a significant reduction in cardiac death (hazard ratio 0.91, 95 % confidence interval (CI) 0.85–0.98). The effects on serum lipids have been minimal, with no reported effects on total, LDL or HDL, cholesterol levels, and a lowering of TGs by about 5 % in trials that did or did not report beneficial effects on CV outcomes (Table 21.1). Hence, whatever CV benefit is associated with *n*-3 FA treatment does not appear to derive from their lipid-lowering effects which require much larger doses than have been used in most clinical end point trials.

Conclusions

The clearest effect of *n*-3 FAs is on serum lipids is the reduction in TG levels. Effects on LDL and HDL levels are minor at best. Since there were minimal effects on TG levels in the major *n*-3 RCTs with clinical end points, the CV benefits of these FAs were apparently not dependent on their lipid-lowering effects. A more reasonable hypothesis is that anti-inflammatory effects, derived from the production of a wide variety of *n*-3 FA metabolites (resolvins, protectins, maresins, etc.) and effects on membrane physiochemical properties, are responsible for their clinical efficacy.

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Polyphenols for Cholesterol Management

22

Sanne M. van der Made and Ronald P. Mensink

Introduction

Coronary heart disease (CHD) is still a major health problem in many countries. It is well known that an increased serum concentration of low-density lipoprotein (LDL) cholesterol is a powerful risk factor for CHD. Epidemiological studies also suggest that high serum concentrations of high-density lipoprotein (HDL) cholesterol may protect against CHD, although results from recent intervention studies with drugs do not support a causal relationship between HDL and CHD risk. Traditionally, reducing saturated and *trans*-fatty acids intake has been the cornerstone in the management of dyslipidemia. However, in recent years, many other dietary components have attracted much interest. This has led, with different degrees of success, to the search and testing of specific foods and food components that may help to improve the serum lipoprotein profile. However, evidence is emerging that diet also affects other risk markers for CHD, such as endothelial function, blood pressure, inflammation, and platelet function.

This chapter reviews the role of polyphenols in dyslipidemia management. In addition, the relation between polyphenols and endothelial function is briefly addressed. Focus is on flavonoids from cocoa and the stilbene *trans*-resveratrol, as flavonoids from cocoa have been extensively studied in the past, while recent studies have ascribed possible cardioprotective effects to *trans*-resveratrol. Metabolism of these polyphenols is discussed as well.

History

Polyphenols are metabolites produced by higher plants and are important for pigmentation, reproduction, growth, and protection against pathogens. It has for long been recognized that foods rich in polyphenols may possess healthy properties, such as anti-oxidative, antibacterial, antihypertensive and anti-inflammatory effects. In humans, the potential beneficial effect of polyphenols on cardiovascular disease has generated a great amount of scientific interest during the past decades. Hertog et al. were among the first to suggest a strong protective effect of flavonoids, a subgroup of polyphenols found in tea, onions, and apples, on CHD-related deaths [1]. The French paradox, the observation that the incidence of CHD is low in the French population, despite a high dietary intake of saturated fat, also supported the notion that polyphenols may have beneficial effects on cardiovascular disease. This association has been attributed to increased intakes of resveratrol, a polyphenol found in foods such as red wine and grapes. This French paradox has formed the basis for numerous papers on the relation between polyphenol intake, and, more specifically, polyphenols from grapes, and

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cardiovascular health. Also studies in the Kuna Indians of the San Blas Islands of Panama supported the idea that polyphenols may improve cardiovascular health. This population consumed on average three 10-ounce cups of cocoa beverage each day. The prevalence of hypertension among Kuna Indians was very low (2.2%), blood pressure did not increase with age, and a lower rate of myocardial infarction and stroke was found as compared to mainland Panamanians [2]. In another study, an inverse relation was found between cocoa intake and cardiovascular mortality in men aged 65–84 years from Zutphen, a city in the Netherlands [3]. Men in the highest tertile of cocoa intake consumed on average 4.2 g cocoa daily, which is equal to a daily intake of 10 g dark chocolate, and had a 50% lower risk than men in the lowest tertile, who did not consume cocoa at all. An inverse relation between cocoa intake and systolic and diastolic blood pressure was also found, but this was not the main explanation for the observed lower cardiovascular risk in this group. In another epidemiologic study in survivors (45–70 years of age) of an acute myocardial infarction, an inverse association was found between chocolate consumption and cardiac mortality [4]. These examples have certainly contributed to the immense growth of scientific interest in the relation between polyphenol intake, dyslipidemias, and cardiovascular health during the past decades.

Chemical Structure

Each structure that includes several hydroxyl groups on aromatic rings can be defined as a polyphenol. Polyphenolic compounds can be divided into distinct groups based on the number of phenol rings and by the structural elements attached to these rings. In this way, four main polyphenol groups have been identified: (1) phenolic acids, (2) flavonoids, (3) stilbenes, and (4) lignans (Fig. 22.1).

Several products rich in these compounds and their estimated daily intakes are listed in Table 22.1 [5].

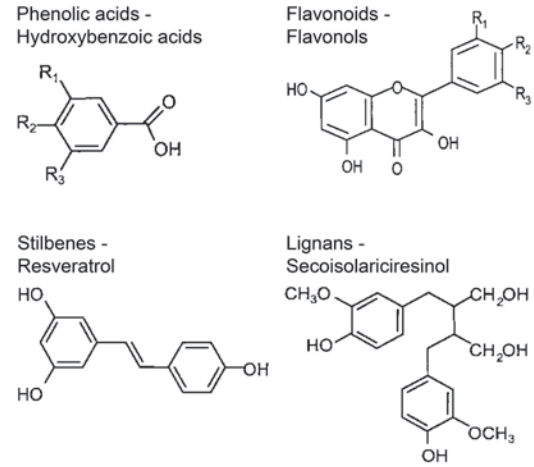

Fig. 22.1 Chemical structure of the four main polyphenolic compounds. Hydroxybenzoic acids is drawn as an example of a subgroup of phenolic acids, the flavonols as the subgroup of the flavonoids, resveratrol belongs to the stilbenes, and secoisolariciresinol is an example of the lignans

Phenolic Acids

Phenolic acids can be classified into derivatives of benzoic acid or derivatives of cinnamic acid. Hydroxybenzoic acids are found in only a few plants eaten by humans. Caffeic acid is the major representative of a hydroxycinnamic acid and occurs in foods mainly as an ester with chlorogenic acid. Coffee consumers have an intake of 0.5–1 g of chlorogenic acid. Hydroxycinnamic acid is also found in amounts varying from 0.5 to 2 g/kg fresh weight, mainly in the skin of mature fruits. Wheat grain contains on average 0.8–2.0 g of ferulic acid—also a hydroxycinnamic acid—per kilogram dry weight. Phenolic acids are also present in rice, wheat, and oat flour in quantities of 70–90 mg/kg fresh weight, whereas corn flour may contain up to 300 mg/kg fresh weight.

Flavonoids

The flavonoids are divided into six subclasses, depending on the oxidation status and saturation of the heterocycle group that is part of the

Table 22.1 Products rich in polyphenols and their estimated daily intakes. (Based on ref. [5])

Main compound	Subgroup	Products rich in this compound	Estimated daily intake (mg/day)
Phenolic acids	Benzoic acids	Red fruits, black radish, onions, tea leaves	
	Cinnamic acids	Grains and seeds, coffee, apples, blueberries, cherries, kiwis, and plums	
Flavonoids	Flavonols	Tea, onions, curly kale, leek, broccoli, tomatoes	13
	Flavones	Parsley, chamomile tea, celery, tangerines	1.6
	Isoflavones	Soybeans, soy milk, tofu, tempeh	USA/Netherlands: 1.2 Asia: 25–50
	Flavanones	Grapefruits, oranges, lemons, tomatoes, mint	14.4
	Anthocyanidins	Cranberries, blackberries, eggplant, cabbage, beans, onions, radishes	3.1
	Flavanols	Tea leaves, cocoa beans, dark chocolate, apples, blueberries, grapes, apricots	156
Stilbenes		Red wine, black grapes	
Lignans		Cereals, berries, vegetables, flaxseed	

flavonoid skeletal structure: (1) flavonols, (2) flavones, (3) isoflavones, (4) flavanones, (5) anthocyanidins, and (6) flavanols. These subclasses share a common structure that consists of two aromatic rings, bound by three carbon atoms that form an oxygenated heterocycle. Flavonoids naturally occur mostly as glycosides rather than as aglycones.

Flavonol The most widely known flavonols are quercetin and kaempferol. Flavonols mainly accumulate in the skin and leaves of vegetables, due to the fact that the biosynthesis of flavonols is stimulated by light. Therefore, the flavonol content of the same species can be very different; cherry tomatoes for instance have a higher flavonol content than regular tomatoes, caused by the different ratios of skin to whole fruit.

Flavones are present in herbs such as parsley, but also chamomile tea, celery, tangerines, and some other citrus fruits contain flavones.

Isoflavones Soybeans are a main source of isoflavones, whereas its content in other beans and peas like kidney beans, black beans, and chickpeas is low. The level of the isoflavones genistein and daidzein in soybeans varies and depends on the geographic zone where the beans are cultivated. Growing conditions and processing also influence the isoflavone content of soybeans, which varies between 580 and 3800 mg/kg fresh weight. In soy milk, this content lies between 30 and 175 mg/L. The intake of isoflavones differs widely around the world. In Asian countries, more soy products are consumed compared to Western countries, which is reflected in a higher estimated daily intake, as indicated in Table 22.1.

Flavanones are mainly found in the solid parts and the membranes separating the segments of citrus fruits. Therefore, a five times higher flavanone content is found in whole fruits compared to juice.

Anthocyanidin is present in fruits and vegetables that have red, blue, and purple pigments. Black currants and blackberries contain about 2–4 g of anthocyanidin per kilogram fresh weight. The amount of anthocyanidins in a food product relates to its color intensity. Furthermore, anthocyanidin is found in fruit skin and its content becomes higher as a fruit ripens.

Flavanol/flavan-3-ol The main flavanols are catechin and epicatechin, which are present in cocoa beans, dark chocolate, and green tea. A cup of green tea can provide up to 200 mg catechins. Several processes and conditions affect

the flavanol content of cocoa, such as the variety and country of origin. Also the fermentation and roasting process, that has been applied, determines the flavanol content of cocoa beans.

Stilbenes

The most widely known and studied stilbene is *trans*-resveratrol, which is found in low quantities in red wine and in the skin of black grapes. The average *trans*-resveratrol content of red wines is 1.9 mg/L, varying from nondetectable levels up to 14.3 mg/L. *Trans*-resveratrol is thought to play a role in explaining the "French paradox."

Lignans

A rich dietary source of lignans is flaxseed (>300 mg/100 g). Other sources are cereals, grains, fruits, and certain vegetables. Lignan content of grain products varies from 7 to 764 mg/100 g [6].

Effects of Polyphenols on Lipid and Lipoprotein Metabolism

In the following paragraphs, effects of chocolate and cocoa on lipid and lipoprotein metabolism are reviewed. Chocolate and cocoa are main sources of flavanols, such as epicatechin. In addition, attention will be paid to the stilbene resveratrol, a polyphenol with supposed cardioprotective effects. Finally, the effects of tea catechins and soy isoflavones are discussed.

Flavonoids

In a recent meta-analysis, effects of flavanol-rich cocoa products or dark chocolate on the serum lipid profile were summarized [7]. Ten controlled intervention studies were identified, including 320 subjects. Daily flavanol intake between the studies varied widely (88–963 mg). Compared with control, it was estimated that consumption of the cocoa products or dark chocolate for 2 weeks significantly decreased LDL cholesterol by −0.15 mmol/L (95% confidence interval, CI: −0.27, −0.03 mmol/L). No significant effect on LDL cholesterol was observed in longer-term studies (4–12 weeks). Total cholesterol concentrations were significantly reduced by 0.16 mmol/L (95% CI: −0.30, −0.02 mmol/L). In both short-term and longer-term studies, no significant effects on serum HDL cholesterol and triglyceride levels were observed (Fig. 22.2).

A possible dose–effect relationship was examined by dividing the studies based on the intake of flavanols (either <500 mg or >500 mg flavanol daily). Surprisingly, a daily flavanol

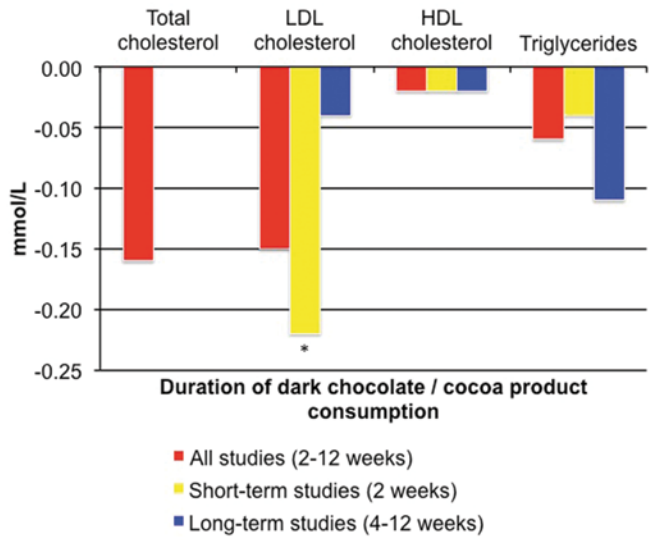

Fig. 22.2 Effects of dark chocolate/cocoa product consumption on serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations. A statistically significant (*) reduction in serum LDL cholesterol was found in short-term studies, while other lipids and lipoproteins did not change. In longer-term (4–12 weeks) studies, effects did not reach statistical significance. Effects of study duration on serum total cholesterol levels were not reported. *LDL* low-density lipoprotein, *HDL* high-density lipoprotein. (Results are from ref. [7])

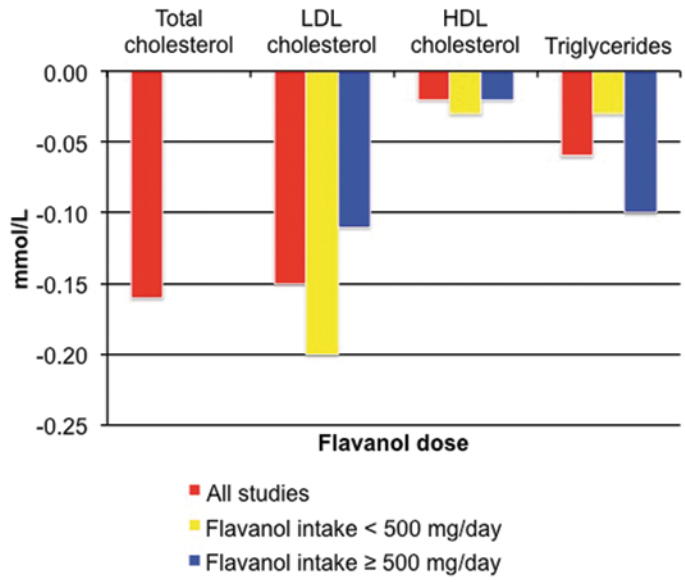

Fig. 22.3 Effects of flavanol intake on serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations. Differences in effects between the two levels of intakes did not reach statistical significance. Effects of different intake level on serum total cholesterol levels were not reported. *LDL* low-density lipoprotein, *HDL* high-density lipoprotein. (Results are from ref. [7])

intake of < 500 mg appeared to lower LDL cholesterol more efficiently than intakes ≥ 500 mg (−0.20 mmol/L vs. −0.11 mmol/L, respectively), but the differences between these effects were not statistically significant (Fig. 22.3).

A comparable LDL cholesterol-lowering effect of −0.07 mmol/L (95% CI: −0.14, 0.00 mmol/L) was found in a more recent meta-analysis that included 21 studies and 986 subjects [8]. In studies that lasted maximally 3 weeks, a mean effect on serum LDL cholesterol of −0.22 mmol/L was observed. However, no effect was observed in longer-term studies (3–26 weeks), which raises questions on the clinical usefulness of these observations. Without considering study duration, HDL cholesterol was increased by 0.03 mmol/L (95% CI: 0.00, 0.06 mmol/L). Beneficial effects on HDL cholesterol were more pronounced in longer-term trials (> 3–26 weeks; Fig. 22.4). In this meta-analysis, the effects of epicatechin (and not of total flavanols) were also examined, but no significant effects on serum total, LDL, and HDL cholesterol were found.

Human studies have also shown an increased in vitro resistance of LDL to oxidation after intake of polyphenol-rich fractions from cocoa [9, 10]. Furthermore, polyphenol-rich cocoa fractions increased fecal cholesterol excretion in rats [11].

Trans-resveratrol

The most widely known stilbene, resveratrol, is thought to exert several cardioprotective effects, among others the modulation of lipid and lipoprotein metabolism. Indeed, many in vitro or animal studies suggest that resveratrol has positive effects on proteins that are involved in reverse cholesterol transport. These proteins include peroxisome proliferator-activated receptor gamma (PPARγ), liver X receptor alpha (LXRα), 27-hydroxylase, and ATP-binding cassette A1 (ABCA1) [12], which are involved in cholesterol efflux. In hamsters, resveratrol may in vitro protect LDL against oxidation, downregulate 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and increase the apolipoprotein A-I (apoA1) to apoB ratio [13]. Furthermore, resveratrol might enhance cholesterol efflux by preventing HDL particles from oxidation (Fig. 22.5) [14]. The possible anti-atherosclerotic effect of resveratrol might also be related to lower a reduced transfer of cholesteryl esters from HDL to very-low-density lipoprotein (VLDL) and LDL through an inhibitory effect on cholesteryl ester transfer protein (CETP) activity [15].

In humans, a recent meta-analysis using seven studies showed no statistically significant

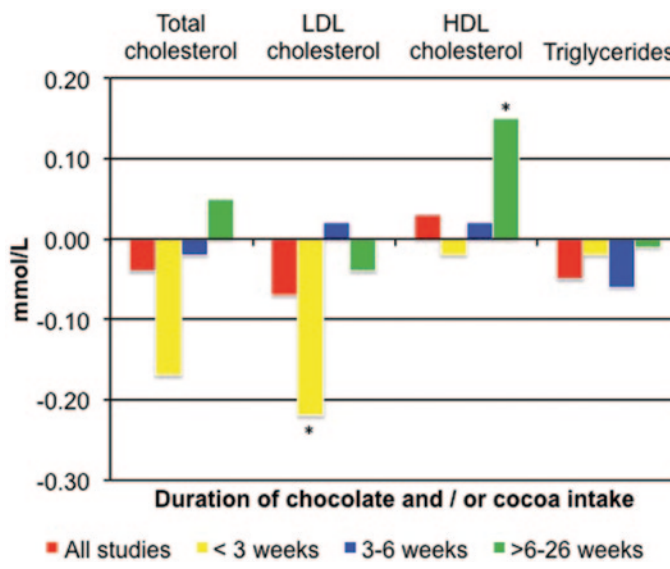

Fig. 22.4 Effect of duration of chocolate and/or cocoa intake on serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations. Statistically, significant differences between the groups with different study durations are indicated with an asterisk. *LDL* low-density lipoprotein, *HDL* high-density lipoprotein. (Results are from ref. [8])

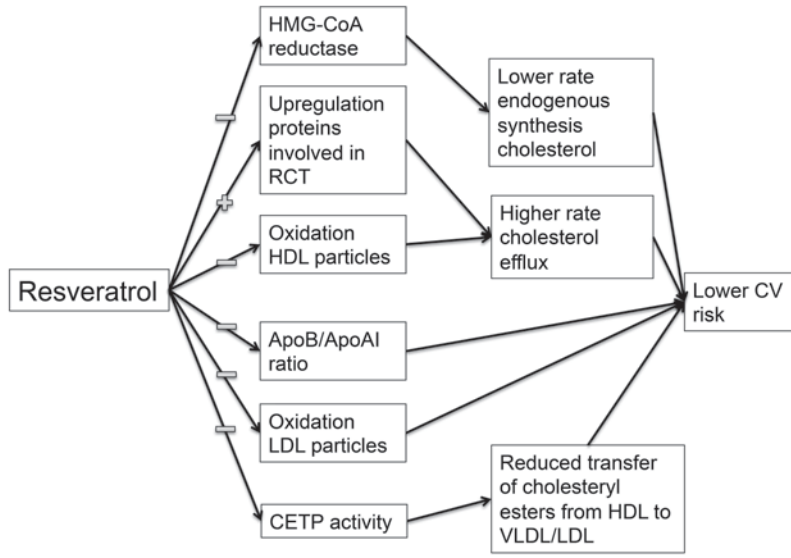

Fig. 22.5 Proposed effects of resveratrol on cholesterol and apolipoprotein metabolism and cardiovascular risk as seen in animals. + stimulating effect, − inhibiting effect, *RCT* reverse cholesterol transport, *HDL* high-density lipoprotein, ApoB apolipoprotein B100, ApoAI apolipoprotein AI, *LDL* low-density lipoprotein, *CETP* cholesteryl ester transfer protein, *VLDL* very-low-density lipoprotein, *CV* cardiovascular

effect of the intake of purified *trans*-resveratrol and extracts containing resveratrol on serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations [16]. Results were not related to the dose of resveratrol used, study duration, or cardiovascular risk profile of the participants. However, the number of studies may have been too few to examine these potential sources of heterogeneity into detail. Also, none of the studies was specifically designed to examine the effects of resveratrol on the serum lipoprotein profile. Finally, in some of the studies, subjects were on statin therapy, which may have masked any potential effects of

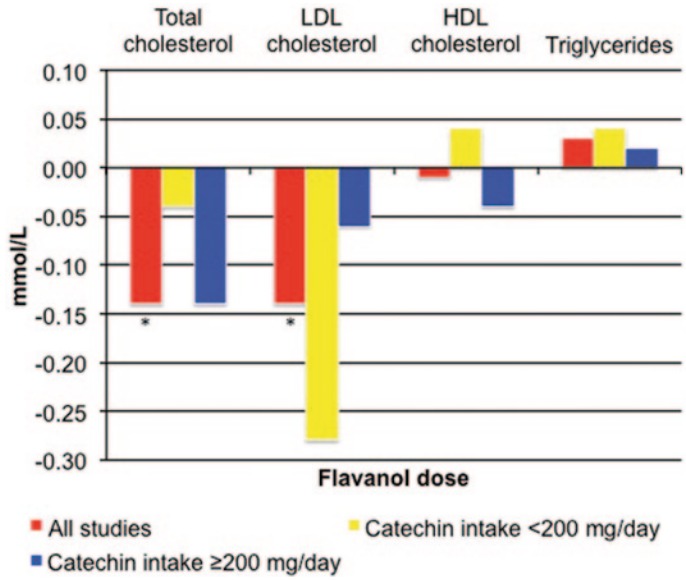

Fig. 22.6 Effects of catechin intake on serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations. A statistically significant (*) reduction in serum total and LDL cholesterol was found in all studies, while HDL and triglyceride concentrations did not change. Differences in effects between the two levels of intakes did not reach statistical significance. *LDL* low-density lipoprotein, *HDL* high-density lipoprotein. (Results are from ref. [17])

resveratrol. Thus, before more definitive conclusions can be drawn, more powerful trials on the effects of *trans*-resveratrol supplementation on lipid metabolism in hyperlipidemic subjects are needed.

Catechins and Isoflavones

Animal studies have shown positive effects on lipid metabolism of green tea catechins, of which epigallocatechin is the most abundantly present. These effects include reducing intestinal lipid absorption, promoting fecal cholesterol excretion, and inhibiting enzymes involved in hepatic cholesterol synthesis.

In humans, a significant reduction in total and LDL cholesterol after green tea catechin intake was found in a meta-analysis, including 1415 subjects in 20 trials. This meta-analysis included studies with green tea catechin doses ranging between 145 and 3000 mg, while study duration varied between 3 and 24 weeks. Green tea catechins significantly lowered total cholesterol (−0.14 mmol/L; 95% CI: −0.25, −0.03 mmol/L), and LDL cholesterol (−0.14 mmol/L; 95% CI: −0.26, −0.02 mmol/L). No significant effects on HDL cholesterol and triglycerides were observed ([17]; (Fig. 22.6).

Regarding the effects of isolated soy isoflavones (in which the soy protein is absent) on lipid metabolism, a meta-analysis with 10 studies and 959 subjects did not suggest that these components do affect serum lipoprotein concentrations [18]. However, soy protein containing intact isoflavones showed a beneficial effect on the lipid profile in two meta-analyses [19, 20].

Effects of Polyphenols on Endothelial Function

The main focus of this paragraph is on the polyphenol epicatechin, because this flavonol from cocoa has been studied most widely with respect to endothelial function. Furthermore, many recent studies have focused on the stilbene *trans*-resveratrol. Therefore, effects of this compound on endothelial function are discussed as well.

Flavonoids

Regarding the positive effects of flavanols on endothelial function, a recent meta-analysis found a beneficial effect of chocolate, cocoa, and/or cocoa flavanols on flow-mediated dilatation (FMD) [8]. An acute effect—2 h after intake of chocolate or cocoa—of on average 3.19% (95% CI: 2.04%, 4.33%) on FMD was reported. Epicatechin dose

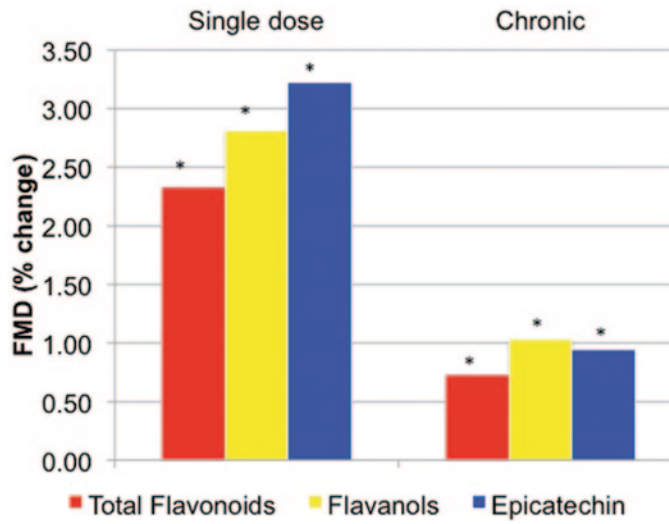

Fig. 22.7 Effect of single-dose and chronic (minimum of 2 weeks intervention) flavonoid intake on % change in FMD. All results were statistically significant (*). *FMD* flow-mediated dilation. (Results are from ref. [22])

might play a role in this beneficial effect, as an increased epicatechin dose (>100 mg/day) from the experimental products showed a more pronounced effect on acute FMD compared to lower epicatechin doses. A mean difference in FMD of 1.34% (95% CI: 1.00%, 1.68%) was observed after chronic intake (3–26 weeks) of chocolate/cocoa.

Another meta-analysis also reported beneficial effects of the intake of flavonoid-rich cocoa on several cardiovascular risk factors. In this study, an increase of 1.53% (95% CI: 0.67%, 2.40%) in FMD after chronic (2–18 weeks) consumption of flavonoid-rich cocoa was found [21]. One meta-analysis examined the effect of flavonoid subclasses on FMD. The acute mean effect of epicatechin on FMD was 3.22% (95% CI: 1.94%, 4.50%), whereas for total flavonoids (the sum of flavanols, catechol flavonoids, procyanidins, epicatechin, and catechins) an acute effect of 2.33% (95% CI: 1.58%, 3.08%) was reported. Also, in studies that reported the longer-term effects (≥2 weeks intervention), epicatechin intake from the experimental products showed a somehow larger improvement (0.94%; 95% CI: 0.47%, 1.42%) on FMD than total flavonoid intake (0.73%; 95% CI: 0.17%, 1.30%) (Fig. 22.7) [22].

The mechanisms to explain the beneficial effect of cocoa polyphenols in general or of epicatechin in particular on FMD have not been elucidated yet. It has been suggested that epicatechin enhances nitric oxide (NO) bioavailability and therefore exerts a positive effect on FMD. This increase in NO might be the result of an increased expression and/or activity of endothelial nitric oxide synthase (eNOS), but also of changes in NO bioavailability or changes in the expression of eNOS-related proteins. This eNOS activation is likely to be mediated via the calcium/calmodulin pathway, as described in Fig. 22.8 [23].

Other suggested pathways that positively influence endothelial function include the antioxidant capacity of flavonoids, which leads to lower oxidative stress levels in vivo and a reduced endothelial adhesion molecule expression in vitro [24]. Furthermore, cocoa and chocolate showed positive effects on fasting insulin and homeostatic model assessment-insulin resistance (HOMA-IR) [8][25]. This finding might be related to the positive effect of chocolate and cocoa on endothelial function, as these processes share common pathways [25].

The upregulation of NO production has several positive effects on the vasculature. First, NO exerts antihypertensive effects through vasodilation. Second, the antithrombotic effect of NO is reflected by the inhibition of platelet aggregation. This effect might be mediated by the inhibition of cyclooxygenase-2 (COX-2), which catalyzes the synthesis of prostaglandin E_2 (PGE_2). Decreas-

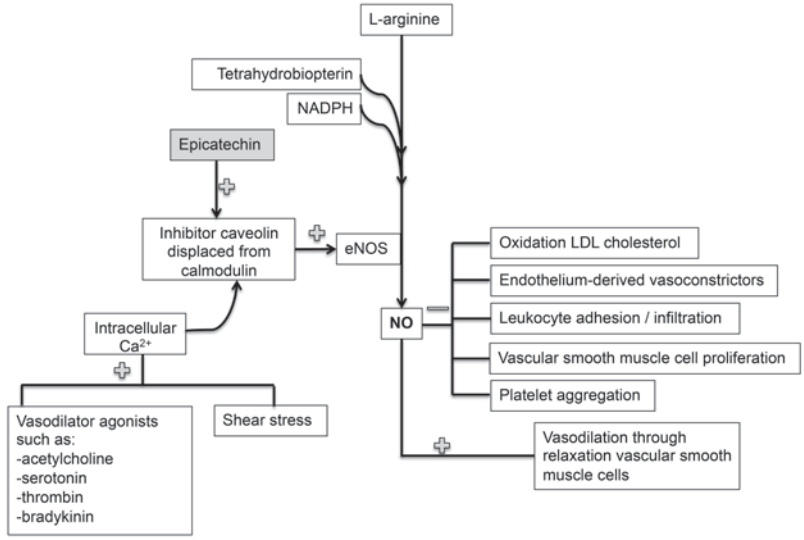

Fig. 22.8 Effects of NO on endothelial function and the possible role of epicatechin in this process. *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide, *NADPH* nicotinamide adenine dinucleotide phosphate, *LDL* low-density lipoprotein. (Based on ref. [23])

ing PGE_2 synthesis has a beneficial effect on platelet aggregation. Finally, the ability of NO to prevent leukocyte adhesion to the endothelium, the reduction of LDL oxidation, and the inhibition of vascular smooth muscle cell proliferation may contribute to the anti-atherosclerotic effects of NO production.

Trans-resveratrol

A recently published article showed an acute, dose-dependent, FMD-improving effect of resveratrol [26]. Here, resveratrol was given to 19 overweight or obese men or postmenopausal women who were borderline hypertensive (systolic blood pressure between 130 and 160 mmHg or diastolic pressure between 85 and 100 mmHg), but did not receive any treatment. Subjects received six capsules containing in total 30, 90, or 270 mg *trans*-resveratrol. The study had a double-blind, randomized crossover design and subjects were asked to consume the indicated doses and a placebo at weekly intervals.

Several in vitro and animal studies have shown that resveratrol may stimulate eNOS and improve NO bioactivity [27]. Except for the effects of resveratrol on NO production, the compound is also thought to exert positive effects on endothelial dysfunction by inhibiting NFκB, leading to lower cytokine production and less vascular inflammation [27]. Furthermore, platelet aggregation might be prevented by resveratrol through prevention of eNOS acetylation and sirtuin type 1 activation.

Polyphenol Metabolism

Flavonoids

Flavonoids, except for catechins, are present in the diet as β-glycosides. Flavonoid glycosides, but not glucosides, are thought to pass the small intestine, followed by hydrolysis to aglycones by enterobacteria in the cecum and colon [28]. Absorption of flavonoid aglycones in the large intestine is facilitated through their lipophilicity by passage across the phospholipid bilayer of the cellular membranes. After entering the circulation, the flavonoid aglycones are further metabolized in the liver (*O*-methylation,

glucuronidation, and/or sulfation). Part of the metabolites will be excreted in the bile, followed by a return to the intestinal lumen, where they might either be reabsorbed by intestinal cells or excreted into feces. Glucosides are thought to be absorbed from the small intestine, which leads to higher plasma values because of the higher absorption efficiency. Catechins are present in foods as aglycones or esterified with gallic acid. Both forms are absorbed from the small intestine.

Bioavailability of flavonol glycosides differs among the separate classes. Time to reach plasma peak concentrations vary between less than 0.5 and 9 h, with the highest bioavailability of quercetin glucosides from onions [29]. Flavonols that are particularly present in cocoa, (epi)-catechin and procyanidin, reach a peak concentration after 2 h [30].

Trans-resveratrol

After oral administration, *trans*-resveratrol is mainly absorbed in the duodenum and, to a lesser extent, the jejunum. After that, metabolic conversion via intestinal and hepatic conjugation starts. Both intestinal subcellular fractions and liver cells are capable of glucuronidation and sulfation of resveratrol, although glucuronidation prevails over sulfation in the liver. This results in low plasma levels of free resveratrol, whereas the major metabolites (*trans*-)resveratrol-3-*O*-sulfate, (*trans*-)resveratrol-3-*O*-glucuronide, and (*trans*-)resveratrol-4'-*O*-glucuronide are mainly found in plasma after resveratrol intake.

A second resveratrol and resveratrol-metabolite peak is observed 6 h after resveratrol intake. This is explained by enterohepatic recirculation of conjugated resveratrol metabolites. During this process, resveratrol is metabolized in the liver and its conjugates are excreted in the bile. This is followed by reabsorption of the conjugates in the small intestine and subsequent return to the liver or excretion via feces (Fig. 22.9).

Resveratrol absorption is at least 70%, and the compound and its metabolites are mainly excreted via urine. The absorption is delayed when resveratrol is taken with foods, especially with a high-fat meal.

Dosing Regimen and Adverse Effects

A recent scientific opinion by the European Food Safety Authority (EFSA) stated that 200 mg of cocoa flavanols should be consumed daily in order to "maintain endothelium-dependent vasodilation, which contributes to normal blood flow" [31]. This amount of flavanols can be consumed through 10 g of high-flavanol dark chocolate. For the other components discussed in this chapter, no effective doses have been formulated by health authorities.

Conclusions

Scientific interest in the relation between polyphenol intake and cardiovascular health has considerably grown during the past decades. Polyphenols can be found in numerous products of which flavonoids from cocoa, the stilbene *trans*-resveratrol from black grapes, green tea catechins, and soy isoflavones have been most widely studied.

Green tea catechins were shown to improve the serum lipid profile by decreasing total cholesterol and LDL cholesterol. Isolated soy isoflavones did not affect serum lipoprotein concentrations, whereas soy protein containing intact isoflavones may have a beneficial effect on the lipid profile. Consumption of cocoa products or dark chocolate for 2 weeks showed a significant decrease in LDL cholesterol. This effect was not found after 4–12 weeks consumption of these products, which raises questions on the clinical usefulness of this finding. HDL cholesterol showed an increase in longer-term studies. Next to this, beneficial effects of flavonoids from cocoa on FMD were reported, which underlines the positive role of these compounds in cardiovascular risk reduction. *Trans*-resveratrol does not affect serum lipid and lipoprotein concentration, but may improve vascular health. However, more studies are needed to substantiate these findings. Several mechanisms underlying these effects of the various polyphenols have been proposed, but an unambiguous explanation cannot be given yet.

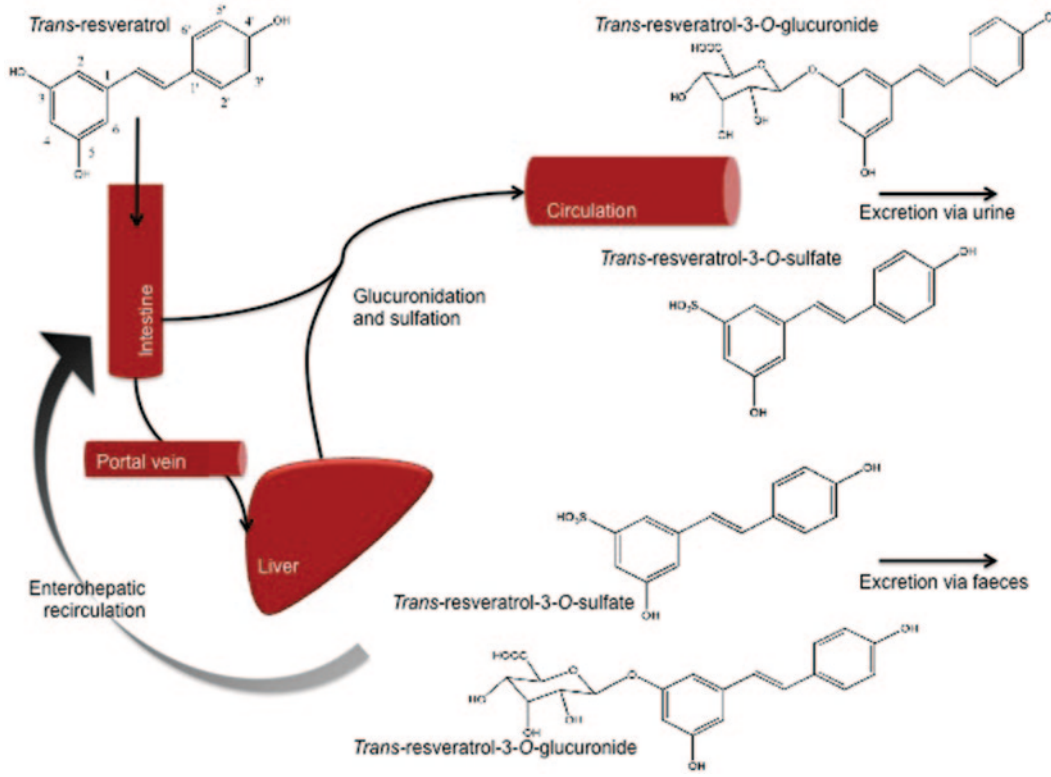

Fig. 22.9 *Trans*-resveratrol is absorbed in the intestine, followed by glucuronidation and sulfation by liver and intestinal subcellular fractions, which results (among others) in the formation of *trans*-resveratrol-3-*O*-glucuronide and *trans*-resveratrol-3-*O*-sulfate. These conjugates can be excreted via urine or undergo enterohepatic recirculation. During this process, *trans*-resveratrol conjugates are returned to the liver via the bile duct, intestines, and portal vein and can either be excreted via feces or reenter the circulation and excreted via urine

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Dietary Supplements for Cholesterol Management

23

Jaime P. Almandoz

Introduction

The United States Dietary Supplement and Health Education Act categorizes the use of botanical or natural medicines, including those used for the treatment of hypercholesterolemia, as "dietary supplements" [1]. Patients tend to seek out alternative or complimentary therapies for one of three reasons. First, they may be dissatisfied with conventional or prescription medications that have been ineffective, harmful, too costly, or technologically oriented. Second, the selection of alternative therapies may give the patient a greater sense of autonomy and empowerment with respect to their healthcare decisions. Third, and most commonly, alternative therapies are more compatible with the patient's beliefs, values, and healthcare philosophy [2].

There is a perception by the general public that botanical products are inherently safe because they are natural and have been used as traditional folk remedies. Little attention is paid to the lack of evidence of their efficacy or safety in well-designed controlled trials. Consumers do not consider that these products may be adulterated with prescription medications or contaminated with harmful substances, as there is a lack of regulation and standardization for composition, biological activity, safety, and reporting of adverse events [3, 4].

Around 20% of the US population take botanical supplements with the highest consumption in older non-Hispanic white women [5]. The use of supplements in those over 65 years of age is increasing and it is worth noting that almost 30% of people in this age range also take five or more prescription medications [6, 7]. As less than half of patients disclose the use of supplements to their physician and <1% to their pharmacist, there is significant potential for medication interactions [6].

Numerous dietary supplements are taken to lower cholesterol; however, many do not demonstrate efficacy or safety in well-designed clinical trials, and struggle with the limitations listed above. The evidence for the more commonly used supplements is reviewed in this chapter while polyphenols, isoflavones, and plant sterols are reviewed elsewhere.

Red Yeast Rice

Red Yeast Rice (RYR) is a traditional Asian food item that is used to flavor, color, and preserve food (Fig. 23.1). The medicinal value of RYR was first promoted during the Tang dynasty, around AD 800, to aid digestion and circulation [8]. RYR consists mainly of nonglutinous rice, red yeast (*Monascus purpureus*), and fermentation by-products comprising of polyketides

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Fig. 23.1 Red yeast rice: nonglutinous rice fermented with *Monascus purpureus*. (Courtesy of Robin Kok)

known as monacolins, fatty acids, and trace elements. In 1979, Endo discovered monacolin K, a polyketide that inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an early rate-limiting step in cholesterol synthesis [9, 10]. Monacolin K, also known as mevinolin or lovastatin, accounts for around 90% of the total monacolin fraction in RYR. Monacolin K and its hydroxyl acid form, monacolin KA, are the predominant active ingredients in most commercially available formulations of RYR, but RYR also contains plant sterols, isoflavones, and *cis*-monounsaturated fatty acids, which are less potent cholesterol-lowering agents [8, 11, 12].

RYR supplements are widely available over the counter and contain unpredictable concentrations of the active constituents. When Gordon et al. analyzed 12 RYR products, there was remarkable variability in the levels of total monacolins (0.31–11.15 mg/capsule), monacolin K (0.10–10.09 mg/capsule), and monacolin KA (0.00–2.30 mg/capsule) [13]. Also, one third of the products contained elevated levels of citrinin, a toxic byproduct of fermentation that is known to be mutagenic and nephrotoxic [14, 15]. The US Food and Drug Administration (FDA) has ruled that RYR is a drug and not a dietary supplement because it contains monacolin K (lovastatin), so manufacturers were told to modify their products so that they do not contain this active component [16, 17]. In spite of this, RYR supplements continue to be sold in the USA with varying but sometimes significant amounts of monacolin K [8].

Multiple clinical trials have evaluated the efficacy of RYR in lowering cholesterol. In one study, 83 subjects with hypercholesterolemia were randomized to 2.4 g/day of RYR (0.4% monacolins by weight) or placebo. After 12 weeks of treatment, as compared to the placebo, RYR significantly reduced total cholesterol (16.1%), low-density lipoprotein (LDL) cholesterol (22.4%), and triglycerides (11.3%). There was no significant effect on high-density lipoprotein (HDL) cholesterol concentration. The treatment was well tolerated with no hepatic or renal function abnormalities noted in any of the participants. However, there was a single report of musculoskeletal chest pain at week 12 in the treatment group [18].

RYR has demonstrated utility in those who have difficulty tolerating conventional cholesterol-lowering therapy with HMG-CoA reductase inhibitors (statins) due to statin-induced myalgia, myopathy, elevated liver transaminases, and gastrointestinal upset. In a placebo-controlled trial, 62 patients with a history of statin-induced myalgia were randomized to receive 3.6 g/day of RYR (6.48 mg monacolins/day) for 24 weeks. There was no increase in liver transaminases, creatine kinase (CK), or pain scores, but significant reductions in total cholesterol (14.9%) and LDL-cholesterol (21.3%) were achieved [19].

Investigators from China randomized 4870 subjects with a history of coronary artery disease (CAD) and dyslipidemia to receive either a placebo or RYR extract containing 12 mg of monacolin K daily for 4.5 years. When compared to placebo, the treatment group had significant reductions in total-cholesterol (10.9%), LDL-cho-

lesterol (17.6%), non-HDL cholesterol (16.6%), and triglycerides (14.6%), and there was a small but significant increase in HDL-cholesterol of 4.2%. There was a 45% relative reduction in coronary events in addition to 30 and 33% reductions in cardiovascular and total mortality, respectively. Total adverse events and study discontinuation were reported as similar in both groups. There were also minor transient changes in liver transaminase and CK levels of unreported severity or duration in both groups [20].

As monacolins are HMG-CoA reductase inhibitors, it is not surprising that RYR shares the potential adverse effects of prescription statin medications, including myalgia, myopathy, rhabdomyolysis, and elevated liver transaminases [21–24]. Patients should be cautioned not to use RYR preparations in conjunction with statins or medications that affect their metabolism, such as those that utilize the cytochrome P450 3A4 pathway. Allergy to RYR appears to be rare but there is a case report of an anaphylactic reaction in a German butcher who used RYR as an ingredient in sausages [25].

RYR products that contain the active monacolins are effective in lowering total cholesterol, LDL-cholesterol, and triglycerides. The monacolin content varies between brands and even batches of commercially available preparations, which leads to inconsistencies in the dose and efficacy. It may be useful as an alternative lipid-lowering agent in patients who are intolerant to prescription statin therapy, but similar precautions should be taken, as it is an HMG-CoA reductase inhibitor with a similar side effect profile. RYR may also be useful in the secondary prevention of those with established cardiovascular disease but additional research and outcomes data are needed.

Soluble Dietary Fiber (SDF)

The FDA recommends the addition of 3 g/day of β-glucan or 7 g/day of SDF to a low saturated fat, low-cholesterol diet in order to reduce cholesterol and the risk of coronary heart disease (CHD). The Administration issued this recommendation after reviewing 33 clinical studies that evaluated the effect of supplementary dietary fiber on lipid levels [26].

Soluble fiber is thought to lower total and LDL-cholesterol concentrations through several mechanisms. Intestinal absorption of cholesterol is reduced in the presence of soluble fiber, which may be due to viscous soluble fiber forming a physical barrier to absorption, alteration in the emulsification of dietary fat, reduction in the formation of small mixed micelles that are more efficiently absorbed, or a combination [27, 28]. In human studies, soluble fiber increases the fecal excretion of bile acids and cholesterol, resulting in greater bile acid synthesis from circulating cholesterol in the blood [29, 30]. Soluble fiber reduces postprandial glucose absorption and insulin levels, which may reduce cholesterol synthesis, as insulin is a stimulator of HMG-CoA reductase [31–33]. Finally, diets high in fiber can lead to changes in gut microbiota that may have multiple beneficial effects on glucose and lipid metabolism beyond the intestinal lumen [34]. Fermentation of soluble fiber by gut flora produces short-chain fatty acids such as acetate, propionate, and butyrate, which may suppress hepatic cholesterol production [35, 36].

β-glucans are the principal components of the endosperm cell walls in cereals such as oats and barley. They are highly viscous, soluble, nonstarch polysaccharides consisting of linear glucose chains with varying molecular weights depending on the method of extraction [37]. There have been multiple studies and meta-analyses that have evaluated the effect of oat β-glucan on cholesterol concentrations. Brown et al. [38] evaluated data from 25 controlled trials and found that an average of 5 g/day of soluble oat fiber significantly reduced total, HDL, and LDL-cholesterols. There was a significant dose–response effect that for every gram of soluble oat fiber consumed per day, the total cholesterol decreased by 1.4 mg/dL, LDL cholesterol 1.2 mg/dL, and HDL-cholesterol 0.07 mg/dL, but there was no effect on triglyceride concentrations.

A more recent review assessed data from 20 clinical trials, one systematic review and the above meta-analysis. Of the studies reviewed,

Fig. 23.2 Psyllium seed husks from *Plantago ovate*. (Courtesy of Cary Bass)

70% reported a significant reduction in circulating cholesterol concentrations and the authors concluded that daily doses of at least 3 g/day of β-glucan result in 5–10% reductions in total cholesterol and LDL-cholesterol in normocholesterolemic or hypercholesterolemic individuals. The authors found that β-glucan is more effective at lowering LDL-cholesterol when given in a liquid form than when it is delivered in solid form like a muffin [39]. For example, men with moderate hypercholesterolemia experienced a 6% lowering in both total cholesterol and LDL-cholesterol after drinking oat milk containing 3.8 g of β-glucan per day [40]. Whereas, Kerckhoffs et al. found no significant reduction in cholesterol in either group when they randomized 48 subjects to bread and cookies with wheat fiber or 5.9 g of β-glucan per day. In a subsequent experiment reported in the same paper, when the subjects were given orange juice containing 5 g/day of β-glucan or wheat fiber, the β-glucan group experienced a 7% reduction in total cholesterol compared with the controls [41]. It is proposed that the cholesterol-lowering efficacy of the baked products may be attenuated because exposure to heat reduces the molecular weight and viscosity of β-glucan polymers [42].

Psyllium is another popular soluble fiber that is the mucilaginous seed husk of the *Plantago ovata* plant (Fig. 23.2). The active cholesterol-lowering component of psyllium is believed to be arabinoxylan, a polysaccharide with a xylose backbone and arabinose side chains [43]. As early as 1965, Garvin et al. reported a 9% reduction in total cholesterol in participants consuming 9.3 g/day of psyllium hydrocolloid for 3 weeks [44]. When Brown et al. [38] analyzed data from 12 trials with a daily dose range of 2–10 g of psyllium, they found that for every gram taken per day, there was a 1.4 mg/dL reduction in total cholesterol, and in the four studies evaluating LDL-cholesterol, a 2.6 mg/dL reduction. The decrease in HDL-cholesterol was trivial (0.15 mg/dL per gram of psyllium) but significant, whereas there was no significant effect on triglycerides.

The meta-analysis that included 21 studies by Wei et al. [45] reported that the use of psyllium (3–20.4 g/day) is associated with a lowering of total cholesterol by 14.5 mg/dL and LDL-cholesterol by 10.8 mg/dL in subjects with mild-to-moderate hypercholesterolemia. Based on their findings, the authors calculated that the consumption of psyllium 5, 10, 15 g/day could result in 5.6, 9.0, and 12.5% decreases in LDL-cholesterol, respectively. As opposed to β-glucan, the form in which psyllium was consumed, e.g., bulk laxative or enriched food, did not significantly affect the degree of cholesterol lowering. Similar to studies described earlier, the authors noted a significant but minimal reduction in HDL-cholesterol and no effect on triglycerides. These findings are similar to those of Anderson et al. [46] who included data from three unpublished studies in a meta-analysis of eight controlled trials. They found that consuming 10.2 g of psyllium per day, as part of a low-fat diet, reduced total cholesterol by 4%, LDL cholesterol by 7%, and the ratio of apolipoprotein B to apolipoprotein A-I by 6%.

The cholesterol-lowering effects of dietary fiber appear to be additive when used in conjunction with a low-fat diet and statin therapy. In a placebo-controlled study of hypercholesterolemic patients with a mean baseline LDL-cholesterol of 173 mg/dL, 8 weeks of treatment with 10 mg of simvastatin plus 15 g of psyllium husk (Metamucil®) per day reduced LDL-cholesterol levels by 63 mg/dL (36%), which was the same amount as 20 mg of simvastatin alone [47].

Soluble fiber is generally well tolerated but should be introduced gradually to avoid gastrointestinal upset. Anaphylaxis and allergies to SDF preparations, separate from food intoler-

ances, are rare but have been reported in isolated cases along with hypersensitivity in healthcare workers with occupational exposures to psyllium [48–50]. Certain prescription medications, such as oral contraceptives or antidepressants, should be taken at a different time to the SDF supplements as the fiber can interfere with their rate of absorption and the total absorbed dose [51].

SDF works in multiple ways to beneficially reduce total cholesterol and LDL-cholesterol as part of a low-fat diet. Liquid or unheated forms may be more effective because the reduction in molecular weight and viscosity that occurs with cooking may decrease its cholesterol-lowering efficacy. SDF may be useful in patients who are unable to tolerate other cholesterol-lowering agents, as an add-on for those on maximal doses of other agents who are not at goal, or to minimize the dose of statin used and its potential side effects. The two most popular forms of SDF are β-glucan and psyllium. If patients would like to incorporate these fibers into their diet, they should aim for at least 3 g/day of β-glucan and 15 g/day of psyllium to achieve significant cholesterol-lowering.

Nuts

Nuts are a recognized sources of poly- and *cis*-monounsaturated fatty acids, dietary fiber, vitamins, minerals, and bioactive compounds like phytosterols and polyphenols [52]. In particular, walnuts have a very high ratio of polyunsaturated fatty acids, and almonds are rich in *cis*-monounsaturated fatty acids [53]. Although the majority of trials have evaluated the cholesterol-lowering effects of walnuts and almonds, other nuts, e.g., pistachios and macadamias, have also been shown to reduce cholesterol levels [53–56]. Recent evidence suggests that consuming >3 servings of nuts/week as part of a Mediterranean diet is associated with a 30–55% lower rate of cardiovascular events and mortality. The protective effect of nuts is thought to be due in part to their beneficial effects on lipid metabolism [57, 58].

Zambon et al. carried out a randomized crossover trial with 55 hypercholesterolemic Spanish men and women consuming a Mediterranean diet as the control treatment. In half of the patients, 35% of the dietary energy from *cis*-monounsaturated fat was replaced with walnuts (41–56 g/day) for 6 weeks. When compared with the control group, the walnut group experienced a decrease of 4.1% in total cholesterol of and 5.9% in LDL cholesterol [59].

A study of almond supplementation in hyperlipidemic subjects found a significant dose–response reduction in cholesterol levels. Groups receiving 37 g/day experienced 3.1% reduction in total cholesterol and 4.4% reduction in LDL-cholesterol, while the group that received 73 g/day experienced reductions of 5.6 and 9.4%, respectively. There was a significant increase in HDL-cholesterol of 4.6% in the low-dose and 3.8% in the high-dose almond groups but no significant changes in triglycerides [60].

A recent pooled analysis of 25 trials evaluated the effect of nut consumption on lipid levels in subjects with normal and elevated cholesterol levels. The authors found that the lipid-lowering effect of nuts was dose-related but similar between different varieties of nuts. Cholesterol reduction was greatest in those consuming a Western diet with higher baseline LDL-cholesterol and lower body mass index. An average daily intake of 67 g of nuts corresponded to a reduction in total cholesterol by 10.9 mg/dL (5.1%) and LDL-cholesterol by 10.2 mg/dL (7.4%). In those with triglycerides >150 mg/dL, plasma levels were reduced by 20.6 mg/dL (10.2%). When triglyceride levels were <150 mg/dL, nut consumption did not have a significant effect on HDL-cholesterol or on triglyceride levels [61].

Patients should be encouraged to incorporate nuts as part of a balanced calorie-controlled diet. As nuts are calorie-dense, they should be used to displace less healthy foods instead of an additional source of high-fat calories. While the magnitude of cholesterol reduction is small, the numerous nutritional components in nuts (monounsaturated fatty acids, fiber, etc.) may provide additive health benefits to complement other cholesterol-lowering strategies.

Fig. 23.3 Flaxseeds from *Linum usitatissimum*. (Courtesy of Tiia Monto)

Flaxseed

Flax (*Linum usitatissimum*) is a flowering crop that bears golden-brown seeds (Fig. 23.3). It has been cultivated since 6000 BC and the ancient Greeks and Romans valued the seeds for their laxative effects. Today, flaxseed is consumed as whole seeds, ground (meal or powder), or as an expressed oil [62]. Whole flaxseed is comprised of 41% fat, 28% dietary fiber, and 21% protein. It has a unique fatty acid profile of 73% polyunsaturated fatty acids, 18% monounsaturated fatty acids, and 9% saturated fatty acids. Linoleic acid, an omega-6 fatty acid, makes up approximately 16% of the total fatty acids, and alpha-linolenic acid (ALA), an ω-3 fatty acid, constitutes around 57%. Flax is also rich in both soluble and insoluble fiber along with the plant lignan, secoisolariciresinol diglycoside (SDG) [63].

The dietary supplementation of whole flaxseed, flaxseed oil, and lignans has been shown to reduce blood cholesterol in animal studies [64–67]. Flaxseed is thought to lower cholesterol through several mechanisms. First, the SDF component may reduce intestinal cholesterol absorption and promote excretion of bile acids [68]; second, SDG and other lignans have been shown to modulate the activity of 7alpha-hydroxylase and acyl CoA cholesterol transferase [69]; and finally, ALA displaces saturated fats from the diet and may augment LDL-cholesterol catabolism [70].

Many human trials have evaluated the effects of whole flaxseed or its components on cholesterol levels. In a study of 199 postmenopausal Canadian women, Dodin et al. randomized the participants to either 40 g of flaxseed or wheat germ placebo daily for 1 year. At the end of the trial, there was no reduction in total cholesterol or LDL-cholesterol in the flaxseed group compared to the baseline. However, when compared with the placebo group there was a modest but statistically significant reduction in total cholesterol of 7.7 mg/dL and an increase in HDL-cholesterol of 3.1 mg/dL [71].

In contrast, 38 postmenopausal women with hypercholesterolemia were given 38 g of either whole flaxseed or sunflower seeds baked into a muffin every day for 6 weeks. The investigators found that there were significant reductions in total cholesterol in both the flaxseed (6.9%) and the sunflower seed (5.5%) groups. Notably, only the flaxseed group saw significant reductions in LDL-cholesterol (14.7%) and lipoprotein(a) (7.4%) when compared to the baseline levels [72]. As compared to whole flaxseed, the addition of 30 g/day of ground flaxseed did not lower total cholesterol and LDL-cholesterol more than a low-fat diet in a study of 161 prostate cancer patients [73].

The effect of dietary flaxseed lignan (300 or 600 mg SDG daily) was tested in hypercholesterolemic subjects with a baseline LDL-cholesterol >140 mg/dL. After 8 weeks of treatment, significant reductions in total cholesterol and LDL-cholesterol were observed in both treatment groups. In the 600 mg/day group, total cholesterol decreased by 22% and LDL-cholesterol 24%. Plasma concentrations of lignan metabolites increased in both treatment groups, and these levels were significantly correlated with reductions in cholesterol [74]. By contrast, when the same approach was taken in patients with type 2 diabetes who had LDL-cholesterol >160 mg/dL, no significant effects on lipid levels were observed with 360 mg/day of SDG after 12 weeks of treatment [75].

Since one of the bioactive components of flaxseed is proposed to be polyunsaturated fatty acids, some studies have evaluated the effects of providing these fatty acids on lipid reduction. In a study by Harper et al., participants received

either 3 g per day of ALA or olive oil. At the end of 6 months, the adjusted total cholesterol level in the ALA group was 17 mg/dL higher than in the olive oil group ($p=0.03$), but the other lipid fractions as well as the particle sizes were unchanged [76]. Similarly, Paschos et al. compared the effect of 15 mL flaxseed oil (8.1 g ALA) to 15 mL safflower oil (11.2 g linoleic acid) in 35 men with untreated dyslipidemia (total cholesterol > 240 mg/dL), and found no changes in serum lipid concentrations after 12 weeks [77].

To investigate the effects of flaxseed fiber on plasma lipids, Jenkins et al. performed a randomized crossover study in hyperlipidemic subjects using 50 g of partially defatted flaxseed or wheat germ baked into muffins. After 3 weeks, there were significant reductions in total cholesterol (4.6%), LDL-cholesterol (7.6%), apolipoprotein A-I (5.8%), and apolipoprotein B (5.4%). In spite of the decrease in apolipoprotein A-I, there was no significant change in HDL-cholesterol [78].

Evaluating the body of evidence, a recent meta-analysis concluded that flaxseed supplementation does lower total and LDL-cholesterol levels without significant effects on HDL-cholesterol or triglycerides. The authors found reductions in total cholesterol when either whole flaxseed (7.3 mg/dL) or flaxseed lignans (10.8 mg/dL) was given. The reduction in LDL-cholesterol was of similar magnitude (6.2 mg/dL) for both whole flaxseed and lignans. Their analysis suggested that flaxseed oil does not have any beneficial effects on cholesterol levels [79].

Overall, flaxseed supplementation is well tolerated with the principle side effect being increased bowel movements [62]. Anaphylaxis from flaxseeds is rare but case reports are present in the literature [80–82].

The data suggest that supplementing the diet with whole, ground, or defatted flaxseed leads to reductions in total cholesterol and LDL-cholesterol. The lack of effect observed from flaxseed oil suggests that the cholesterol-lowering power of flaxseed may be related to its fiber, lignans, or a combination. The cholesterol-lowering effect of isolated flaxseed lignans is dose dependent and more efficacious in patients with higher cholesterol levels. If flaxseeds are added to the diet to reduce cholesterol, patients should strive to consume at least 40 g of whole or ground seeds per day. There are only a few trials that have evaluated the effect of flax lignans, and although a daily dose of 600 mg SDG shows some promise, the evidence is not strong enough to support the use of isolated lignans.

Soy Protein

Soy Protein (SP) is derived from the soybean, *Glycine max,* in a multistep process that serves to isolate the protein from soybeans by extracting the lipid and fibrous components. This results in an isolated SP concentrate or soy flour that can be further processed into texturized products [83].

There are epidemiological studies that show lower incidences of hypercholesterolemia and ischemic heart disease in Asian countries where greater quantities of soy products are consumed [84, 85]. Interest in the ability of SP to lower cholesterol began when scientists noted that substituting casein (a common milk protein) with SP in atherogenic, but cholesterol-free, diets prevented hypercholesterolemia and atherosclerosis in rabbits [86]. Sirtori et al. then demonstrated that replacing dietary animal protein with SP in hypercholesterolemic patients reduced total and LDL-cholesterol levels, which was subsequently confirmed in a multicenter trial [87, 88]. The evidence is such that the FDA has recommended a daily intake of > 25 g (four servings) of SP as part of a low-fat, low-cholesterol diet to reduce total and LDL-cholesterol levels [89].

Several mechanisms have been proposed for how dietary SP lowers cholesterol. Altered bile acid metabolism and increased gastrointestinal excretion of cholesterol are alleged to be mediated by soy peptides, heat-stable saponins, or trypsin inhibitors that promote cholecystokinin secretion and biliary outflow [89, 90]. However, this has not been supported by clinical studies looking at excretion of fecal neutral steroid or bile acid outputs [91].

It is suggested that phytic acid decreases cholesterol by chelating zinc in the intestine. This results in a higher ratio of copper to zinc,

which favors lower cholesterol levels [92, 93]. In vitro studies have demonstrated an increase in LDL-receptor activity that is mediated by storage proteins contained in soy, particularly the 7S globulin [94, 95]. Clinical studies have shown that LDL-receptor activity and LDL-cholesterol degradation by mononuclear cells are increased by SP-enriched diets in patients with hypercholesterolemia [96].

Soy isoflavones are discussed elsewhere in this publication, and the readers are referred to Chap. 22 for further information. Briefly, soy isoflavones are bioactive molecules that are removed from SP preparations during processing with alcohol. Carefully controlled studies designed to determine whether the cholesterol-lowering effect is from the SP or the isoflavones have concluded that the LDL-cholesterol-reducing effect of SP, although modest, is independent of isoflavones [97].

In 1995, Anderson et al. [98] published a meta-analysis of 29 controlled studies, which concluded that SP lowers cholesterol levels proportional to the degree of hypercholesterolemia and not by the quantity consumed, which ranged from 18 to 124 g/day. Total cholesterol levels were reduced by 20% in those with baseline cholesterol values >335 mg/dL, 7% in those 259–333 mg/dL and there was no significant effect in those with total cholesterol levels <255 mg/dL. However, when they analyzed the data within groups receiving SP, they found a significant dose-related reduction in cholesterol: total cholesterol was reduced by 8.9 mg/dL in the group receiving 25 g SP/day, 17.4 mg/dL in the 50 g SP/day group , and 26.3 mg/dL in the 75 g SP/day group. In this analysis, the type of SP (isolate or textured), diets (usual, low-fat control, etc.), and the age of the subjects did not influence the magnitude or dose dependency of cholesterol reduction.

A more recent meta-analysis by Jenkins et al. [99] analyzed 11 studies with balanced macronutrient profiles and consumption of 20–133 g SP/day. The authors demonstrated that the cholesterol-lowering properties of SP are attributable to both intrinsic and extrinsic factors. They determined that 3.6–6% of LDL-cholesterol lowering is due to the extrinsic displacement of saturated fats and dietary cholesterol from foods when consuming 13–58 g of SP/day. A further 4.3% reduction in LDL-cholesterol was attributed to SP's intrinsic effects.

SP is largely well tolerated but contains at least 16 potential allergens, e.g., soy hydrophobic protein. As a result, soy is on the UN Food and Agriculture Organization's list of the eight most significant food allergens and is felt to be an under-recognized cause of food-related anaphylaxis [100, 101]. Based on the limited data available, SP products that contain isoflavones do not appear to have a negative impact on health in pregnancy or in hormone-dependent malignancy states such as breast or prostate cancer [102]. However, it would be prudent to exercise vigilance and moderation with intake.

In conclusion, SP modestly lowers total cholesterol and LDL-cholesterol through extrinsic and intrinsic mechanisms when it replaces animal protein from the diet. It is most effective in those with higher baseline cholesterol levels and when more than half of the daily protein requirement (>25 g/day) is comprised of SP. The beneficial effects of soy on lipids appear to be from the protein component and not isoflavones, which are not recommended for cholesterol lowering because of a lack of evidence.

Garlic

Garlic (*Allium sativum*) has been widely used in cooking and as a traditional medicine for thousands of years. An ancient Egyptian manuscript, the *Codex Ebers,* cites it as a treatment for heart disorders, tumors, and numerous other complaints [103]. Garlic is comprised mainly of water (65%) and its dry weight is made up of fructose-containing carbohydrates, sulfur compounds, fiber, protein, vitamins, minerals, and saponins. The majority of compounds from garlic are water soluble and less than 1% are oil soluble. As such, it is often difficult to compare studies utilizing different garlic preparations as the content of active compounds will vary depending on whether

it is raw whole garlic, garlic powder, garlic oils, or other extracts [104].

In vitro studies using isolated rodent and human hepatocytes have demonstrated that garlic inhibits cholesterol synthesis in a dose-dependent manner without significant toxicity [105–107]. Work by Gebhardt et al. suggests that the reduction in cholesterol synthesis occurs at the level of HMG-CoA reductase, but at higher doses lanosterol and 7-dehydrocholesterol will accumulate, suggesting effects further downstream in the cholesterol synthesis pathway [106]. More recent work proposes that organosulfur compounds in garlic, e.g., cysteine sulfoxides like *S*-allyl-L-cysteine or allicin, inhibit cholesterol synthesis via 4α-methyl oxidase. Allicin is formed by the action of the heat-sensitive enzyme alliinase on alliin, a sulfur-containing amino acid, when raw garlic is cut or chewed [108]. Water-soluble compounds like *S*-allyl-L-cysteine can inhibit cholesterol synthesis by up to 60% but are cytotoxic at higher concentrations [109, 110]. However, it may be that these in vitro cholesterol-lowering effects are mainly due to cytotoxicity.

The German Association of General Practitioners performed the largest multicenter trial to evaluate garlic as a cholesterol-reducing agent by administering a commercially available garlic powder supplement [111]. They randomized 261 patients with type IIa or IIb hyperlipoproteinemia and total cholesterol and/or triglyceride levels > 200 mg/dL to receive either 800 mg of garlic powder (1.3% alliin content) or placebo daily for 16 weeks. The investigators found a mean decrease in total cholesterol of 12% (from 266 to 188 mg/dL) and a decrease of 17% in triglycerides (226–188 mg/dL). The greatest cholesterol lowering was observed in patients with baseline cholesterol levels of 250–300 mg/dL. In this study, 21% of the treatment group and 9% of the placebo group complained of a mild garlic smell.

Zhang et al. conducted an 11-week study in which 51 healthy subjects received either 8.2 mg per day of garlic oil (containing allyl sulfides) or placebo. A further 27 volunteers received a garlic powder preparation containing 7.8 mg of allicin per day. There was no significant difference in lipid levels seen in the garlic-treated group as a whole at the end of the interventions with either garlic preparation. However, there was a significant improvement in HDL-cholesterol in women, with an increase of 6.2 mg/dL, and a reduction in total to HDL-cholesterol ratio following the garlic oil treatment specifically [112].

Studies on the use of aged garlic extract (AGE) are very limited. The AGE is made by soaking raw garlic in aqueous ethanol for 20 months at room temperature. The filtered extract is reduced until the final product contains 1.47 g/L of *S*-allyl-L-cysteine. Macan et al. [113] assessed the safety of using an AGE (Kyolic®) in 52 subjects on oral anticoagulation therapy. Volunteers were randomized to receive 5 mL of AGE twice daily or placebo for 12 weeks. The authors did not comment on the use of lipid-lowering medications in the study population at baseline, but did report that 9% of subjects in the treatment group and 15% in the placebo group had a history of hypercholesterolemia. The mean total cholesterol concentration at baseline was 184 mg/dL in both groups and the LDL-cholesterol was 104 mg/dL in the treatment group and 108 mg/dL in the placebo group. Following treatment, there were no significant differences in total cholesterol, LDL-cholesterol, or triglyceride concentrations between groups or within groups. There was, however, a modest but significant increase in mean HDL-cholesterol concentration by 2.9 mg/dL in the group that received AGE.

In contrast to the above study, Lau et al. used the same preparation of AGE and randomized 32 participants with untreated hypercholesterolemia (mean total cholesterol 306 mg/dL) to receive 4 mL of AGE or placebo daily for 6 months. In the AGE group, there was an increase in total cholesterol in almost all subjects for the first 3 months. However, by the end of the study, 11 of 15 subjects achieved > 10% reduction in total cholesterol. In another experiment, the authors evaluated 14 subjects with total cholesterol levels < 200 mg/dL and found no significant cholesterol-lowering effect after 6 months of the treatment. Finally, when

ten participants with baseline cholesterol within 240–380 mg/dL range were treated with AGE, six of ten experienced > 10 % cholesterol lowering at 6 months [114].

A study from India evaluated the effect of eating 10 g of raw garlic after breakfast every day on cholesterol levels in 50 medical students. After 2 months of treatment, there was a significant reduction in total cholesterol of 15.5 % compared to the control group. However, an increase in clotting time and fibrinolytic activity was also seen in these otherwise healthy young volunteers. No comment was made by the authors on how well the therapy was tolerated or if any participants dropped out [115].

A meta-analysis by Reid et al. [116] is the most comprehensive to date, and evaluated 39 primary garlic trials. They concluded that garlic supplements are effective in reducing total cholesterol by 17 mg/dL and LDL-cholesterol by 9 mg/dL in those with cholesterol levels > 200 mg/dL. The magnitude of cholesterol lowering was larger in trials of longer duration and in subjects with higher baseline cholesterol levels. The largest total cholesterol reduction was observed with AGE treatment, while the greatest LDL-cholesterol lowering was seen with garlic powder preparations. There was a small but significant increase in HDL-cholesterol (1.5 mg/dL) but no effect on triglycerides. While generally well tolerated, 60 % of the 39 trials reported side effects. Garlic breath, odor, or taste was most frequently reported in the treatment groups receiving raw garlic or garlic powder. However, gastrointestinal side effects were not more prevalent compared to placebo groups and no abnormalities were observed in hepatic or hematological factors.

The current data suggest that garlic, especially garlic powder, is effective at lowering LDL-cholesterol by 10 % or more in patients with hypercholesterolemia who are supplemented > 3 months. There is also a modest but significant increase in HDL-cholesterol but no change in triglyceride levels. There are no data to demonstrate any benefit of garlic supplements in patients who are already taking conventional lipid-lowering therapy.

Fig. 23.4 Rhizomes of *Coptis chinensis,* from which berberine is extracted. (Courtesy of Akiyoshi Matsuoka)

Berberine

Berberine is an isoquinoline alkaloid, originally isolated from the rhizomes of the plant *Coptis chinensis* (Fig. 23.4), which has been used in Asia to treat gastrointestinal infections and diabetes for centuries [117]. The extract has been shown to improve insulin resistance, glucose control, and body weight in several in vitro, animal, and human studies [118–122]. In vitro studies suggest that berberine affects cholesterol metabolism by inhibiting cholesterol synthesis through multiple pathways, including activation of adenosine monophosphate-activated protein kinase, upregulating LDL-receptors through LDL-receptor messenger-ribonucleic acid stabilization in an extracellular signal-regulated kinase (ERK)-dependent manner, increasing transcription of the LDL promoter using the c-Jun N-terminal kinases (JNK) pathway, and reducing proprotein convertase subtilisin/kexin type 9 (PCSK9) mRNA and protein levels [123–126].

In two small studies, obese subjects taking 1.5 g/day of berberine hydrochloride had a non-significant 12 % reduction in total cholesterol; while patients with type 2 diabetes had a significant 13 % decrease in total cholesterol after 3 months [127, 128]. More convincingly, a randomized controlled trial of 144 hypercholesterolemic Caucasian subjects showed that consuming 500 mg of berberine twice daily significantly decreased total cholesterol, LDL-cholesterol, and

triglycerides by 11.6, 16.4 and 21.2%, respectively, and increased HDL cholesterol by 9.1% [129]. When 116 patients with type 2 diabetes and dyslipidemia were randomized to the same dose of berberine (1000 mg/day) for 3 months, there were significant improvements in glucose tolerance and body weight, in addition to significant reductions in total cholesterol (18.1%), LDL-cholesterol (21.1%), and triglycerides (35.9%) [122].

One study evaluated the addition of berberine to conventional statin therapy in a single-center trial. In this study, 63 treatment-naive subjects with hypercholesterolemia were randomized to receive berberine hydrochloride (500 mg twice daily), simvastatin (20 mg once daily), or both for 2 months. There were significant reductions in total cholesterol (9.1, 21.8, 29.1%), LDL cholesterol (14.3, 23.8, 31.8%), and triglycerides (11.4, 22.1, 38.9%) in the berberine, simvastatin, and combination groups, respectively. The cholesterol and triglyceride reductions in the combination group were significantly greater when compared to the simvastatin and berberine monotherapy groups. HDL-cholesterol was not significantly changed in any of the three groups [130]. The addition of berberine to simvastatin therapy appears to be safe and well tolerated. The additional LDL-cholesterol lowering achieved with the combination suggests that berberine could be used with low-dose statin therapy to reduce the dose of statin required as well as potential side effects or toxicities.

Overall, berberine appears to be well tolerated and does not cause elevations in liver transaminases or creatine kinase. The most commonly reported adverse reactions to berberine are gastrointestinal in nature, consisting of self-limiting constipation, flatulence; and in rare instances headache [122, 127, 129, 131]. Berberine appears to be safe for use in patients with chronic liver disease, including chronic hepatitis B, hepatitis C, and alcoholic liver cirrhosis, as evaluated at a single Chinese center. In this study, subjects experienced significant reductions in total cholesterol, LDL-cholesterol, and triglycerides without elevations in liver transaminases or other side effects [132]. In addition to cholesterol-lowering, berberine is known to have antiarrhythmic and vasodilatory effects on the cardiovascular system [133, 134]. There is a case report of a man who was taking berberine for hypercholesterolemia and developed a junctional bradycardia, which reverted to normal sinus rhythm within 10 days of the supplement being discontinued [135].

Fig. 23.5 Resin of the guggul tree (*Commiphora mukul*). (Courtesy of Jacopo Koushan)

In conclusion, berberine appears to be a relatively safe agent that moderately reduces total cholesterol, LDL-cholesterol, and triglycerides by around 10–20%. It may also improve blood glucose control and reduce body weight, which also have beneficial effects on hyperlipidemia. Though data are limited, the available evidence suggests that berberine further lowers cholesterol levels when used in conjunction with statins. Further studies are needed to confirm this effect and the safety of combination therapy before berberine is recommended as a supplemental therapy to conventional lipid-lowering agents or as an alternative in patients with statin intolerance.

Guggul

The *Commiphora mukul,* also known as the guggul tree, is native to arid parts of the Indian subcontinent. Medicinal use of its gum resin (Fig. 23.5) has been described in ayurvedic texts since 600 BC for the treatment of inflammatory conditions, obesity, and atherosclerosis [136]. The lipid-lowering properties of guggul were first evaluated in the 1960s and commercial

preparations have been marketed for this purpose since the late 1980s [137].

The active isomers, E- and Z-guggulsterone [*cis*- and *trans*- 4,17(20)-pregnadiene-3,16-dione], are available in ethyl extracts of the resin [138]. One mode of cholesterol-lowering action is thought to be though the inhibition of the farsenoid X receptor (FXR), which is a nuclear hormone receptor activated by bile acids. For example, FXR-null mice do not exhibit the significant decrease in cholesterol when treated with guggulsterone that is observed in wild-type mice fed with a high-cholesterol diet [139]. There is also evidence that guggulsterone increases hepatic LDL-cholesterol uptake, fecal excretion of sterols and bile acids, LDL-cholesterol catabolism, and inhibits HMG-CoA reductase [140–143].

There are early trials from India that demonstrate significant reductions in LDL-cholesterol with guggulsterone therapy, but many of these suffer from flawed study designs [137, 144, 145]. A randomized, double-blind, placebo-controlled trial with a standardized, commercially available guggul extract (Guggulipid) was performed in 103 adults with primary hypercholesterolemia who were eating Western diets. In this trial, Guggulipid increased mean LDL cholesterol by 4–5% in both the standard-dose and high-dose treatment groups. Only 18% of participants treated with Guggulipid experienced a 5% or greater reduction in LDL cholesterol [146]. Further, Guggulipid caused a hypersensitivity drug rash in 3% of the standard dose and 15% of the high-dose recipients. While one study reporter headache in 71% of treated subjects [147], gastrointestinal upset appears to be the most frequently reported side effect [137]. There has been one case of rhabdomyolysis reported in an Italian man who had been taking *C. mukul* capsules for 2 weeks to treat hypercholesterolemia. He had previously developed elevated serum creatine kinase while on simvastatin therapy, which had normalized before starting *C. mukul* [148].

It appears that guggulsterones do not reduce serum cholesterol in Western populations consuming a Western diet. Despite plausible biological mechanisms for lowering cholesterol, predominantly from rodent models, rigorous studies of guggulsterone therapy in humans have not been able to replicate the early data from Indian trials. This extract also appears to cause an excess of hypersensitivity skin rashes and gastrointestinal side effects requiring cessation of therapy. Therefore, at this time guggulsterone therapy is not recommended for patients looking for alternative cholesterol-lowering therapies.

Policosanol

Policosanol is a mixture of naturally occurring alcohols extracted from the wax of purified sugar cane (*Saccharum officnarum L.*). The extract was initially developed in Cuba where it was first approved for use in 1991. The principal components of policosanol are the higher aliphatic primary alcohols octocosanol (CH_3–$CH_{2(26)}$–CH_2–OH), triacontanol, and hexacosanol [149].

The mechanism of action of policosanol in humans is unknown. In vitro experiments suggest that policosanol affects cholesterol synthesis at a level upstream of mevalonate formation, enhances LDL-particle uptake and degradation [150]. Animal models suggest that increased clearance of LDL-cholesterol is the primary mode of cholesterol lowering as opposed to reduced cholesterol synthesis [151, 152]. Other experimental models suggest that policosanol prevents lipoprotein peroxidation, has antiplatelet effects, and attenuates the development of atherosclerosis [153–156].

The initial studies on policosanol were performed in Cuba by one consortium. This group reported that doses of 10–20 mg/day reduced total cholesterol by ~20% and LDL-cholesterol up to 31% in a dose-dependent manner [157–160]. Significant increases in HDL-cholesterol of 24–29% were also observed in several early studies [160–163].

More robust randomized, placebo-controlled studies have failed to replicate the levels of LDL-cholesterol lowering that were initially reported. These studies have shown no significant lipid

Fig. 23.6 Globe artichoke (*Cynara scolimus*) in bloom. (Courtesy of Magnus Manske)

reductions in patients of several phenotypes, including primary hypercholesterolemia, heterozygous familial hypercholesterolemia, and combined hyperlipidemia with 8–12 weeks of policosanol treatment, ranging in dose from 10 to 80 mg/day [164, 165].

A comparative study evaluated the lipid-lowering effects of policosanol (20 mg/day) compared to atorvastatin (10 mg/day) for 12 weeks. The authors found that policosanol did not significantly reduce total cholesterol or LDL-cholesterol levels, nor did it provide any additional cholesterol lowering when given in combination with atorvastatin [166].

In spite of promising early reports from Cuban researchers, rigorously conducted trials performed elsewhere have shown that policosanol is ineffective at treating dyslipidemia and should not be recommended to patients.

Artichoke Leaf Extract

The globe artichoke (*Cynara scolymus*) is a member of the daisy family and is native to the Mediterranean region (Fig. 23.6). Artichoke leaf extract (ALE) has been used medicinally since the Ancient Egyptian times as an aid to digestion and to treat hangovers, jaundice, and snake bites [167]. Since the 1930s, there have been reports suggesting that ALE has favorable effects on cholesterol plaques and lipid metabolism [168]. Up to 4% of ALE is made of sesquiterpene lactones; up to 2% consists of phenolic acids such as chlorogenic acid, caffeic acid, and cynarin; and around 1% of ALE is flavonoids, including luteolin, cynaroside, and scolymoside [167]. Experiments in cell cultures and animal models have demonstrated that ALE decreases cholesterol synthesis through luteolin, which is an intermediate below HMG-CoA reductase in the cholesterogenic pathway, and increases biliary excretion [169–173].

Very few randomized controlled trials have evaluated the effect of ALE on lipoprotein metabolism. Petrowicz et al. [174] published the results, in abstract format, of a randomized controlled trial in 44 subjects with average total cholesterol levels of 204 mg/dL. Although subjects took 640 mg of ALE three times daily, there was no effect on lipid concentrations. However, in a subgroup of 24 participants with baseline total cholesterol levels >200 mg/dL, there was a reduction in cholesterol that was attributed to ALE, which was dependent on the baseline cholesterol level—the degree of reduction was not disclosed.

A multicenter study by Englisch et al. randomized 143 participants with total cholesterol levels >280 mg/dL to 1800 mg/day of ALE or placebo for 6 weeks. Compared to baseline values, the ALE group experienced an 18.5% reduction in total cholesterol and a 22.9% decrease in LDL-cholesterol with no significant changes in HDL-cholesterol or triglyceride levels [175].

The most recent randomized trial enrolled 131 subjects to receive either 1280 mg/day of a standardized ALE or placebo for 12 weeks. In the treatment group, there was a modest but significant decrease in total cholesterol (4.2%) but no significant changes in LDL-cholesterol, HDL-cholesterol, or triglycerides [176].

Finally, a study of 17 subjects with familial hypercholesterolemia specifically evaluated the effect of Cynarin, the 1,5-dicaffeyl ester of quinic acid, which is a phenolic acid found in ALE. The intervention failed to produce any significant changes in cholesterol or triglyceride concentrations after 3 months of treatment [177].

None of the studies reported any significant adverse events or laboratory test abnormalities as a result of ALE treatment. However, there are scant reports of transient gastrointestinal effects such as constipation and flatulence [168, 176]. In conclusion, the current evidence, reinforced by a recent Cochrane Database Systematic Review, does not support the use of ALE to lower cholesterol [178].

Conclusions

Healthcare consumers are free to choose from a multitude of readily available and well-promoted dietary supplements. Many patients see these supplements as safer alternatives, which are more aligned with their philosophy on healthcare. However, caution must be exercised as these dietary supplements frequently contain active compounds that are not standardized or regulated, and have the potential to interact with other medications. Patients should be encouraged to discuss the use of all dietary supplements with their physicians and pharmacists to reduce the risk of adverse effects.

There is a distinct role for the use of dietary supplements in patients who are unable or unwilling to take conventional lipid-lowering agents. However, supplements should only be recommended once there is convincing evidence for their safety and efficacy, either as lipid-lowering monotherapies or adjuncts to standard treatments like statins. Reasonable data exist to support the use of several supplements for cholesterol lowering, including RYR, SDF, nuts, flaxseed, SP, garlic, and berberine. In addition to inherent metabolic properties, several of these agents, including SDF, nuts, and soy protein, reduce plasma cholesterol levels simply by displacing lipid-rich or cholesterogenic foods from the diet. Patients should be encouraged to modify their lifestyles and consume a low-fat and low-cholesterol diet as part of any cholesterol-reducing therapy plan, including the use of dietary supplements.

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Statins: Risk-Benefits and Role in Treating Dyslipidemias

24

Antonio M. Gotto and Jennifer E. Moon

Introduction

Statins are recommended as first-line therapy for the management of lipid disorders, and particularly elevations in low-density lipoprotein cholesterol (LDL-C) [1, 2]. Numerous clinical trials in primary and secondary prevention, conducted in men, women, the elderly, patients with diabetes or hypertension, and other subgroups, have established the efficacy and safety of statin therapy for the prevention and treatment of atherosclerotic vascular disease. Currently available statins include atorvastatin (Lipitor), fluvastatin (Lescol, Lescol XL), lovastatin (Mevacor), pitavastatin (Livalo), pravastatin (Pravachol, Lipostat), rosuvastatin (Crestor), and simvastatin (Zocor); many are now available in generic form [3–9]. In large part, due to statin therapy, the percentage of US adults with high total cholesterol has declined substantially in the past decade (Fig. 24.1) [10]. Coronary heart disease mortality in the USA has also declined steadily beginning in the 1970s, although cardiovascular disease remains the leading cause of death [11]. Optimization of cardiovascular risk factors including dyslipidemia thus remains a public health priority.

History

The development of the statin class of lipid-lowering drugs marked a major turning point in the evolution of the lipid hypothesis and in the management of dyslipidemia [12]. In 1976, the biochemist Akira Endo, working at the Sankyo Company, isolated a factor from the fungus *Penicillium citrinum,* which he identified as a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenyzme A reductase (HMG-CoA reductase) [13]. This substance, which he called compactin or mevastatin, was the first statin to be administered to humans. Compactin was soon being studied in clinical trials in Japan, as well as experimentally around the world. Sankyo then terminated development of this agent in 1980 for reasons that have never been published. However, Merck Research Laboratories decided to pursue the development of statin drugs after isolation of its own fungal agent from *Aspergillus terreus* by Al Alberts and colleagues in 1978. Coincidentally, Dr. Endo had independently identified the same compound, called lovastatin, mevinolin, or monacolin K, within a year of Alberts' discovery. On September 1, 1987, lovastatin became the first statin to be approved in the USA by the Food and Drug Administration (FDA). Since that time, various other statins both derived from fungus and produced synthetically have been introduced in countries around the world.

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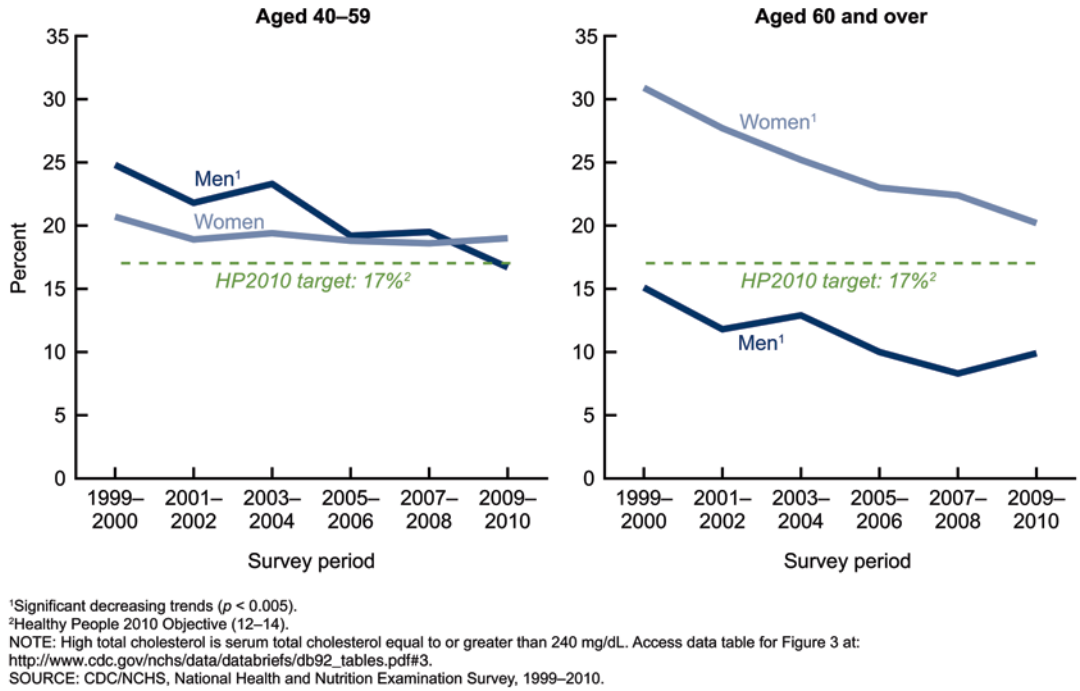

¹Significant decreasing trends ($p < 0.005$).
²Healthy People 2010 Objective (12–14).
NOTE: High total cholesterol is serum total cholesterol equal to or greater than 240 mg/dL. Access data table for Figure 3 at: http://www.cdc.gov/nchs/data/databriefs/db92_tables.pdf#3.
SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 1999–2010.

Fig. 24.1 Trends in percentage of adults aged 40–59 and 60 and above with high total cholesterol. (United States 1999–2010). Reprinted from Centers for Disease Control [Internet], Total and high-density lipoprotein cholesterol in adults: National Health and Nutrition Examination Survey, 2009–2010, 2012 April [cited 5 Sept 2012];92. Available from: www.cdc.gov/nchs/data/databriefs/db92.htm

Chemical Structure

Lovastatin, pravastatin, and simvastatin are fungal derivatives with structures that differ from each other only by a methyl or a hydroxyl side group (Fig. 24.2) [14]. Atorvastatin, fluvastatin, rosuvastatin, and pitavastatin are synthetic compounds with considerable variations in chemical structure that define their solubility properties and are thought to affect their relative potency. All statins have a moiety that resembles HMG-CoA and that may be present in an active, open (hydroxy acid) form or an inactive, closed (lactone) form. Lovastatin and simvastatin are prodrugs that are administered in the inactive lactone form and converted to the active drug form within the body. The other statins are administered in an open-acid structure.

Statins vary markedly in their solubility, with pravastatin and rosuvastatin considered hydrophilic statins [15]. The other statins are characterized by different degrees of lipophilicity; lovastatin and simvastatin are the most lipid soluble. Increased lipid solubility is thought to facilitate passive diffusion of the statin across cell membranes. Pharmacokinetic properties of the statins are listed in Table 24.1.

An important difference among the various statins concerns their metabolism, which affects their potential for drug–drug interactions. Lovastatin, simvastatin, and atorvastatin are metabolized via the cytochrome P450 (CYP) 3A4 pathway, while fluvastatin and rosuvastatin are metabolized by the CYP2C9 pathway. Coadministered inhibitors or substrates for CYP3A4 and CYP2C9 can interact with statins metabolized via these isoenzymes (see sections on dosing regimen and drug interactions and compatibilities). Interaction with CYP3A4 inhibitors is particularly problematic and can increase statin

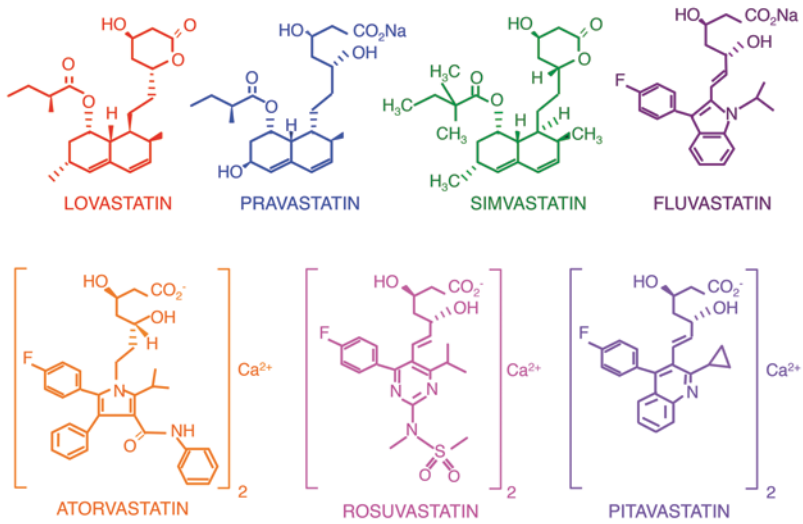

Fig. 24.2 Chemical structures of statins

Table 24.1 Pharmacokinetic properties of statins [3–9, 15]

	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
Origin	Synthetic	Synthetic	Microbial	Synthetic	Semi-synthetic	Synthetic	Semi-synthetic
Solubility	Lipophilic	Lipophilic	Lipophilic	Lipophilic	Hydrophilic	Hydrophilic	Lipophilic
T_{max} (h)	1.0–2.0	0.5–1.0	2.0–4.0	1	1.0–1.5	3.0–5.0	4.0
Absorption (%)	30	98	30	80	34	40–60	60–80
Bioavailability (%)	14	24	<5	51	17	20	<5
Protein binding (%)	>98	98	>95	>99	50	88	95
CYP-mediated metabolism	CYP3A4	CYP2C9 (75%), CYP3A4 (20%), CYP2C8 (5%)	CYP3A4	CYP2C9 (marginal), CYP2C8 (less than marginal)	Not CYP-mediated	CYP2C9	CYP3A4
Renal excretion (%)	<2	<6	10	15	20	10	13
Half-life (h)	14 (active), 20–30 (metabolites)	IR: 3, ER: 9	1.1–1.7	12	2	19	1.4–3.0

CYP cytochrome P450, *ER* extended release, *IR* immediate release

plasma concentrations, leading to increased risk for myotoxicity [16]. Pitavastatin is marginally metabolized by the CYP2C9 pathway, and pravastatin is not significantly metabolized by the CYP pathway. The primary route of metabolism for pitavastatin is glucuronidation, a process that can be inhibited by gemfibrozil to potentially increase risk for myotoxicity [17]. Pravastatin predominantly undergoes isomerization and enzymatic ring hydroxylation.

Mechanism of Action

Statins act by competitively and reversibly inhibiting HMG-CoA reductase, the rate-limiting enzyme that catalyzes the conversion of HMG-CoA to mevalonate in the cholesterol biosynthesis pathway (Fig. 24.3) [14]. Mevalonate is a precursor to all the isoprenoids and sterols produced by the body, including cholesterol. Binding of the HMG-CoA-like moiety on the statin to the enzyme induces a conformational change in the rest of the statin. Numerous bonds come into play to maintain the interaction, and the strength and number of bonds may determine the relative potency of the statin [15]. Inhibition of HMG-CoA reductase decreases intrahepatic cholesterol levels and leads to subsequent upregulation of LDL receptors in the liver. Increased clearance of apolipoprotein B-containing LDL and very low-density lipoprotein (VLDL) particles by the LDL receptor has the overall effect of decreasing plasma total cholesterol and LDL-C levels. It is thought that sustained inhibition of cholesterol biosynthesis additionally decreases synthesis of VLDL by the liver, resulting in reductions in triglycerides and increases in high-density lipoprotein cholesterol (HDL-C) levels, although this process is not well understood. Statins, however, do not reduce chylomicron levels and may not be the drugs of choice for those with severe hypertriglyceridemia.

Pleiotropic effects of statins unrelated to LDL-C reduction have also been proposed, based primarily on the results of in vitro experimental studies. While it is difficult to completely separate lipid from non-lipid effects, some researchers have proposed that statins may help improve endothelial function and myocardial ischemia; stabilize atherosclerotic plaques; exert antioxidant effects; and reduce macrophage activity, thrombosis, and inflammation, independent of their cholesterol-lowering actions [18]. Clinical evidence for the anti-inflammatory properties of statins was suggested in the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) Study, which showed that simultaneous reductions in the inflammatory marker high-sensitivity C-reactive protein (hs-CRP) and LDL-C by rosuvastatin in individuals without elevated LDL-C resulted in significantly decreased cardiovascular risk [19]. Reductions in inflammation may also help explain the benefit of statins following acute coronary syndromes [20]. In general, however, the putative pleiotropic effects of statins are thought to be explained primarily by their efficacy in reducing LDL-C levels [21]. Hypotheses that statins might be beneficial for the treatment of non-atherosclerotic conditions, including arrhythmias, Alzheimer's and neurodegenerative disease, cancer, and autoimmune diseases, have not been validated clinically.

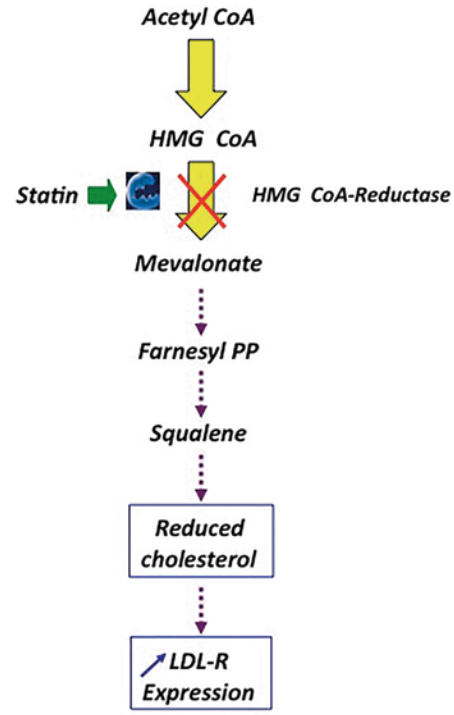

Fig. 24.3 Cholesterol biosynthesis pathway. *HMG-CoA* 3-hydroxy-3-methyl-glutaryl-coenyzme A reductase, *LDL* low-density lipoprotein

Pharmacodynamics

Statins act primarily to reduce plasma LDL-C concentrations, and decreases of approximately 20–63 % from baseline may be expected, depending on the dose and specific agent (Table 24.2).

Table 24.2 Relative lipid-modifying efficacy of statins [3–9, 25]

	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
% LDL-C decrease by statin dose							
30	–	40 mg	20 mg	1 mg	20 mg	–	10 mg
38	10 mg	80 mg	40 or 80 mg	2 mg	40 mg	–	20 mg
41	20 mg	–	80 mg	4 mg	80 mg	5 mg	40 mg
47	40 mg	–	–	–	–	10 mg	80 mg[a]
55	80 mg	–	–	–	–	20 mg	–
63	–	–	–	–	–	40 mg	–
Range of % decreases in triglycerides across doses							
	17–53	12–25	2–27	13–22	9–24	10–43	8–41
Range of % increases in HDL-C across doses							
	5–9	3–11	1–10	1–8	2–12	8–22	7–16

[a]The US Food and Drug Administration recommends that no new patients should be prescribed the 80-mg dose of simvastatin, and only patients taking this dose for 12 or more months without evidence of muscle toxicity should be maintained on simvastatin 80 mg.
HDL-C high-density lipoprotein cholesterol; *LDL-C* low-density lipoprotein cholesterol

Variations in individual responses to statin therapy may in part be genetically determined [22]. Comparison of four statins in the Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin (STELLAR) Trial found that percent reductions in LDL-C and LDL-C goal attainment were greatest with rosuvastatin, followed by atorvastatin, simvastatin, and pravastatin [23]. Across dose ranges, rosuvastatin reduced LDL-C by a mean of 8.2% more than atorvastatin, 12–18% more than simvastatin, and 26% more than pravastatin ($p < 0.001$ for all three comparisons).

In addition to lowering LDL-C, statins reduce triglycerides by approximately 10–37%. Modest increases in HDL-C in the range of 5–15% may also be observed. Reductions in non-HDL-C levels typically mirror the efficacy of the different statins in reducing LDL-C. For example, in the STELLAR trial, the greatest reduction in non-HDL-C levels was achieved with rosuvastatin at 42–48%, followed by atorvastatin at 34–48%, simvastatin at 26–42%, and pravastatin at 19–27% [23].

Indications

The FDA-approved indications for statins vary according to the specific agent (Table 24.3). In general, the statins are licensed for the treatment of dyslipidemias and to reduce cardiovascular risk as an adjunct to dietary therapy. Differences pertain to their effects on lipid fractions, use in primary versus secondary prevention, and treatment of specific dyslipidemias. All of the statins are indicated to reduce total cholesterol and LDL-C in patients with primary hypercholesterolemia; all except lovastatin may also be used to reduce apolipoprotein B and triglycerides and to increase HDL-C in patients with mixed dyslipidemias. Pitavastatin does not have an indication to reduce cardiovascular morbidity. Rosuvastatin is unique for having an indication for primary prevention based on elevated levels of hs-CRP.

Dosing Regimen

Lifestyle interventions, including reduced intake of saturated fats and cholesterol, increased consumption of plant stanols/sterols and fiber, increased physical activity, weight reduction, and smoking cessation, should be instituted in all patients with elevated LDL-C levels [1]. High-risk patients may be started on statins concurrently with the initiation of therapeutic lifestyle changes; in others, statin therapy may be warranted if LDL-C levels do not fall significantly after an initial trial of lifestyle modification [2].

Lovastatin and simvastatin should be administered in the evening, and lovastatin should

Table 24.3 US Food and Drug Administration indications for statins [3–9]

Indication	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
Primary prevention of CHD in patients with HC, multiple risk factors, and/or diabetes	X Patients with multiple risk factors		X Patients with average to moderately elevated TC and LDL-C, plus low HDL-C		X Patients with HC	X Men ≥50 year and women ≥60 year, with hs-CRP ≥2 mg/L, plus one risk factor	X Patients with diabetes, peripheral vascular disease, or cerebrovascular disease
Reduce TC, LDL-C, apo B, and triglycerides and increase HDL-C	X	X		X	X	X	X
Reduce TC and LDL-C only in patients with primary HC			X				
Reduce triglycerides in patients with hypertriglyceridemia and primary dysbetalipoproteinemia	X				X	X	
Slowing progression of atherosclerosis		X	X		X	X	
Secondary prevention of CHD	X	X			X		X
Treatment of adolescent patients with heterozygous FH	X	X	X		X	X	X
Treatment of homozygous FH	X					X	X

CHD coronary heart disease; *FH* familial hypercholesterolemia; *HC* hypercholesterolemia; *HDL-C* high-density lipoprotein cholesterol; *hs-CRP* high-sensitivity C-reactive protein; *LDL-C* low-density lipoprotein cholesterol; *TC* total cholesterol

be taken with the evening meal to enhance its absorption. The other statins may be taken at any time of day with or without food. Dosages to achieve LDL-C reductions in the range of 30–45%, which is considered moderate-intensity statin therapy, are atorvastatin 10–20 mg/day, fluvastatin 40–80 mg/day, lovastatin 40 mg/day, pitavastatin 2–4 mg/day, pravastatin 40 mg/day, rosuvastatin 10 mg/day, and simvastatin 20–40 mg/day. A meta-analysis of patients on atorvastatin, simvastatin, and rosuvastatin found that doubling the statin dose resulted in a further 4–6% decrease in LDL-C levels [24].

The standard dosing ranges, starting doses, and dose limitations for the different statins are listed in Table 24.4. Starting and maximum doses of statins may be reduced for patients with renal disease and in pediatric or adolescent patients. In Asian patients, a reduced starting dose of rosuvastatin should be considered since pharmacokinetic studies have demonstrated a twofold increase in median exposure to rosuvastatin compared to Caucasians [8]. The FDA has restricted use of the 80-mg/day dose of simvastatin based on the results of the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) trial, which found significantly more cases of myopathy in patients receiving 80 mg/day of simvastatin compared to those receiving 20 mg/day [25, 26]. Only patients who have already received 80 mg/day for more than 12 months without muscular symptoms should be prescribed this dose. Due to increased risk of drug–drug interactions leading to muscle toxicity, there may be dosing limitations when statins are coadministered with gemfibrozil and a number of agents metabolized by the CYP3A4 and CYP2C9 pathways, including certain azole antifungals (fluconazole), macrolide antibiotics (erythromycin, clarithromycin), human immunodeficiency virus (HIV) protease inhibitors (atazanavir, darunavir, fosamprenavir, lopinavir, nelfinavir, ritonavir, saquinavir), calcium-channel blockers (amlodipine, diltiazem, verapamil), amiodarone, rifampin, danazol, ranolazine, and cyclosporine. For contraindications with these and other drugs, see the section on Drug Interactions and Compatibilities below.

Risks and Precautions

Statins are contraindicated in patients with active liver disease or unexplained persistent elevations of serum transaminases. They are also contraindicated in women who are pregnant or planning to become pregnant, as well as in nursing mothers, since cholesterol is essential to fetal development and statins are excreted in breast milk.

Adverse Effects

Extensive clinical experience indicates that statin therapy may be initiated and maintained over the long term with a high degree of safety. Risks for cancer and death by nonvascular causes are not affected by statin treatment. A meta-analysis from the Cholesterol Treatment Trialists' (CTT) Collaboration, which included 170,000 patients from 26 randomized trials, found no increase in cancer incidence (relative risk (RR) 1.00, 95% confidence interval (CI) 0.96–1.04; $p=0.9$) or in nonvascular mortality (RR 0.97, 95% CI 0.92–1.03; $p=0.3$) per 1 mmol/L reduction in LDL-C with statin therapy [27]. Clinical trials have demonstrated that reducing LDL-C levels from average to below-average levels improves cardiovascular outcomes, with no increased risk of death from nonvascular causes [19, 28–30]. In addition, follow-up studies of randomized trials show a lack of long-term hazards associated with statin use [31–33].

The most common adverse events associated with statin therapy are elevations in liver enzymes and muscular side effects. All of the statins may produce initial elevations in serum alanine and aspartate transaminases, usually within the first 3–4 months of therapy, which resolve spontaneously or with statin discontinuation or dose reduction [34]. Clinically relevant elevations in liver enzymes are defined as levels exceeding three times the upper limit of normal and occur in less than 1% of patients. The FDA recommends pretreatment liver function tests when starting statin therapy, but recently terminated its prior recommendation for routine monitoring of hepatic enzymes since lasting liver damage rarely

Table 24.4 Statin dosing, interactions, and contraindications [3–9]

	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
Dosage forms	10, 20, 40, 80 mg/day	IR: 20, 40 mg/day; ER: 80 mg/day	IR: 10, 20, 40 mg/day; ER: 10, 20, 40, 60 mg/day	1, 2, 4 mg/day	10, 20, 40, 80 mg/day	5, 10, 20, 40 mg/day	5, 10, 20, 40, 80[a] mg/day
Recommended administration time	Any time of day	Any time of day	With evening meal	Any time of day	Any time of day	Any time of day	Evening
Usual starting dose	10–20 mg/day for patients with hyperlipidemia or mixed dyslipidemia; 40 mg/day for reductions greater than 45%	40–80 mg/day for LDL-C reductions ≥25%; 20 mg/day for lesser reductions	20 mg/day	2 mg/day	40 mg/day	10–20 mg/day; 5 mg/day for Asian patients	10–20 mg/day; 40 mg/day if high risk for CHD
Renal impairment	No dose adjustment	Use caution with doses >40 mg/day with severe impairment	Use caution with doses >20 mg/day with severe impairment	Limit to 2 mg/day with moderate and severe impairment	Start 10 mg/day for significant renal impairment	5 mg/day up to 10 mg/day with severe impairment	Start at 5 mg/day and closely monitor
Starting and maximum doses for pediatric/adolescent patients	10 mg/day up to 20 mg/day for ages 10–17 with heterozygous FH	20 mg/day for ages 10–16 with heterozygous FH, titrated up to 80 mg/day at 6-week intervals	10 mg/day up to 40 mg/day for ages 10–17 with heterozygous FH	N/A	Limit to 20 mg/day for ages 8–13 years; start and limit to 40 mg/day for ages 14–18	5 mg/day up to 20 mg/day for ages 10–17 with heterozygous FH	10 mg/day up to 40 mg/day for ages 10–17 with heterozygous FH
Dose limitations	20 mg/day in HIV patients on saquinavir/ritonavir, darunavir/ritonavir, fosamprenavir, or fosamprenavir/ritonavir; 40 mg in HIV pts on nelfinavir	20 mg/day with cyclosporine or fluconazole	20 mg/day with danazol, diltiazem, verapamil; 40 mg/day with amiodarone	1 mg/day with erythromycin; 2 mg/day with rifampin	20 mg/day with cyclosporine; 40 mg/day with clarithromycin	5 mg/day with cyclosporine; 10 mg/day with gemfibrozil (avoid) and lopinavir/ritonavir and atazanavir/ritonavir	10 mg/day with diltiazem or verapamil; 20 mg/day with amiodarone, amlodipine, ranolazine

Table 24.4 (continued)

	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
Specific contra-indications	–	–	Itraconazole, ketoconazole, posaconazole, HIV protease inhibitors, boceprevir, telaprevir, erythromycin, clarithromycin, telithromycin, and nefazodone	Cyclosporine	–	–	Itraconazole, ketoconazole, posaconazole, HIV protease inhibitors, boceprevir, telaprevir, erythromycin, clarithromycin, telithromycin, nefazodone, gemfibrozil, cyclosporine, and danozol
Specific warnings	Avoid cyclosporine, tipranavir/ ritonavir, and telaprevir; use caution with lopinavir/ritonavir	Monitor PT/INR with coumarin anticoagulants; monitor with phenytoin and glyburide; use caution with colchicine	Monitor with colchicine and ranolazine	Avoid gemfibrozil; monitor PT/INR with warfarin when initiating statin	–	Monitor PT/INR with coumarin anticoagulants; avoid gemfibrozil; use caution with ritonavir	Use caution with colchinine; monitor with digoxin or coumarin anticoagulants

[a]The US Food and Drug Administration recommends that no new patients should be prescribed the 80-mg dose of simvastatin, and only patients taking this dose for 12 or more months without evidence of muscle toxicity should be maintained on simvastatin 80 mg.

CHD coronary heart disease, *ER* extended-release, *FH* familial hypercholesterolemia, *HIV* human immunodeficiency virus, *INR* international normalized ratio, *IR* immediate-release, *LDL-C* low-density lipoprotein cholesterol, *PT* prothrombin time

occurs [35]. Most of the statins carry warnings indicating that patients who consume large quantities of alcohol or who have a history of liver disease should be prescribed statins only with caution. However, the National Lipid Association Liver Expert Panel has published recommendations indicating that patients with chronic liver disease, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis may safely receive statin therapy, based on evidence from case–control studies showing that these patients are not at higher risk of statin hepatotoxicity [36]. Others have argued that patients with preexisting liver disease, including hepatitis C, cirrhosis, liver transplants, and hepatocellular carcinoma, may also benefit from statin treatment without increased risk of side effects [37]. Still, larger studies are needed to confirm these results, and caution in the long-term treatment of patients in these populations is advisable since the risk of statin-induced liver damage over a longer period of time remains unclear.

Myalgia, or a diffuse muscle pain or soreness, may be experienced by some patients initiating statin therapy. A meta-analysis of 20 randomized trials estimated the rate of mild muscle pain as 190 cases per 100,000 person-years [38]. Rates in everyday clinical practice may be higher, affecting approximately 5% of patients [39]. Myopathy, a more serious condition, is muscular pain accompanied by creatine kinase (CK) levels exceeding ten times the upper limit of normal. Rarely with statin therapy, myopathy can progress to life-threatening rhabdomyolysis, which can cause myoglobinuria and potential renal failure when CK levels exceed 40 times the upper limit of normal [34]. Clinical trial and cohort study data indicate rates of approximately 11 cases of myopathy and three cases of rhabdomyolysis per 100,000 patient-years with statins [38]. One of the statins, cerivastatin, was withdrawn from the market in 2001 due to increased risk of fatal rhabdomyolysis.

Risk for myotoxicity is increased by treatment with high statin doses; combination therapy with niacin and fibrates, particularly gemfibrozil; concurrent treatment with CYP3A4 inhibitors in patients receiving lovastatin, simvastatin, or atorvastatin; conditions including renal impairment and hypothyroidism; and advanced age, female sex, small body size, or Asian race [39]. Increased risk for statin-induced myopathy may in part be genetically determined [40, 41]. A variant in the *SLCO1B1* gene, which helps regulate the hepatic uptake of statins by coding for the organic anion transporting polypeptide 1B1 (OATP1B1) transport protein, was associated with increased risk for statin-induced myopathy in a genome-wide association study of participants in a large randomized trial of simvastatin and in a follow-up pharmacogenetics study of statin safety. For patients who experience unexplained muscular pains or weakness after initiating statins, physicians may consider continuing therapy at a reduced dosage, switching to a different statin, or ceasing statin use, based on CK measurements [38]. Statin therapy should be discontinued if CK levels exceed ten times the upper limit of normal, if muscular symptoms become intolerable, or if other potential causes for the symptoms have been ruled out.

It is unclear exactly how statins cause muscular side effects. The *SLCO1B1* polymorphism may play a role by reducing the uptake of statins by hepatic tissues, thus increasing statin blood levels. Statins decrease the formation of coenzyme Q, also called ubiquinone, which is a metabolite of the HMG-CoA reductase pathway. A proposed mechanism of statin-induced myopathy is coenzyme Q10 deficiency; another is low vitamin D levels since patients with vitamin D deficiency have myalgia and poor muscle function. However, clinical trials with coenzyme Q10 or vitamin D supplementation have not been found to be efficacious in alleviating myopathy. Other hypotheses for statin-induced myopathy include reduction of the cholesterol content of the plasma membrane of skeletal muscle cells, leading to their instability or rupture, induction of myocyte apoptosis by reducing isoprenoid levels, and impairment of intracellular calcium homeostasis through interference with the mitochondrial respiratory chain [15, 42].

Revised labeling by the FDA draws attention to a newly recognized increase in the risk for incident diabetes and for glycated hemoglobin

(HbA_{1c}) and/or fasting plasma glucose elevations with statin therapy. This finding was sparked by results from JUPITER, which showed a 27% increase in the risk for new-onset diabetes with rosuvastatin [19]. Subsequently, a meta-analysis of 13 statin trials found that statins increased the risk for incident diabetes by 9% over 4 years [43]. This increase in risk corresponds to one additional case of diabetes for every 255 people treated with a statin for 4 years, as compared to a reduction in major coronary events of 5.4 events. One analysis of JUPITER and two other trials found that the risk with rosuvastatin may be higher at 18% [43], although a different analysis of the JUPITER cohort suggested that the risk of developing diabetes with rosuvastatin is strongly related to the presence of preexisting diabetes risk factors [44]. Another meta-analysis of five trials found a 12% increase in the risk for new-onset diabetes over 5 years, compared to a 16% reduction in major cardiovascular events, associated with intensive versus moderate-dose statin therapy [45]. Additionally, a study from the Women's Health Initiative reported a significant increase in diabetes risk in postmenopausal women [46]. However, patients who do develop diabetes would continue to experience an equivalent degree of clinical benefit as those without diabetes [47]. Collectively, the body of evidence lends support to a statement by the FDA that the risk for incident diabetes is outweighed by the demonstrated clinical event reduction with statins [35].

Another recent change in FDA labeling concerns rare cognitive adverse events that have been identified in post-marketing reports. According to the FDA, post-marketing adverse event reports have described individuals over the age of 50 years who have experienced notable, but nonserious and ill-defined cognitive impairment (e.g., memory loss, forgetfulness, amnesia, memory impairment, and confusion) associated with statin use [35]. Symptoms have appeared between 1 day and years following initiation of statin therapy, and they disappear after statin discontinuation, typically after a median of 3 weeks. According to the FDA, these rare occurrences of cognitive impairment are not believed to lead to clinically significant cognitive decline.

Drug Interactions and Compatibilities

All statins carry a warning regarding increased risk for myopathy with coadministration of erythromycin, cyclosporine, niacin, or fibrates. Coadministration of statins with these agents may be specifically contraindicated or the statin dose limited (Table 24.4); if not, caution and careful monitoring are recommended. Erythromycin and cyclosporine may interact with statins via the CYP3A4 pathway and by interfering with OAT1B1, a membrane transporter that helps regulate drug influx to the liver; cyclosporine additionally affects P-glycoprotein, a gastrointestinal transporter [48]. These interactions can significantly increase systemic statin concentrations. Gemfibrozil increases risk for muscular toxicity by interacting with statins via multiple mechanisms, including glucuronidation [17]. In theory, co-therapy with other fibrates may also increase this risk, but the combination of fenofibrate and simvastatin was used in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial in patients with type 2 diabetes without causing an increase in rates of myopathy/myositis/rhabdomyolysis compared to treatment with simvastatin alone (0.1% vs. 0.1%; $p = 1.00$) [49]. Some cases of muscular toxicity with the niacin/statin combination have been reported, but the level of evidence for niacin-induced myopathy in general is relatively weak, when niacin is used as both monotherapy and in combination with statins [50].

Statins metabolized by the CYP3A4 pathway, including lovastatin, simvastatin, and atorvastatin, can interact with inhibitors of CYP3A4 to cause elevations in statin plasma concentrations and increase risk for myopathy. Some CYP3A4 inhibitors, including macrolide antibiotics and protease inhibitors, also inhibit OATP1B1 to increase systemic statin exposure [15]. Statin dose limitations when coadministered with CYP3A4 inhibitors and major drug interactions, including contraindications, are shown in Table 24.4. In general, atorvastatin undergoes less metabolism by CYP3A4 than lovastatin and simvastatin, so it may be subject to fewer drug interactions by this pathway [48]. Daily consumption of one glass of

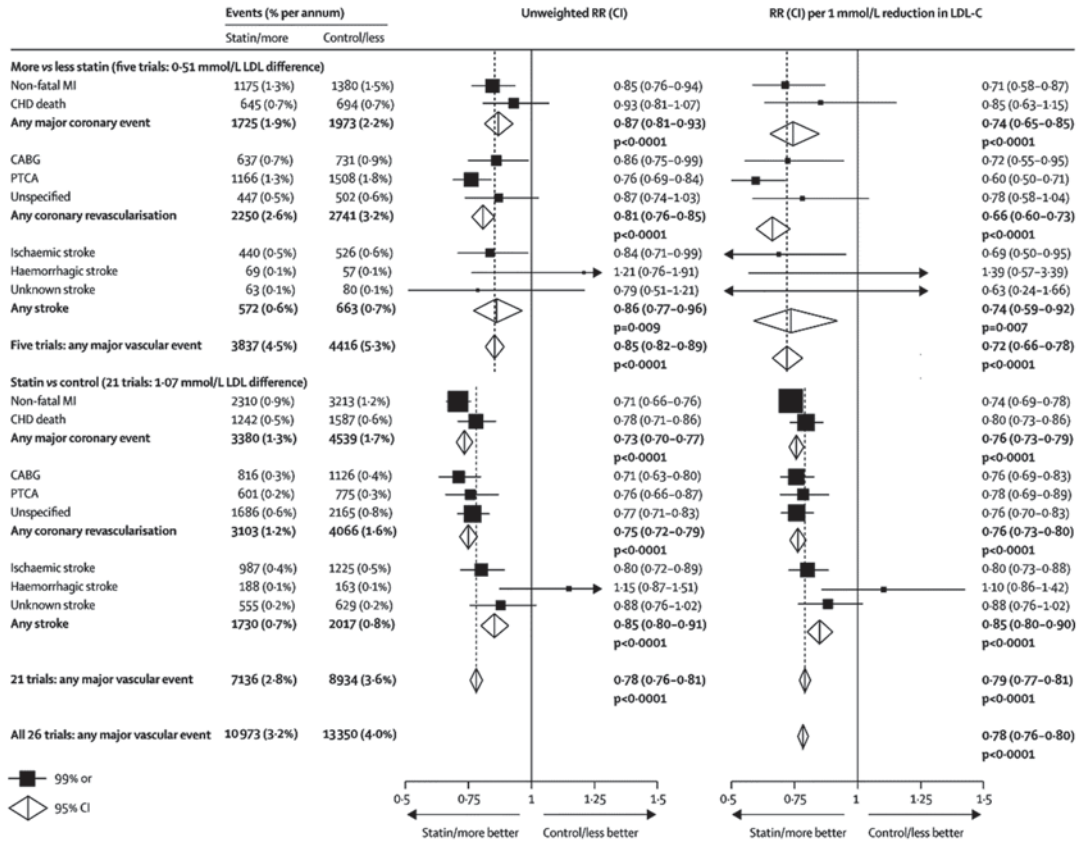

Fig. 24.4 Cholesterol Treatment Trialists' Collaboration: effects on each type of major vascular event. In the *left* panel, unweighted rate ratios (RRs) are plotted for each comparison of first event rates between randomly allocated treatment groups. In the *right* panel, RRs are weighted per 1.0 mmol/L (39 mg/dL) LDL cholesterol (LDL-C) difference at 1 year. RRs are shown with horizontal lines denoting 99% CIs or with open diamonds denoting 95% CIs. *CI* confidence interval, *MI* myocardial infarction, *CHD* coronary heart disease, *CABG* coronary artery bypass graft, *PTCA* percutaneous transluminal coronary angioplasty. (Reprinted from Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomized trials. Lancet. 2010;376(9753):1670–81, Copyright 2010, with permission from Elsevier)

grapefruit juice, a CYP3A4 inhibitor, has been shown to increase exposure to lovastatin, simvastatin, and atorvastatin, and quantities greater than one quart per day are not recommended [16].

Statins metabolized by the CYP2C9 pathway, including fluvastatin and rosuvastatin, carry a lower risk for myopathy when coadministered with CYP3A4 inhibitors but may interact with coumarin anticoagulants. Prothrombin times or international normalized ratio (INR) should be monitored in these cases. Pravastatin and pitavastatin are not metabolized by the CYP3A4 pathway, so risk for interactions with strong CYP3A4 inhibitors is reduced. Due to its lack of significant drug interactions and its demonstrated efficacy in individuals aged 70–82 years, pravastatin is considered a safe agent of choice especially for elderly patients [51].

Clinical Trials

The evidence linking statin-induced reductions in LDL-C with improved clinical outcomes is robust. The CTT meta-analysis of 26 randomized statin trials showed reduced risk for all-cause mortality and for every type of major vascular event except for hemorrhagic stroke (Fig. 24.4)

[27]. For every 1 mmol/L reduction in LDL-C, the relative risk for all-cause mortality was reduced by 10% ($p<0.0001$) and for major vascular events by 22% ($p<0.0001$). This degree of clinical benefit was observed regardless of baseline LDL-C levels and was maintained when intensive statin therapy was used to achieve low LDL-C levels. The estimated absolute event reduction in major vascular events and in vascular deaths over 5 years at various levels of risk is shown in Fig. 24.5. Another meta-analysis confirmed that reductions in cardiovascular events and in all-cause mortality were similar in women and men [52]. In addition to cardiovascular outcomes trials, numerous imaging trials have demonstrated slowing in the progression of atherosclerotic lesions, as well as plaque regression, with statin treatment. The clinical significance of trials using surrogate endpoints has not yet been established, although the effects of such vascular alterations are likely to be positive.

Individuals with established atherosclerotic vascular disease are at high risk for a future cardiac event. Secondary prevention trials, such as the Pravastatin or Atorvastatin Evaluation and Infection Therapy—Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) and Treating to New Targets (TNT) studies, have established that treatment to low LDL-C levels results in improved clinical outcomes, or "lower is better." PROVE IT-TIMI 22 demonstrated a 16% reduction in the risk for death or a major cardiovascular event ($p=0.005$) in patients treated with intensive statin therapy for 2 years immediately following acute coronary syndromes [53]. Patients receiving atorvastatin 80 mg/day achieved a median LDL-C level of 62 mg/dL, compared to 95 mg/dL in the group receiving pravastatin 40 mg/day. Similarly, the TNT trial showed a 22% relative reduction in major cardiovascular events ($p=0.0002$) in patients with stable coronary heart disease treated with intensive (80 mg/day) compared to moderate (10 mg/day) doses of atorvastatin for 5 years [54]. Patients in the intensive-dose arm achieved a mean LDL-C level of 77 mg/dL, compared to 101 mg/dL in the moderate-dose arm. The CTT meta-analysis also found that intensive treatment to LDL-C levels between 1 and 2 mmol/L (39–77 mg/dL) was associated with a significant 15% greater reduction in major vascular events ($p<0.0001$), compared to more moderate regimens that achieved LDL-C levels that were on average about 0.5 mmol/L (19 mg/dL) higher [27]. Clinical guidelines, including those from the American Heart Association and American College of Cardiology, support aggressive treatment to low LDL-C levels in all patients with coronary heart disease or other forms of atherosclerotic vascular disease [55, 56].

Clinical trial evidence clearly demonstrates the benefit of primary prevention. The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) and JUPITER trials have shown that substantial clinical benefit can be obtained in low-risk populations with LDL-C levels that are not considered elevated. The AFCAPS/TexCAPS trial with lovastatin demonstrated a 37% reduction in first acute major coronary events ($p<0.001$) over 5 years in patients who also had low levels of HDL-C [30]. At baseline, patients had a mean LDL-C of 150 mg/dL, and mean HDL-C levels were 36 mg/dL in men and 40 mg/dL in women. The JUPITER study enrolled individuals with LDL-C levels <130 mg/dL and elevated hs-CRP (≥2 mg/L) [19]. Treatment with rosuvastatin over 1.9 years decreased LDL-C levels to a median of 55 mg/dL and reduced hs-CRP levels by 37%, resulting in a 44% relative reduction in major cardiovascular events ($p<0.00001$) and a 20% reduction in all-cause mortality ($p=0.02$). The results indicate that patients with elevated hs-CRP may benefit from statin therapy, despite having LDL-C levels that are not elevated. A meta-analysis of ten primary prevention trials reported a 12% reduction in all-cause mortality and a 30% reduction in major coronary events associated with statin use [57]. Similarly, a Cochrane review, which included 14 randomized primary prevention trials, found that statins reduced all-cause mortality by 17% and major cardiovascular events by 30% [58]. Nevertheless, the authors of the Cochrane review questioned the widespread use of statins in low-risk primary prevention, in part because of its cost-effectiveness. However, the increasing availability of low-cost generic statins has the potential

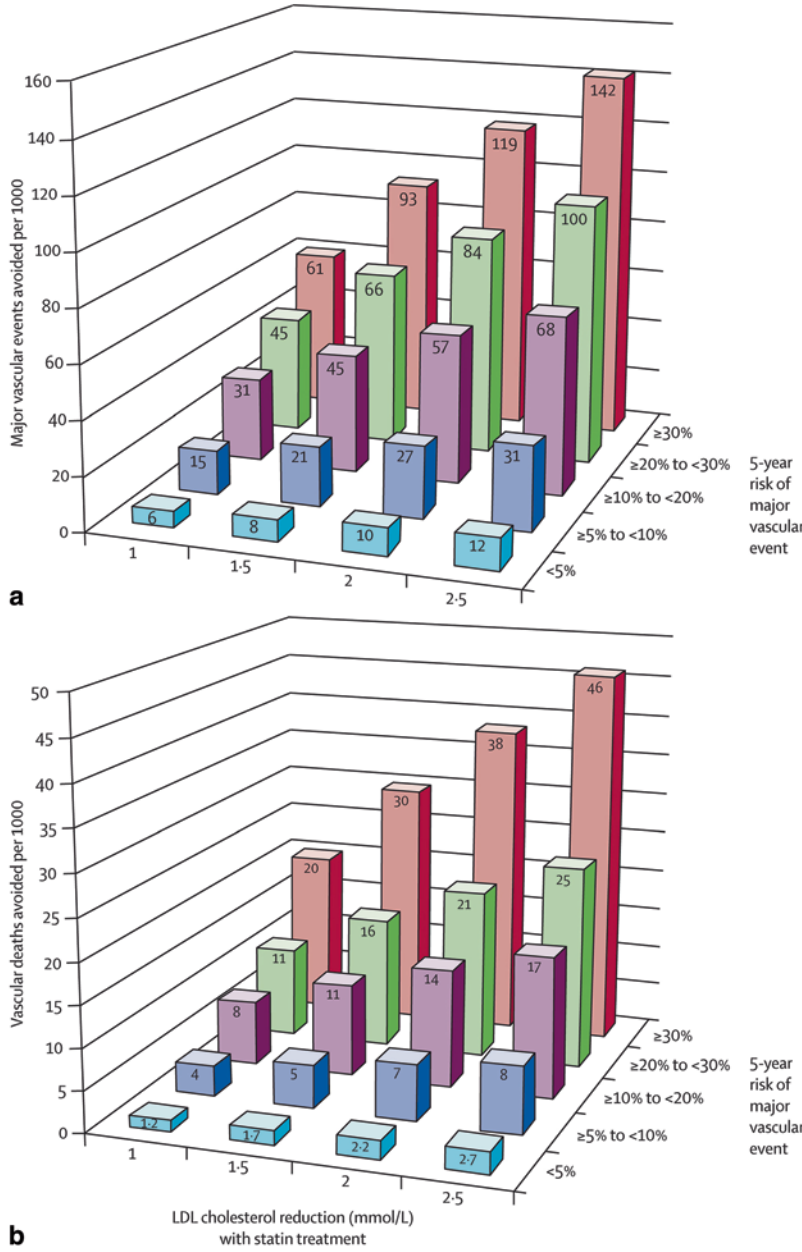

Fig. 24.5 Predicted 5-year benefits of LDL cholesterol reductions with statin treatment at different levels of risk: (**a**) major vascular events and (**b**) vascular deaths. Life table estimates using major vascular event risk or vascular death risk in the respective risk categories and overall treatment effects per 1.0 mmol/L (39 mg/dL) reduction in LDL cholesterol with statin. (Reprinted from Cholesterol Treatment Trialists' (CTT) Collaboration. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomized trials. Lancet. 2012;380(9841):581–90, Copyright 2012, with permission from Elsevier). *LDL* low-density lipoprotein

to make widespread statin use cost-effective, even possibly cost saving, in primary prevention [59]. Finally, a CTT meta-analysis found that in low-risk individuals, every 1 mmol/L reduction in LDL-C was associated with an absolute reduction in major vascular events of about 11 per 1000 over 5 years, far outweighing the potential hazards of statin therapy [60]. In general, the collective evidence provides ample support for the preventative treatment with statins of individuals without established coronary heart disease.

Major trials have established the clinical or lipid-lowering efficacy of each of the individual statins in various populations. AFCAPS/TexCAPS was the definitive study with lovastatin. With pravastatin, the West of Scotland Coronary Prevention Study (WOSCOPS), which enrolled men with severely elevated LDL-C levels, was the first primary prevention trial to show reductions in coronary events and mortality [61]. Two secondary prevention trials conducted with pravastatin in patients with LDL-C levels in the average range demonstrated reductions in recurrent events and, in one trial, significant reductions in coronary and all-cause mortality [29, 62]. Use of pravastatin in elderly individuals aged 70–82 years was associated with a 15% reduction in the primary composite endpoint of coronary death, nonfatal myocardial infarction, and stroke ($p=0.014$) and a 24% reduction in coronary mortality ($p=0.043$) [47].

Significant reductions in the relative risks for all-cause mortality and coronary events in secondary prevention were observed with simvastatin in the pivotal Scandinavian Simvastatin Survival Study (4S), which showed a 30% reduction in all-cause mortality ($p=0.0003$) over 5.4 years in patients with a history of myocardial infarction or angina [63]. The Heart Protection Study (HPS) mega-trial with simvastatin, which included 20,000 high-risk patients, including a large number of female (25%) and elderly individuals, demonstrated a 13% reduction in total mortality ($p=0.0003$) and an 18% reduction in deaths from coronary heart disease ($p=0.0005$) [28]. Fluvastatin was shown to reduce the risk of recurrent cardiac events by 22% ($p=0.013$) when initiated immediately following percutaneous coronary intervention, and an angiographic trial found that it slowed the progression of atherosclerosis [64, 65].

Atorvastatin has been extensively evaluated in clinical trials, including the secondary prevention PROVE IT-TIMI 22 and TNT studies. Within primary prevention, atorvastatin reduced the risk for coronary death or myocardial infarction by 36% ($p=0.0005$) in high-risk hypertensive patients in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid-Lowering Arm (ASCOT-LLA) after a period of 3.3 years; significant reductions in the risks for stroke and for cardiovascular and coronary events were also observed [66]. In a placebo-controlled trial with high-risk diabetic patients, participants receiving atorvastatin 10 mg/day attained a median LDL-C of approximately 77 mg/dL and experienced a significant 37% reduction in major cardiovascular events ($p=0.001$) over 4 years [67]. Subgroup analyses of the ASCOT-LLA and TNT trials in patients with diabetes also demonstrated significant reductions in major cardiovascular events with atorvastatin treatment to LDL-C levels around 80 mg/dL [68–69]. In patients with a history of stroke or transient ischemic attack but without coronary heart disease, the 5-year risk for recurrent stroke was reduced by 16% ($p=0.05$) and for major cardiovascular events by 20% ($p=0.002$) with intensive atorvastatin therapy (80 mg/day); a slight increase in the incidence of hemorrhagic stroke was observed, consistent with the results of the CTT meta-analysis [70]. Intensive atorvastatin therapy was shown to halt atherosclerotic plaque progression in a trial using intravascular ultrasound, whereas standard treatment with pravastatin did not [71].

The JUPITER trial established the efficacy of rosuvastatin in primary prevention. In addition, rosuvastatin has demonstrated atherosclerotic regression in an intravascular ultrasound trial with high-risk patients [72], as well as slowing in the progression of carotid intima-media thickness in low-risk, asymptomatic individuals [73]. Clinical trials have not yet established the clinical efficacy of pitavastatin, which has been available in Japan since 2003 and in the USA since 2010. Lipid endpoint studies indicate that pitavastatin's effects on lipid measures are comparable to those

obtained with equivalent doses of atorvastatin and simvastatin [74, 75]. Pitavastatin has been shown to induce atherosclerotic plaque regression [76] and has demonstrated safety and tolerability in a large Japanese population over a period of 2 years [77].

Short-term studies indicate that statin therapy is safe and efficacious in high-risk children. Guidelines from the National, Heart, Lung, and Blood Institute and the American Academy of Pediatrics recommend a full lipid profile in children between the ages of 1 and 4 if there is a family history of cardiovascular disease or dyslipidemia or if the child has other risk factors [78]. All children should be screened between the ages of 9 and 11 years. Diet and physical activity management are essential for children and adolescents with elevated LDL-C levels. After a trial of lifestyle modification, statin therapy may be warranted in children beginning at age 8 if they have LDL-C ≥190 mg/dL and additional risk factors. Familial hypercholesterolemia should be suspected in children with LDL-C ≥190 mg/dL [79]. The initial goal of treatment for children receiving statins is to lower LDL-C levels ≤130 mg/dL or to achieve LDL-C reductions ≥50%. Children receiving statin therapy should be monitored for growth (height, weight, and body mass index), in addition to liver- and muscle-related adverse events [15].

Importantly, statins should not be used in women who are pregnant, planning to become pregnant, or breastfeeding since cholesterol is essential to fetal and infant development. If planning to become pregnant, women should stop statins approximately 6 months before conception, and statins should be discontinued if a patient becomes pregnant while taking them. Available data on the risk of teratogenicity with statin use is scarce and may be smaller than previously thought; nevertheless, use of statins is contraindicated during pregnancy [80].

Conclusion

Over the past several decades, the advent of statin therapy has dramatically altered strategies for the management of dyslipidemia and for the prevention and treatment of atherosclerotic vascular disease. Statins are the mainstay of lipid-lowering therapy, and multiple clinical trials have demonstrated that LDL-C reduction with statins leads to improvements in cardiovascular morbidity and mortality in patients with or at risk for coronary heart disease. Side effects associated with statin use are outweighed by their proven benefits in clinical event reduction.

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Fibrates: Risk Benefits and Role in Treating Dyslipidemias

25

Min Jun and Vlado Perkovic

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality worldwide [1]. The World Health Organization reported that more than 17 million deaths worldwide in 2008 were attributable to cardiovascular death [1]. The identification and targeting of the many risk factors for cardiovascular disease through appropriate interventions are thus critically important.

Derangements in lipid concentrations have long been associated with an elevated risk of cardiovascular disease. As such, pharmaceutical interventions aimed at normalizing abnormal lipid profiles have been widely researched and implemented. Among these, lipid-lowering strategies targeting low-density lipoprotein (LDL) cholesterol with statins, the recommended first-line therapy in the treatment of dyslipidemia, have proven to be particularly effective [2, 3]. However, a high residual risk still remains, pointing to the limited power of a single mode of intervention aimed at reducing LDL-cholesterol concentrations. Additional alternative lipid-lowering strategies are therefore needed to prevent the excess cardiovascular events still observed in a substantial proportion of patients receiving statin therapy.

Fibrates are a class of drugs which are effective at improving lipid profiles and have been shown to be particularly effective in lowering triglyceride (TG) and elevating high-density lipoprotein (HDL) cholesterol concentrations [4]. As such, fibrate therapy has been suggested for many years to be an ideal lipid-lowering strategy in addition to LDL-cholesterol lowering interventions. However, outcome trials of fibrate therapy have produced varied results [5–8]. In addition, although rare, the risk of potential adverse effects when combined with statins [9] has substantially limited the utility of fibrates as cardioprotective agents. However, recent studies have identified a significantly greater beneficial effect of fibrates in a specific subset of patients, which include those with hypertriglyceridemia and low HDL-cholesterol concentrations [5, 10]. This chapter reviews the available randomized controlled trial evidence on the potential benefits and harms associated with fibrate therapy.

History of Fibrates

Fibrates are one of the most well-studied pharmacological compounds, with origins dating back to 1962 [11]. The effects of this drug class were first noted when preclinical studies in rats

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demonstrated that the then-novel compound (reported then to be a combination of androsterone and chlorophenoxyisobutyric ester (CPIB)) significantly reduced serum cholesterol concentrations [12]. This compound, which was initially referred to as Atromid and Atromid-S, and eventually clofibrate, was shown to be effective, when delivered orally, at reducing serum cholesterol, TG, and uric acid in humans [13]. After the publication of some of the early preclinical and clinical studies reporting clofibrate as effective lipid-lowering agents, subsequent confirmatory studies were published in relation to the effects of the newly developed compound [14–16]. With the emergence of high serum cholesterol as a potential risk factor for cardiovascular disease, the value of effective pharmaceutical interventions aimed at reducing risk as part of a chronic disease prevention strategy became increasingly important. Clofibrate was approved for use in the USA in 1967 and became one of the most widely used lipid-lowering drugs [17]. In the early 1970s, data from large-scale, multicenter, randomized controlled trials assessing the effects of clofibrate on the primary and secondary prevention of cardiovascular endpoints, particularly coronary events, were made available [8, 18–22].

The largest of these trials was the World Health Organization Cooperative Trial, a primary prevention trial which randomized 10,627 participants to clofibrate (1.6 g/day) or an olive-oil-based placebo [8]. The results of this landmark trial showed a 20% reduction in first major coronary events among participants receiving clofibrate compared to those in the control group. However, the trial also showed a significant increase in non-cardiovascular mortality in the group which received clofibrate [8]. The use of clofibrate for the treatment of dyslipidemia declined as a result, and newer fibrates including gemfibrozil, fenofibrate, bezafibrate, and ciprofibrate have been developed. Fenofibrate is the newest formulation of the fibric acid derivative and its efficacy on major cardiovascular events has been evaluated recently in large-scale international trials.

Mechanism of Action of Fibrates

Pharmacology

Fibric acid derivatives, or fibrates, belong to a class of drugs which activate the hormone-activated nuclear receptors, particularly peroxisome proliferator-activated receptor α (PPAR α). Fibrates also act on the remaining two known PPAR subtypes, β/δ and γ, but to a comparatively lesser extent [23, 24]. As a group, PPARs belong to a family of nuclear hormone receptor proteins that function as transcription factors and are major regulators of gene expression in relation to many areas such as metabolism and cell differentiation. PPARs achieve this regulation of gene expression by binding to specific sites (known as response elements) on the DNA as heterodimers with a retinoid X receptor. The regulatory role of PPARs in gene expression highlights their critical physiological role as lipid sensors and regulators of lipid metabolism. The three known PPAR subtypes each have distinct patterns of gene expression and are localized across different tissue types in differing levels of concentration [25]. PPAR α is highly expressed in liver, heart, kidney, muscle, and adipose tissue [25–27] and plays a critical role in activating fatty acid catabolism which consequently results in lower concentrations of circulating TGs and reductions in lipid storage [28]. PPAR β was thought to be ubiquitously expressed [29]; however, additional reports have demonstrated that it is particularly highly expressed in the skin (differentiated keratinocytes) [30, 31]. PPAR γ has shown to be expressed in white and brown adipose tissue, the gut, and immune cells [23, 32]. PPAR γ has been reported to be involved in adipocyte differentiation, lipid storage, [33, 34], and also glucose metabolism [35]. Activation of PPARs can be initiated by endogenous molecules such as fatty acids but also by fibrates. The reduction of TG concentrations is achieved through the activation of PPARs, which subsequently stimulates the oxidation of free fatty acids and induces the expression of lipoprotein lipase, the enzyme responsible for breaking down TGs and phospholipids.

Fibrates also suppress the transcription of the apoC-III gene which subsequently results in the decreased production of hepatic apoC-III [36]. In addition, fibrates have been reported to reduce apoB, in particular very low-density lipoprotein particles. Taken together, these processes most likely explain the hypolipidemic effects of fibrates [37].

The totality of evidence to date indicates that all fibrates generally have a similar mode of action. Five major mechanisms by which fibrates achieve modulation of lipid profiles had been proposed in 1998: [38]

1. It has been proposed that elevated lipolytic activity through the induction of lipoprotein lipase and the subsequent reduction in TG-rich lipoprotein largely contributes to the total reduction in plasma TG concentrations.
2. Fibrates induce the β-oxidation pathway and decrease fatty acid synthesis which consequently result in a lower availability of fatty acids for TG synthesis and thus ultimately leads to a reduction in TG concentrations.
3. Fibrates induce structural changes in LDL receptors which increase the likelihood of LDL catabolism and results in substantial reductions in LDL-cholesterol levels.
4. Reduction in neutral lipid (cholesteryl ester and TG) exchange may contribute to decreases in TG-rich lipoprotein.
5. "Increase in HDL production and stimulation of reverse cholesterol transport." Fibrate therapy has been proposed to increase HDL concentrations through the elevated production of apoA-I and apoA-II which are the two main apolipoproteins of HDL [39–42].

Effects of Fibrates

Pharmacokinetics

As a class of drugs, fibrates are generally well absorbed from the gastrointestinal tract and have high oral bioavailability, close to 100% (with the exception of immediate-acting fenofibrate, which has an oral bioavailability of approximately 60%) [43]. Fenofibrate is a prodrug that is hydrolyzed to fenofibric acid in vivo, while gemfibrozil and bezafibrate are active compounds [43]. In healthy participants, bezafibrate, [44], fenofibrate, [45], and gemfibrozil [46] have a half-life of around 2, 20, and 1.5 h, respectively. Fibrates are primarily excreted through the kidneys and as such, significant increases in plasma half-life in people with renal impairment have been observed [43].

Effect on Lipid Profiles

Fibrates are a class of lipid-lowering agents which can effectively improve lipid profiles. A meta-analysis of 22 randomized controlled trials assessed the efficacy of fibrates on lipid profiles and showed that all of the fibrates identified by the review including fenofibrate, bezafibrate, and gemfibrozil were effective in improving lipid concentrations, particularly in lowering TG concentrations [4]. Regarding the magnitude of the effectiveness, fenofibrate decreased TG concentrations by a range of 9.3–52.3%, gemfibrozil by a range of 28.2–42.8%, and bezafibrate by a range of 14.6–38.1% (Table 25.1). The proportional, compared to baseline HDL-cholesterol levels, increase in HDL-cholesterol concentrations by fenofibrate, gemfibrozil, and bezafibrate was 2.7–25.3, 6.0–15.8, and 6.1–51.1%, respectively. Fenofibrate reduced LDL-cholesterol concentrations by a range of 4.9–47.0% (with two studies reporting no effect; increased LDL-cholesterol concentration by 16.5% and 35.2%), gemfibrozil by a range of 4.1–16.7% (with one study reporting no effect; increased LDL concentration by 1.4%), and bezafibrate by a range of 4.7–22.9%. Whether a particular type of fibrate demonstrates superior efficacy in improving lipid profiles is unclear. To date, there have been a limited number of published studies directly comparing the effects of different types of fibrates. Many of these studies have been cross-over trials which included a small number of patients, making it difficult to reach a reliable conclusion [47, 48]. A meta-analysis assessing the effects of fibrates on cardiovascular outcomes reported that gemfibrozil tended to be more efficacious at normalizing lipid concentrations than were fenofibrate or clo-

Table 25.1 Effects of fibrates on lipid profiles. (Adapted from publication by Abourbih et al. [4])

		Total cholesterol				Triglyceride				LDL cholesterol				HDL cholesterol			
	Study size	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change* (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)
Fenofibrate																	
ACCORD (2010)	5518	4.53	3.91	3.97	−13.7	1.83	1.66	1.92	−9.3	2.6	2.1	2.07	−19.2	0.99	1.07	1.05	8.1
Keech et al. (2005)	9795	5.03	4.22	4.55	−16.1	1.73	1.47	1.87	−15.0	3.06	2.43	2.6	−20.6	1.1	1.13	1.12	2.7
Vakklianen et al. (2003)	405	5.56	5.01	5.55	−9.9	2.55	1.71	2.33	−32.9	3.37	3.15	3.44	−6.5	1.02	1.08	1.05	5.9
Farnier et al. (2005)	253	6.84	6.14	6.7	−10.2	3.12	1.82	2.63	−41.7	4.27	4.06	4.2	−4.9	1.1	1.31	1.14	19.1
Farnier et al. (2005)	372	6.74	5.27	5.9	−21.8	3.1	1.73	2.75	−44.2	4.14	3.34	3.55	−19.3	1.1	1.31	1.14	19.1
Farnier et al. (2007)	244	6.61	5.64	6.53	−14.7	2.61	1.53	2.53	−41.4	4.2	3.56	4	−15.2	1.17	1.39	1.17	18.8
Farnier et al. (2007)	367	6.56	3.94	4.32	−39.9	2.56	1.3	1.8	−49.2	4.19	2.22	2.27	−47.0	1.16	1.32	1.31	13.8
Knopp et al. (1987)	227	7.9	6.47	8.05	−18.1	2.18	1.31	2.26	−39.9	NR	4.5	5.74	NC	1.23	1.47	1.21	19.5
Davidson et al. (2006)	146	6.27	6.06	6.08	−3.3	5.42	3.44	5.45	−36.5	3.09	3.6	3.1	16.5	0.92	1.06	0.91	15.2
Krempf et al. (2000)	138	7.97	5.91	8.11	−25.8	1.44	0.97	1.55	−32.6	5.84	3.93	5.88	−32.7	1.47	1.55	1.49	5.4
Seidehamel et al. (1989)	147	6.76	5.85	6.94	−13.5	6.94	3.31	7.19	−52.3	2.84	3.84	2.87	35.2	0.79	0.99	0.82	25.3
Nissen et al. (2007)	102	5.35	4.99	5.29	−6.7	2.78	1.8	2.81	−35.3	NR	2.96	2.95	NC	0.97	1.1	0.96	13.4
Bezafibrate																	
BIP (2000)	3090	5.5	5.37	5.51	−2.4	1.64	1.4	1.69	−14.6	3.84	3.66	3.82	−4.7	0.9	1.36	0.99	51.1
Meade et al. (2002)	1568	5.59	NR	NR	NC	2.13	NR	NR	NC	3.38	NR	NR	NC	1.12	NR	NR	NC
Pauciullo et al. (2000)	162	7.36	5.9	6.04	−19.8	2.94	1.82	2.79	−38.1	4.93	3.8	3.79	−22.9	1.06	1.33	1.02	25.5
Elkeles et al. (1998)	164	5.68	5.28	5.79	−7.0	2.16	1.44	2	−33.3	3.82	3.3	3.93	−13.6	0.98	1.04	0.92	6.1

Table 25.1 (continued)

		Total cholesterol				Triglyceride				LDL cholesterol				HDL cholesterol			
	Study size	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change* (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)	Baseline (mmol/L)	Treatment (mmol/L)	Placebo (mmol/L)	Percent change (%)
Gemfibrozil																	
Frick et al. (1987)	4081	6.98	6.38	7.05	−8.6	1.99	1.3	2.01	−34.7	4.88	4.49	4.95	−8.0	1.22	1.32	1.22	8.2
Rubins et al. (1999)	2531	4.53	4.4	4.58	−2.9	1.81	1.3	1.87	−28.2	2.88	2.92	2.92	1.4	0.83	0.88	0.83	6.0
Frick et al. (1993)	628	6.98	6.44	7.27	−7.7	2.07	1.42	2.27	−31.4	4.86	4.66	5.22	−4.1	1.2	1.3	1.18	8.3
Vinik et al. (1993)	442	NR	NR	NR	NC	3.07	2.13	3.1	−30.6	NR	NR	NR	NC	NR	NR	NR	NC
Schaeffer et al. (1996)	305	7.48	6.49	7.44	−13.2	2	1.25	2.1	−37.5	5.34	4.84	5.39	−9.4	0.9	0.99	0.91	10.0
Avogaro et al. (1999)	217	NR	NR	NR	NC	3.58	2.42	4.29	−32.4	NR	NR	NR	NC	NR	NR	NR	NC
Wiklund et al. (1993)	137	7.27	6.19	7.12	−14.9	1.8	1.03	1.83	−42.8	5.14	4.28	5.01	−16.7	1.2	1.39	1.16	15.8

*Percent change between baseline and end of follow-up levels in the treatment group

NR not reported, NC not calculable, LDL low-density lipoprotein, HDL high-density lipoprotein, BIP Bezafibrate Infarction Prevention, ACCORD Action to Control Cardiovascular Risk in Diabetes

fibrate [10]. However, the analysis was limited due to the inclusion of only four trials.

Effect on Clinical Outcomes

Effects in the "General" High-Risk Population

In treating dyslipidemia, fibrates have been shown to clearly improve lipid profiles, lowering TG and elevating HDL-cholesterol concentrations. However, translation of these short-term improvements into long-term cardiovascular benefit has not been consistently demonstrated in large randomized controlled trials evaluating the effects of fibrate therapy on "hard" cardiovascular outcomes over the last four decades. Concerns regarding the toxicity of clofibrate have led to the decline in its use [8] and subsequent fibric acid analogues have been assessed for their efficacy. These fibric acid analogues including bezafibrate, gemfibrozil, and fenofibrate have been extensively studied for their potential cardiovascular benefit, mainly in large, multicenter, secondary prevention trials. The Helsinki Heart Study was a 5-year, double-blind trial where 4081 males (40–55 years of age) with primary dyslipidemia were randomized to gemfibrozil 600 mg twice daily or placebo [49]. The trial assessed the effects of simultaneously increasing HDL-cholesterol and reducing non-HDL-cholesterol on the risk of coronary heart disease. Gemfibrozil therapy compared to placebo, reduced serum TG by 35.4% and raised HDL cholesterol by 8.9%; LDL-cholesterol levels declined only by 9.3%. (Gemfibrozil significantly and consistently improved the HDL:LDL-cholesterol ratio throughout the trial.) Overall, the trial reported clear benefit from gemfibrozil therapy, showing a 34% reduction in the incidence of coronary heart disease (95% CI: 8.2–52.6%). Similarly, the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) specifically aimed to answer the question of whether raising HDL-cholesterol and lowering TGs would reduce the risk of cardiovascular disease. The trial randomized 2531 men with coronary heart disease and low HDL-cholesterol (≤40 mg/dL or 1.0 mmol/L) and LDL (≤140 mg/dL or 3.6 mmol/L) cholesterol to gemfibrozil or placebo over a median follow-up of 5 years. At 1 year, the mean HDL cholesterol was increased by 6% and TG level was 31% lower with no change in LDL cholesterol in the gemfibrozil group than in the placebo group. The results showed a 22% reduction (95% CI: 7–35%) in the risk of the primary outcome (nonfatal myocardial infarction or death from coronary heart disease) in the group which received gemfibrozil. The Bezafibrate Infarction Prevention (BIP) Trial evaluated the effects of bezafibrate on major cardiovascular outcomes (fatal and nonfatal myocardial infarction, or sudden death) by randomizing 3090 participants (91% men, age 45–74 years) with a previous history of cardiovascular disease to bezafibrate or placebo [6]. Bezafibrate increased HDL cholesterol by 18% and reduced TG by 21%. The trial reported no difference in the incidence of the primary outcome between the two groups (13.6% in the bezafibrate group and 15% in the placebo group, $p=0.26$) [6].

The most recent large-scale, multicenter trials have assessed the effects of fenofibrate on cardiovascular outcomes in participants with type 2 diabetes. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial randomized 9795 participants with type 2 diabetes to fenofibrate (200 mg daily) or placebo [7]. The fenofibrate group, compared to the placebo group had 1.2–5.1% increase in HDL cholesterol, 21.0–30.2% lowering of TGs, and 5.8–12.0% lowering of LDL cholesterol. After a follow-up duration of 5 years, the trial reported that fenofibrate therapy did not significantly reduce the incidence of the primary outcome (nonfatal myocardial infarction or death due to coronary heart disease; HR 0.89, 95% CI: 0.75–1.05, $p=0.16$); however, the risk of total cardiovascular disease was reduced by 11% (95% CI: 1–20%, $p=0.035$). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial is currently the most recent large-scale randomized controlled trial assessing the effects of fibrates on cardiovascular disease [5]. The ACCORD trial randomized 5518 participants with type 2 diabetes receiving statin

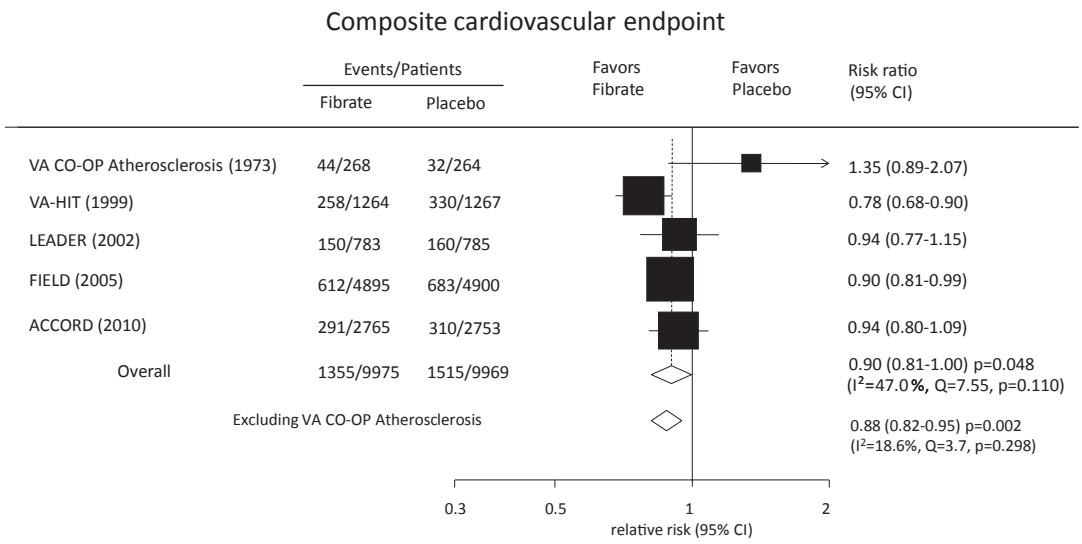

Fig. 25.1 Effects of fibrates on major cardiovascular events defined as a composite of fatal and nonfatal myocardial infarction and all stroke types; figure adapted from publication by Jun et al. [51]

therapy to fenofibrate (160 mg daily) or placebo. Those receiving fenofibrate, compared to those on placebo, had 10.9–21.9% reduction in serum TG, 0.7–4.6% increase in HDL-cholesterol and essentially no change in LDL cholesterol. The trial reported that adding fenofibrate to concurrent statin therapy did not significantly reduce the risk of the primary outcome (fatal or nonfatal cardiovascular outcomes; HR 0.92, 95% CI: 0.79–1.08, $p=0.32$) nor did it reduce the risk of any of its individual components [5].

Synthesizing the available clinical trial evidence to date, a large systematic review and meta-analysis of outcome trials (including the trials mentioned above) assessing the effects of fibrates in a range of populations reported that fibrate therapy produced a 10% relative risk reduction (95% CI: 0–18%) for major cardiovascular events (myocardial infarction and stroke; Fig. 25.1) and a 13% relative risk reduction for coronary events (95% CI: 7–19%) [10] (Fig. 25.2). A cumulative meta-analysis suggests that the observed coronary benefit has remained consistent for more than 30 years (Fig. 25.3). Another important finding from this review included the observation that fibrate therapy significantly reduced the progression of albuminuria by 14% (95% CI: 2–25%; three trials (FIELD, ACCORD, and Diabetes Atherosclerosis Intervention Study (DAIS)) including 15,731 participants and 3859 events). The FIELD and ACCORD trials also separately reported reductions in the rate of diabetic retinopathy progression with the FIELD trial showing a 31% reduction (95% CI: 16–44%) in the need for first laser treatment for diabetic retinopathy and the ACCORD trial reporting a 40% reduction in the rate of progression (95% CI: 13–58%) [50,51].

Effects in People with High TG and Low HDL Cholesterol

While recent large-scale trials have reported mixed outcomes regarding the efficacy of fibrates, they have suggested a subgroup where the utility of fibrate therapy may be most effective. The BIP trial also reported significant risk reductions in the group which had the highest baseline TG (≥2.26 mmol/L,≥200 mg/dL) [6] and the ACCORD trial, in a prespecified subgroup analyses, showed that fenofibrate significantly lowered the risk of the primary outcome in participants with elevated TG (≥2.30 mmol/L;≥204 mg/dL) and lower HDL-cholesterol (≤0.9 mmol/L;≤34 mg/dL) concentrations (12.37% in fenofibrate, 17.32% in placebo) [5]. While not separately statistically significant, similar results were reported

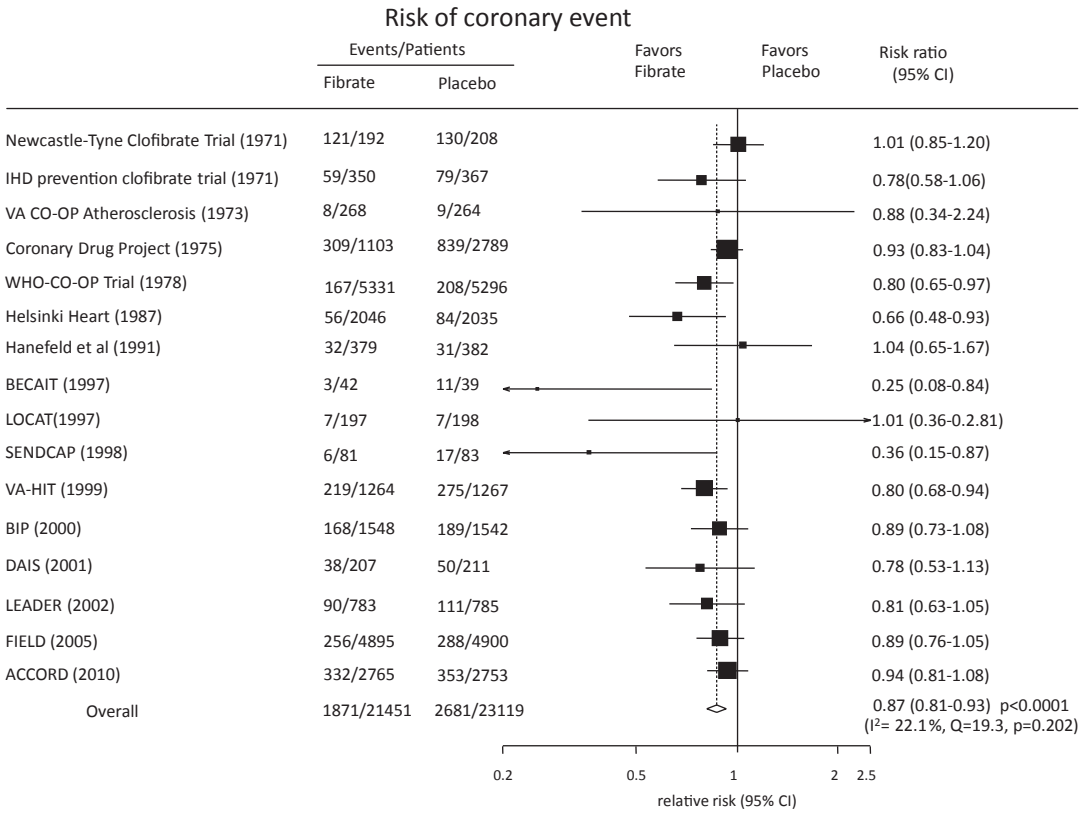

Fig. 25.2 Effects of fibrates on coronary events; figure adapted from publication by Jun et al. [10]

in subgroup analyses of the FIELD trial [7]. In a meta-analysis assessing the effects of fibrates on cardiovascular outcomes, the authors concluded that the overall magnitude of the proportional risk reduction was more modest compared to that observed with statin therapy, but also suggested that individuals with high concentrations of TG may particularly benefit from fibrate therapy. These results were consistent with some of the individual results of the trials which had been included in the meta-analysis [10].

Effects in Diabetes

Most of the large-scale trials conducted exclusively in type 2 diabetes have assessed the effects of fenofibrate [5, 7, 52]. As mentioned previously, the ACCORD and FIELD trials did not report an overall significant cardioprotective effect from fibrate therapy, although the point estimates were in the direction of benefit. The DAIS assessed the effect of fenofibrate on progression of coronary artery disease in type 2 diabetes with the primary outcome being mean lumen diameter. The study was not powered to examine clinical outcomes; however, authors reported that there were fewer clinical outcomes (defined as all-cause mortality, myocardial infarction, coronary angioplasty, coronary bypass operation, and hospitalization due to angina) observed in the fenofibrate group compared to the placebo group (38 vs. 50) [52]. The St. Mary's Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention (SENDCAP) Study randomized 164 type 2 diabetes patients without a history of cardiovascular disease to either gemfibrozil or placebo and the primary outcome was change in carotid intima media thickness [53]. Regarding cardiovascular outcomes, the study reported a significantly lower 3-year cumulative incidence rate of coronary heart disease event in the bezafibrate group compared to the placebo group (7 vs. 23 %, $p = 0.01$) [53].

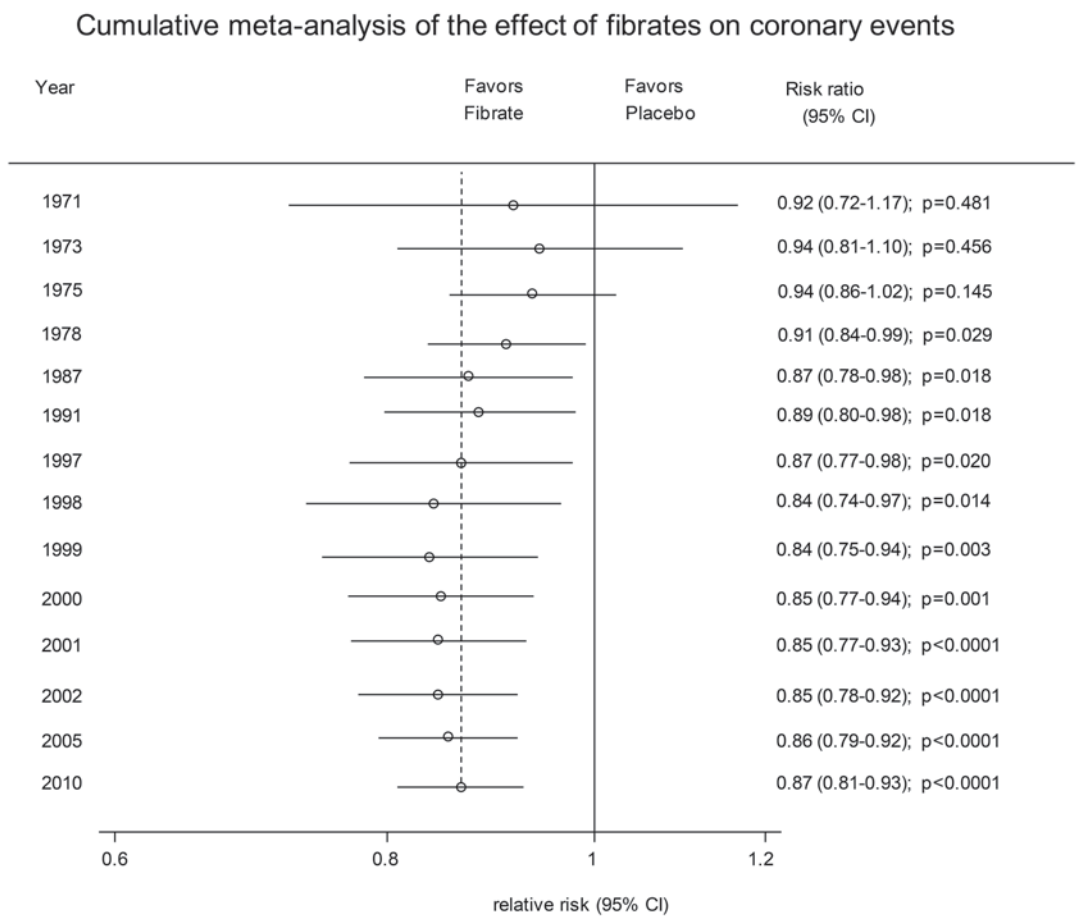

Fig. 25.3 Cumulative meta-analysis of the effect of fibrates on coronary events over time

Effects in Chronic Kidney Disease

Dyslipidemia is a risk factor for progressive kidney disease [54]. However, the use of fibrates in the chronic kidney disease (CKD) population has been limited due to well-documented reports of fibrate-induced acute elevations of creatinine concentrations. Indeed, the National Kidney Foundation (NKF) and the National Lipid Association (NLA) both recommend the cautious use of fibrates in the CKD population [55]. The NKF recommends gradually restricting the dose of fenofibrate, from a glomerular filtration rate (GFR) of 60–90 mL/min and avoiding use in patients with a GFR of < 15 mL/min [55]. There is currently no adequately powered outcome trial of fibrate therapy specifically in the CKD population and large clinical trials have usually excluded patients with CKD. Only very limited data on the effects of fibrates on clinically important outcomes are thus available from subgroup analyses of trials of the broader, high-risk populations. In these large multicenter trials of high-risk populations including type 2 diabetes, fibrate therapy was reported to be more efficacious in individuals with high concentrations of TG and low concentrations of HDL cholesterol [5–7]. This was confirmed in a large meta-analysis which included the results of 18 trials and showed that trials including individuals with high average baseline TG concentrations reported significantly greater proportional risk reductions [10]. Univariate metagression analysis in the study reported that fibrate therapy produced most benefit when TG concentrations were improved [10]. These results suggest that greater magnitudes of benefit from fibrate therapy may be observed in the CKD

population where elevated TG concentrations and decreased HDL-cholesterol are prevalent. Subgroup analyses of large randomized trials including participants at high risk of cardiovascular disease have supported this view. A prespecified, post-trial, subgroup analysis of VA-HIT showed that gemfibrozil reduced the risk of cardiovascular disease (defined as a composite of coronary death or nonfatal myocardial infarction) by 27% (95% CI: 4–44%) in participants with chronic renal insufficiency defined as creatinine clearance of ≤75 mL/min [56]. Similar results were reported in a prespecified, post-trial analysis of the FIELD trial, where fenofibrate therapy reduced the risk of cardiovascular events by 32% (95% CI: 3–53%) in diabetic participants with moderate renal impairment defined as an estimated GFR of 30–59 mL/min/1.73 m^2 [57].

In addition, a recent meta-analysis assessing the effects of fibrates in CKD pooled the results of the VA-HIT and FIELD trial to provide a better estimate of the possible effect of fibrates on cardiovascular disease in this population [58]. The meta-analysis reported that fibrate therapy was effective in reducing the risk of cardiovascular disease in participants with an estimated GFR of >60 mL/min/1.73 m^2 (RR 0.86, 95% CI: 0.77–0.96) and also in participants with further progressed kidney disease defined as an estimated GFR of 30–59 mL/min/1.73 m^2 (RR 0.70, 95% CI: 0.54–0.89). Importantly, the study suggested that the magnitude of benefit may differ according to kidney function, although the *p* value for heterogeneity between subgroups of kidney function did not reach statistical significance ($p = 0.12$) [58].

Much less is known about the effects of fibrates on long-term renal outcomes including end-stage renal disease. Observational analyses from large, multicenter trials have suggested the acute drug-induced elevation in creatinine, which has been discussed as a potential cause for concern, may confer long-term renal benefit [59, 60]. A meta-analysis showed that there was no clear effect of fibrates on end-stage renal disease (RR 0.85, 95% CI: 0.49–1.49). The authors discussed the lack of evidence in this area as they reported that their review only identified two studies (including 9852 participants and 50 end-stage renal disease events) which reported the effect of fibrates on end-stage renal disease.

Combination Therapy

The control of dyslipidemia is primarily achieved through the use of statins which are aimed at reducing LDL-cholesterol concentrations. However, statins are comparatively less effective in elevating HDL-cholesterol and lowering TG concentrations [61]. As such, it has been postulated that the use of statin–fibrate combination therapy may provide additional benefits over statin monotherapy by lowering the residual cardiovascular risk still observed in patients with mixed dyslipidemia at high risk of cardiovascular disease. Conversely, gemfibrozil has been implicated as contributing to the development of rhabdomyolysis or myopathy when combined with statins [9]. So the combined therapy of gemfibrozil and statins has been discouraged due to possible adverse drug–drug interactions [62].

The only trial to specifically address this question was the ACCORD Lipid study, which assessed the effects of adding fenofibrate to statin therapy on the risk of cardiovascular disease, and showed no significant differences in the incidence of the primary endpoint (fatal or nonfatal cardiovascular events; 2.2% in the fenofibrate group and 2.4% in the placebo group) [5]. Of note, the results of this trial were entirely consistent with the results of the other fibrate trials where background statin therapy was not used. Combined with the now-documented modest cardiovascular benefit of fibrates, this suggests that ACCORD may have been negative as a result of inadequate power rather than lack of efficacy.

Risk (Safety and Tolerability of Fibrates)

Fibrate are a class of drugs which are generally well tolerated in most people. However, there are some safety issues which have been reported to date.

Elevation of Serum Creatinine

Fibrate-induced elevations in creatinine have been well documented. For this reason, recommendations advise caution when using fibrates in patients with CKD. While there have been reports of rises in serum creatinine from gemfibrozil, [63, 64], bezafibrate, [65, 66], and ciprofibrate [65, 67], much of the literature regarding this issue has been centered on fenofibrate [5, 7, 68–70]. The mechanism behind fibrate-induced elevations in creatinine is currently not clear. There are, however, several potential mechanisms explaining the generally acute change in creatinine with fibrate therapy. Interference in the generation of vasodilatory prostaglandins by fibrates originating from the activation of PPARs has been proposed as one potential explanation [63]. It has also been postulated that a rise in serum creatinine can result from an increase in the metabolic production of creatinine [71].

The clinical impact of the fibrate-induced elevations in creatinine is also not well elucidated. However, studies have demonstrated that the acute elevation in serum creatinine due to fibrate therapy is reversible [59, 60, 72]. In addition, it also does not appear to translate to longer-term renal harm as the incidence of the end-stage kidney disease was no different in the fenofibrate arm of the FIELD trial compared to the placebo arm (21/4895 vs. 26/4900) [59]. The demonstrated reversibility of the rise in serum creatinine appears to support the view that the drug-induced elevation in creatinine does not reflect a true deterioration of renal function. Furthermore, recent post-trial analyses from large randomized trials [59, 60] have postulated that the acute changes in serum creatinine are not only reversible but may also translate to longer-term renal benefit.

The acute fibrate-induced elevation in creatinine and its subsequent reversibility were confirmed in the FIELD trial as the mean plasma creatinine concentration increased to 10.0 μmol/L (0.11 mg/dL) during the pre-randomization, 6-week fenofibrate run-in period, but returned to baseline levels in participants randomized to the placebo group [59]. In participants randomized to fenofibrate, plasma creatinine remained 10–12 μmol/L (0.11–0.14 mg/dL), higher compared to placebo ($p<0.001$); however, interestingly, the long-term plasma creatinine rise from 4 months to close of study was smaller with fenofibrate than with placebo, suggesting a long-term renal benefit [59]. Furthermore, this was mirrored by slower estimated GFR loss. In addition, in order to assess the longer-term impact of the early, sustained fibrate-induced rise of serum creatinine, a subgroup of the FIELD trial ($n=661$), was reassessed 52 ± 13 days after study close-out. In this "washout" group, plasma creatinine changes were similar to those in the remaining cohort; however, concentrations at approximately 8 weeks after study close out were significantly lower in participants randomized to fenofibrate than in those on placebo ($p<0.001$) [59].

In a similar setting, a separate substudy of the ACCORD trial, the ACCORD Renal Ancillary Study, included 1081 participants from the original cohort to explore the reversibility of drug-induced creatinine elevation [60]. The study categorized participants into three groups: (1) the fenofibrate-treated group which included active fenofibrate participants who had experienced ≥ 20% increase in serum creatinine from trial baseline to month 4; (2) the fenofibrate control group which included active fenofibrate participants who had experienced ≤ 2% increase in creatinine during the same period; and (3) the placebo group which included participants who had been randomized to placebo. There was no restriction on their change in serum creatinine. Similarly to the FIELD substudy, study participants of the ACCORD Renal Ancillary Study were followed up 6–8 weeks after study close out. As expected, the results showed that serum creatinine concentrations were significantly higher in the fenofibrate case group compared to the placebo control group. This observed elevation in creatinine was entirely reversible as serum creatinine concentrations returned to similar levels to that of the placebo control group suggesting no residual loss of GFR after 5 years of therapy. In the fenofibrate control group which had experienced ≤ 2% increase in serum creatinine, the mean serum creatinine was lower than the placebo control group after 51 days of cessation

from therapy, suggesting a net preservation of renal function [60].

The impact of this preservation of renal function observed in the FIELD and ACCORD trials, particularly on clinical outcomes including cardiovascular and end-stage kidney disease, is not clear. However, these results strengthen the view that suggests that the acute fibrate-induced creatinine elevation in type 2 diabetic patients with relatively preserved renal function may confer longer-term renoprotective effects.

Rhabdomyolysis, Creatine Kinase Elevations, Myopathy

Rhabdomyolysis, creatine kinase, and myopathy have been reported to be adverse effects of fibrate therapy, both as monotherapy and in combination with statins [5, 7, 73, 74]. As myopathy can occur in both fibrates and statins when administered as monotherapy, and although rare, its occurrence is of particular concern when used in combination. Indeed, use of combined lipid-lowering agents may increase the risk of myopathy and although infrequent in nature, may lead to rhabdomyolysis in more severe cases [75]. The risk of developing myopathy appears to differ across different fibrate types. An assessment of safety data from the US Food and Drug Administration (FDA) showed that gemfibrozil, when combined with a statin (with the exception of cerivastatin), was associated with around a 15-fold increased risk of rhabdomyolysis when compared to fenofibrate combined with a statin [76].

Elevated Risk of Non-Cardiovascular Mortality

While, overall, fibrates have demonstrated a significant reduction in the risk of major cardiovascular events [10], and have been effective particularly in reducing the risk of coronary events, some earlier studies, especially those assessing the effects of clofibrate, have reported both significant and nonsignificant elevations in the risk of nonvascular mortality [8, 21, 49]. These three trials involving clofibrate ($n=2$) and gemfibrozil ($n=1$) were published between 1975 and 1987. They have largely been responsible for the nonsignificant marginal adverse effect of fibrates on nonvascular mortality observed in a recent meta-analysis [10]. The first of these trials was the Coronary Drug Project which randomized 5011 participants to clofibrate, niacin, and placebo [21] over a mean follow-up of 6 years. The risk of all-cause mortality did not differ between the clofibrate and placebo groups (RR 1.00, 95% CI: 0.89–1.13); however, a nonsignificant rise in the risk of nonvascular mortality and other non-cardiovascular mortality (RR 1.36, 95% CI: 0.87–2.12) caused concern. There were no significant differences according to specific cause of death including cancers and other non-cardiovascular mortality. In 1975, the results of the WHO Co-operative Trial were published, showing a 20% risk reduction in coronary events (RR 0.80, 95% CI: 0.65–0.97) [8]. However, the study concluded that clofibrate could not be recommended as a primary prevention lipid-lowering agent due to their documented observation of a significant rise in nonvascular mortality (RR 1.44; 95% CI: 1.05–1.97). This increase in risk appeared to be largely attributable to deaths due to liver and gall bladder diseases and pancreatitis [8]. The Helsinki Heart Study also reported a nonsignificant excess in nonvascular mortality (RR 1.20, 95% CI: 0.65–2.19); however, the study showed that there were no significant differences between the treatment groups in any of the specific causes of death, including cancer [49]. More recent trials have reported no significant associations between fibrate therapy and elevated nonvascular mortality; however, the FIELD trial reported a nonsignificant excess in nonvascular mortality (RR 1.10, 95% CI: 0.91–1.33) [7]. The authors reported, however, that the observed excess was not associated with any specific cause of death, including invasive cancer, and thus attributed the results to a chance finding. Collectively, more recent studies assessing the effects of gemfibrozil, bezafibrate, and fenofibrate have not shown significant increases in the risk of nonvascular mortality.

Other Adverse Effects

Elevation of Homocysteine

Fibrate-induced elevation in homocysteine concentrations has been well documented [77–80]. Higher concentrations of plasma homocysteine have been identified as a risk factor for disease and all-cause mortality [81, 82]. However, it appears that the magnitude of this increase varies across different types of fibrates. Fenofibrate therapy is associated with a proportional change in homocysteine concentrations ranging from 35 to 55% [77, 78, 80, 83–86] compared to 18 to 19% for the treatment with gemfibrozil and bezafibrate [78, 87, 88]. The long-term clinical impact of the observed elevations in homocysteine remains unclear.

Gall bladder disease including cholecystectomies and biliary disease have been reported in patients receiving fibrate therapy [7, 49, 52, 89, 90]. Whether fibrate therapy increases the risk of gall bladder disease is unclear. In an epidemiological study involving 1754 participants aged ≥ 30 years, the frequency of fibrate use in participants with gallstones was 21%, compared with 11% in participants with no gallstones [91]. In contrast, a meta-analysis assessing the effects of fibrates on cardiovascular disease in large, multicenter randomized controlled trials did not observe an increased risk of gall bladder disease with fibrate therapy (RR 1.19, 0.89–1.60) [10]. The analysis involved seven trials including 27,828 participants and 3948 events (defined as any gallbladder disease, biliary disease, cholecystectomy, and cholelithiasis).

Conclusion

Targeting the reduction of LDL cholesterol in patients with dyslipidemia is the primary, best-proven and most effective lipid-lowering interventional strategy. However, a substantial residual risk of poor clinical outcomes remains in these patients and thus additional strategies to mitigate this risk are of critical importance. Interventions aimed at improving other major lipid subfractions including TG and HDL-cholesterol have been identified as potentially effective lipid-lowering strategies. Fibrates have been shown to clearly lower TG and increase HDL-cholesterol concentrations. However, mixed results from outcome trials of fibrates and the potential risk for adverse effects such as elevated serum creatinine and also rhabdomyolysis when combined with statin therapy have limited its widespread use as a cardioprotective agent. It appears, however, that the magnitude of benefit from fibrate is largely dependent on the patient population. Recent studies have been remarkably consistent in their conclusions showing that the greatest risk reductions may be observed in individuals with elevated TG and decreased HDL concentrations. Furthermore, recent post-trial analyses have not only demonstrated the reversibility of the fibrate-induced elevations in creatinine but also suggested potential long-term renal benefits from the acute rise in creatinine. However, there are currently no trials conducted specifically in this patient population and thus the conduct of such trials would be imperative in confirming the greater beneficial effects of fibrates in this patient group. In the meantime, results to date suggest that the use of fibrates in patients with high concentrations of TG and low HDL concentrations should continue to be warranted.

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Niacin: Risk Benefits and Role in Treating Dyslipidemias

26

John R. Guyton, Kristen Bova Campbell and Wanda C. Lakey

Introduction

Niacin (nicotinic acid), the first medication employed in clinical treatment of dyslipidemia, is at a crossroads today because of two large clinical trials in which niacin failed to reduce major cardiovascular events. Until these trials were reported, niacin appeared to be a valuable anti-atherosclerotic agent especially for patients with low high-density lipoprotein cholesterol (HDL-C). Whether a major role versus a limited role should be assigned to niacin today may depend on better understanding of its nonlipoprotein effects, which could lead to more rational dosing strategies.

History

Nicotinic acid is pyridine-3-carboxylic acid. It is named for its first practical derivation from nicotine by nitric acid oxidation. The vitamin function of nicotinic acid and its amide (together known as vitamin B3) was discovered in 1937–1938 when these compounds effectively treated the skin rash, diarrhea, and neurological symptoms of pellagra [1]. The metabolic cofactors nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) incorporate vitamin B3. NAD and NADP can be generated alternatively from tryptophan, and pellagra can also result from Hartnup disease, an autosomal recessive disorder of tryptophan membrane transport [2].

To promote public acceptance, "niacin" was coined from *ni*cotinic *ac*id vitam*in*. Nicotinic acid and nicotine share no medical properties at all. Historically, "niacin" has referred to as both nicotinic acid and nicotinamide, but nicotinamide does not affect lipoproteins. With evolving usage, "niacin" is becoming the medically appropriate term to refer specifically to nicotinic acid in the context of lipid treatment—a transition that we encourage, as it fosters patient acceptance of a difficult drug.

Altschul and colleagues [3] discovered in 1955 that gram doses of niacin had the ability to decrease serum cholesterol levels in subjects with normal and hyperlipidemic profiles. By 1975, the Coronary Drug Project (CDP) showed that niacin treatment reduced recurrent nonfatal myocardial infarction (MI) in men with prior MI [4]. Niacin is now known to have a dose-dependent, multifactorial effect on plasma lipoproteins, as it decreases triglyceride, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and lipoprotein(a), and increases HDL [5].

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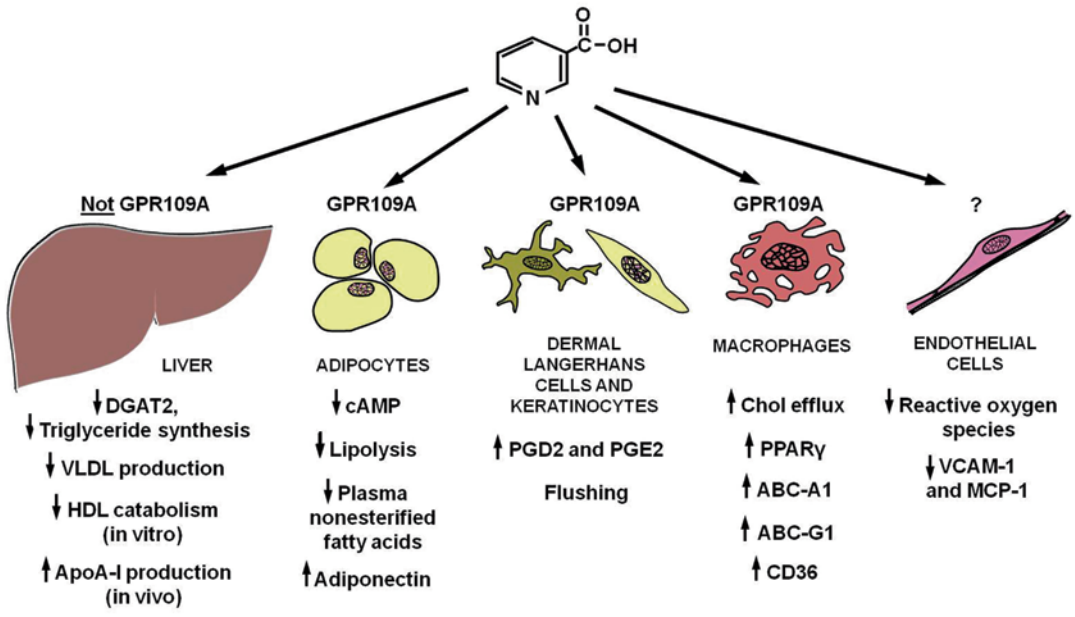

Fig. 26.1 Pharmacological effects of niacin relevant to atherosclerosis. Effects on adipocytes, dermal Langerhans cells, keratinocytes, and macrophages are dependent on a G-protein-coupled receptor, *GPR109A*. The liver, which mediates most of the changes in lipoprotein metabolism, does not express this receptor. Effects demonstrated in endothelial cells are not known to be receptor dependent. Modified from reference [6] and reproduced by permission of The Royal Society of Chemistry. *DGAT2* diacylglycerol acyltransferase-2, *VLDL* very-low-density lipoprotein, *LDL* low-density lipoprotein, *GPR* G-protein-coupled receptor, *HDL* high-density lipoprotein, *cAMP* cyclic adenosine monophosphate, *PGD2* prostaglandin D2, *PGE2* prostaglandin E2, *PPARγ* proliferator-activated receptor γ, *ABC* ATP-binding cassette protein, *VCAM* vascular cell adhesion molecule, *MCP* monocyte chemotactic protein

Mechanism of Action

Pharmacologic effects of niacin in the liver, adipose tissue, skin, and other tissues occur either via a G-protein-coupled receptor (GPR109A) or independent of this pathway (Fig. 26.1) [6]. In the 1960s, Carlson and colleagues [5] showed that plasma nonesterified fatty acid (NEFA) levels fell by 60% or more within minutes of administration of niacin to fasting humans, caused by inhibition of adipocyte lipolysis. Other investigators showed that niacin suppresses the rise in cyclic adenosine monophosphate (cAMP) in adipocytes exposed to epinephrine; eventually, antilipolysis was shown to occur through inhibition of adenylyl cyclase via a G-protein-coupled cell surface receptor of the G_i/G_0 type [7,8]. In 2003, three groups working with candidate orphan GPRs identified GPR109A (initially called HM74A) as the human niacin receptor and PUMA-G as its mouse homolog [9–11]. Expression of messenger RNA (mRNA) for these receptors occurs in the adipose tissue, spleen, adrenal, and lung [10, 11]. Cellular dose–response experiments point toward β-hydroxybutyrate as the endogenous ligand for GPR109A (Fig. 26.2). Increasing concentrations of β-hydroxybutyrate in the setting of starvation can regulate its own production through a homeostatic feedback loop by activating GPR109A to decrease lipolysis and prevent ketoacidosis [12].

Antilipolysis and its consequences, still incompletely understood, might disclose a flaw in the currently favored bedtime dosing regimen for niacin. Bedtime dosing essentially replaced mealtime dosing in the late 1990s with the introduction of prescription extended release (ER) niacin. Figure 26.3a shows data from Carlson's work indicating marked suppression of NEFA following oral administration of 200 mg niacin to three fasting

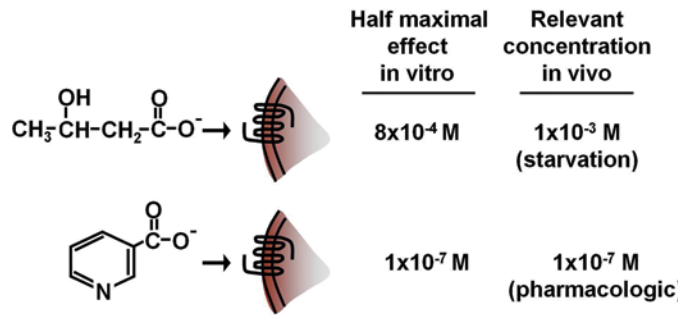

Fig. 26.2 Ligands for GPR109A. Beta-hydroxybutyrate activates the receptor within its natural physiologic range during times of starvation. Niacin shows much greater receptor affinity, but receptor activation requires pharmacologic niacin doses, well above the vitamin dose of approximately 25 mg daily. *GPR* G-protein-coupled receptor

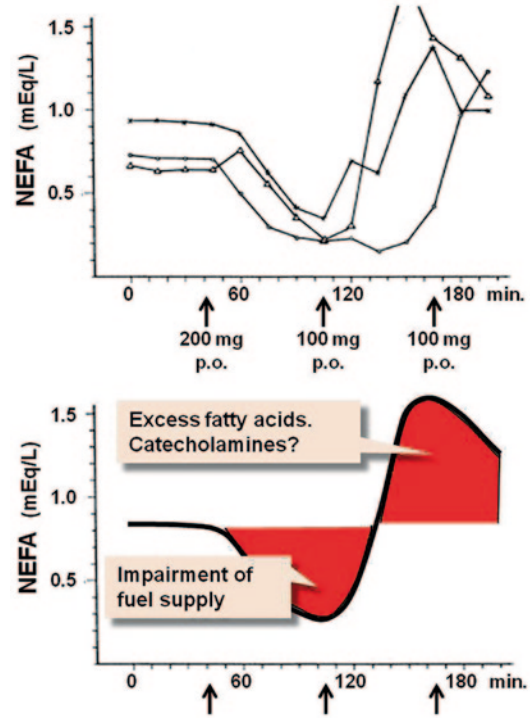

Fig. 26.3 **a** Effect of oral niacin administration on arterial plasma NEFA concentration in three human subjects. Reproduced from Carlson and Orö [13] with permission from John Wiley & Sons, Inc. **b** Possible secondary effects of the NEFA response on cardiovascular events. See discussion in the text. *NEFA* nonesterified fatty acid

subjects [13]. After 1–3 h, recovery and overshoot of plasma NEFA occur despite continued dosing of niacin. Recently, almost identical results were shown with bedtime ER niacin [14]. Figure 26.3b illustrates potential consequences. The initial dip in NEFA represents an impaired fuel supply for the heart. The subsequent overshoot of NEFA might prove arrhythmogenic in itself and is likely due to a surge of counterregulatory hormones including catecholamines [15,16]. The sequence of decreased fuel supply followed by excessive fatty acids and possibly catecholamines could promote cardiovascular events in susceptible patients. At mealtimes, fuel is supplied through intestinal absorption and insulin is increased instead of counterregulatory hormones, making mealtimes perhaps better for niacin administration. Further research in this area is needed.

The major pathway of niacin-induced cutaneous flushing in humans is stimulation of prostaglandin D2 (PGD2) production mediated by GPR109A in dermal Langerhans dendritic cells, with subsequent activation of DP1 PG receptors on vascular and possibly other cells (Fig. 26.4)

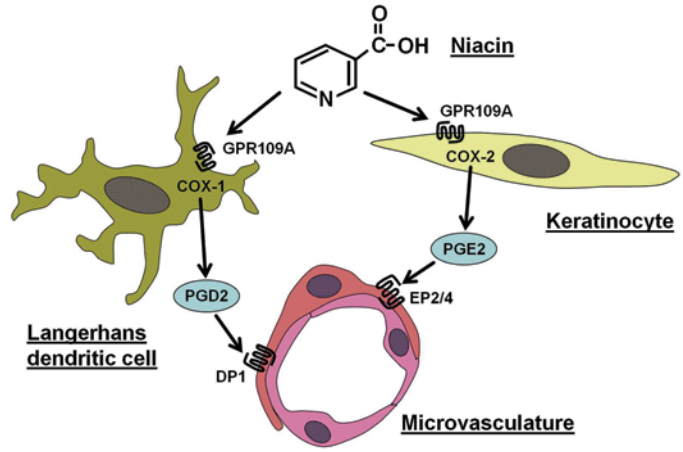

Fig. 26.4 Model of the biphasic flushing response associated with niacin via GPR109A receptors on Langerhans cells and keratinocytes. Niacin binding to GPR109A on Langerhans cells produces mostly prostaglandin D2 (*PGD2*) through the cyclooxygenase-1 (*COX-1*) pathway. PGD2 stimulates receptors on microvascular and other cells to produce an early phase of flushing. Stimulation of GPR109A on keratinocytes produces PGE2 via COX-2, and PGE2 mediates a delayed phase of flushing in mice. *GPR* G-protein-coupled receptor

[17]. The niacin receptor mediates flushing and antilipolysis through distinct postreceptor signal transduction pathways. Flushing is mediated through β-arrestin-1, while antilipolysis occurs through suppression of cAMP [18]. Laropiprant, a DP1 receptor antagonist, is moderately, but not completely effective in blocking flushing and skin symptoms when administered with niacin [19]. In mice, a brief phase of flushing mediated by Langerhans cells and cyclooxygenase-1 (COX-1) is followed by a major delayed phase mediated by keratinocytes with COX-2-dependent production of PGE2 (Fig. 26.4). These mouse data still require translation into human studies [20].

Niacin induces GPR109A-related anti-inflammatory effects in adipocytes and monocytes. In adipocytes, niacin suppressed pro-inflammatory chemokines and upregulated adiponectin, an anti-inflammatory adipokine [21]. Niacin inhibited monocyte adhesion and chemotaxis and reduced cytokine secretion in cell culture models of inflammation [22].

The major effects of niacin on lipoprotein metabolism are not explained by GPR109A, as lipolysis inhibition appears to play only a minor role in serum cholesterol reduction [5]. Multiple GPR109A agonists have suppressed lipolysis in human trials, but failed to modify serum lipoprotein levels apart from minor ($\leq 5\%$) increases in HDL-C [14]. Lipoprotein changes with niacin administration are thought to be mediated in the liver, and hepatocytes do not express GPR109A [9–11].

In a study using [^{3}H]-glycerol administered to humans, niacin was shown to decrease the production of VLDL triglyceride [23]. Niacin added to microsomal preparations of human hepatoblastoma (HepG2) cells noncompetitively inhibited the final step of triglyceride synthesis mediated by diacylglycerol acyltransferase-2 (DGAT2) [24]. Niacin added to the media of HepG2 cell cultures increased intracellular posttranslational apoB degradation. This effect was ascribed to decreased lipidation of nascent apoB peptide in the endoplasmic reticulum [25]. Niacin also decreases plasma lipoprotein(a) levels, presumably by a mechanism similar to the lowering of other apoB-containing lipoproteins [5].

Niacin effectively increases HDL cholesterol levels and may augment reverse cholesterol transport from peripheral tissues to the liver [26]. Cell culture studies have suggested that niacin may increase HDL levels by inhibiting the endocytic uptake of whole HDL particles [27, 28]. The cell surface receptor for HDL endocytic

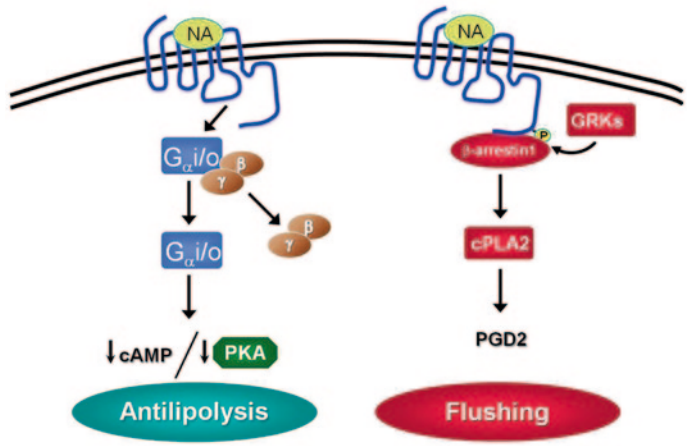

Fig. 26.5 GPR109A postreceptor signaling pathways. In adipocytes, ligand binding leads to exchange of GTP for the bound GDP in the heterotrimeric G-protein complex. The alpha subunit, $G_{\alpha}i/o$, dissociates from the beta and gamma subunits. Free $G_{\alpha}i/o$ inhibits adenylate cyclase leading to reduced cyclic AMP (*cAMP*) and reduced protein kinase A (*PKA*) activity. This in turn downregulates hormone-sensitive triglyceride lipase activity. In dermal Langerhans cells, the activated receptor is phosphorylated by G-protein-coupled receptor kinases (*GRK*s), which lead to binding of beta-arrestin-1. As a result of translocation of beta-arrestin-1 to the receptor complex, cytosolic phospholipase A2 (*cPLA2*) is activated to release arachidonic acid from phospholipids, providing substrate for the formation and secretion of prostaglandin D2, which mediates the flushing response [18]. *GPR* G-protein-coupled receptor, *GTP* guanosine triphosphate, *GDP* guanosine diphosphate

uptake is adenosine triphosphate (ATP) synthase beta chain, which is downregulated by niacin. In HepG2 cells, niacin did not change rates of apolipoprotein A-I (apoA-I) transcription or synthesis [27]. Niacin did not inhibit the selective uptake of HDL cholesteryl esters into the cells, an important step in reverse cholesterol transport mediated by scavenger receptor B1 [27]. In contrast to the cell culture studies, a stable isotope kinetic study in humans showed increased apoA-I production after niacin [29] (Fig. 26.5).

Niacin effects on key receptors and transporters involved in reverse cholesterol transport were demonstrated in monocyte and macrophage cells. COX-dependent activation of the peroxisome proliferator-activated receptor γ (PPARγ) by niacin increased the expression of CD36, the scavenger receptor for the uptake of oxidized lipoproteins in monocytoid cells. ATP-binding cassette protein A1 (ABCA1) expression in these cells, as well as cholesterol efflux, were increased by niacin via inhibition of cAMP/protein kinase A [30]. Lukasova and colleagues demonstrated inhibition of atherosclerosis by niacin independent of lipoprotein alterations in LDL receptor-negative mice. GPR109A knockout mice did not demonstrate this effect, and bone marrow cells transplanted from knockout mice into irradiated animals did not support the anti-atherosclerotic effect of niacin. Niacin promoted cholesterol efflux via the cholesterol transporter ABCG1 and impaired macrophage recruitment to plaques via inhibition of MCP-1 [31].

Pharmacodynamics

Niacin is well absorbed after oral administration. Metabolism depends on whether tablets provide immediate release (IR) or are modified for ER or slow release (SR). IR-niacin gives peak plasma levels by 30–60 min and is largely excreted in the urine as unchanged nicotinic acid [32]. ER- and SR-niacin overlap in terms of release characteristics with peak plasma concentrations at 0.5–5 h or longer [33, 34]. Both undergo extensive first-pass metabolism in the liver including conversion to nicotinuric acid and nicotinamide [34].

Two nonlipoprotein effects of niacin mediated by GPR109A, flushing in dermal cells and antili-

Table 26.1 Percent changes in lipids and lipoproteins associated with 2000 mg total daily dose of different niacin formulations [35,37–42].

Niacin type	Dosing	Total cholesterol	Triglyceride	HDL-C	LDL-C	Lipoprotein(a)
IR	2–3 times daily	−12%	−30%	+25%	−15%	−20% to −25%
ER	HS	−12%	−30%	+25%	−15%	−20% to −25%
SR	2 times daily (a.m./p.m.)	−14%	−30%	+15%	−18%	No data

IR immediate release (also called crystalline or regular niacin), *ER* extended release, *SR*, slow release, *HR* half strength, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

polysis in adipocytes, can be demonstrated with a dose as low as 200 mg orally—which is still much higher than the vitamin dose of 25 mg daily. Lipoprotein effects require higher doses, generally in the range of 0.5–2 g daily, although IR-niacin can be dosed as high as 4.0–4.5 g daily divided into 2 or 3 doses. Niacin reduces the levels of atherogenic lipoproteins—VLDL, β-VLDL, IDL, LDL, small dense LDL, and lipoprotein(a) [5] and raises the levels of HDL and HDL2, associated with protection from atherosclerosis [35]. Niacin formulation and dosing regimen influence lipoprotein effects (Table 26.1). The dose response of lipoproteins to niacin is generally linear up to 2.0–2.5 g daily, unlike the log-linear relationship evident with statins. ER niacin (2 g) and IR niacin (1 g t.i.d.) given to hypertriglyceridemic subjects raised apoA-I by 10–11% and lowered apoB by 7–12% [35,36].

Indications

Regulatory approval in the USA for ER niacin applies to reducing elevated cholesterol, triglyceride, LDL-C, and apoB, and to increasing HDL-C [34]. In patients with prior MI and hyperlipidemia, niacin monotherapy is indicated to reduce the risk of recurrent nonfatal MI. The practitioner might consider restricting this indication to IR-niacin given with meals, which was the dosing regimen in the relevant randomized trial (see below). Niacin's effects on lipoproteins are additive to those of other lipid-modifying drugs. Niacin is indicated as adjunctive therapy for adults with severe hypertriglyceridemia and risk of pancreatitis, but caution should be applied to patients with diabetes mellitus, as niacin-induced worsening of diabetes [43] could aggravate hypertriglyceridemia.

Dosing Regimens

Niacin dosing should aim to reduce skin flushing, which occurs in more than 80% of patients [44]. Treatment should start with low doses, such as 125–250 mg IR niacin twice daily or 500 mg ER niacin at supper or bedtime, and gradually increased over weeks to months. IR and SR niacin should be taken "with food in the stomach," that is, strictly between the middle and the end of the meal. ER-niacin is usually given at bedtime with a low-fat snack; however, because of the potential consequences of antilipolysis previously mentioned, the practitioner may consider suppertime dosing of ER niacin. The first dose of niacin in the day, either the single dose of ER niacin or the morning dose of IR or SR niacin, usually should be preceded by a COX inhibitor such as aspirin 325 mg or ibuprofen 200 mg given 30–60 min prior to niacin to reduce flushing. The 81-mg dose of aspirin does not inhibit flushing well. Inositol hexanicotinate, commonly marketed as "no-flush" or "flush-free" niacin, is not bioavailable and has never been shown to improve lipid levels [33]. A recent innovation in the authors' practice is requesting that patients stop niacin if a serious infection occurs (more than a minor upper respiratory illness) until well, and to stop for a week following surgery. Niacin is re-started gradually to avoid excessive flushing. This was instituted because of niacin's anti-inflammatory actions and because a major trial with niacin plus laropiprant showed increased infections [59].

Risks and Precautions

Adverse Effects Skin flushing, a feeling of prickly heat experienced mostly on the head, neck, and shoulders, often accompanied by erythema, is the chief side effect of niacin. Prolonged flushing might be ameliorated by chewing an aspirin tablet. Experienced health-care providers can help patients achieve a long-term tolerance of niacin in the great majority of cases, mainly by taking advantage of tachyphylaxis to flushing that develops over days to months with regular administration [44]. A gap in niacin treatment for as little as 3 days can cause re-emergent flushing and necessitate re-titration [45]. Acanthosis nigricans occurs idiosyncratically and is dose dependent [46].

Serious hepatic toxicity is almost entirely associated with the use of SR niacin, usually with doses exceeding 1000 mg twice daily [44]. In monitored clinical experience with ER niacin, 1% or fewer patients had hepatic transaminase elevations >3x the upper limit of normal (ULN) [34]. ER niacin has only been studied as a once-daily dose at bedtime; therefore, safety of other dosing regimens is unknown.

Specific deficiencies of clotting factor synthesis have occurred in patients taking niacin, reversible after withdrawing the drug [47]. Niacin can interfere with bilirubin transport, and isolated increases in serum bilirubin <3 mg/dl without signs of hepatotoxicity should not necessarily lead to dose reduction [48].

Neither IR niacin nor ER niacin monotherapy has been associated with the onset of myopathy, but some statin-intolerant patients may also have symptoms with niacin. Theoretically, even mild liver toxicity from niacin with coadministration of a statin could cause myopathy due to decreased hepatic clearance of the statin [44].

At daily doses of 2 g or less, niacin generally is associated with modest increases in fasting glucose around 5 mg/dl and hemoglobin A1c up to 0.3% [49, 50]. During the initial 24 weeks of treatment, glucose elevations may be somewhat higher, followed by a return toward normal even without specific treatment. Most diabetic patients require no or only minor adjustment of antidiabetic therapy after starting niacin [51]. Higher niacin doses, however, cause unequivocal hyperglycemia as well as hyperuricemia which can increase the risk of gout [43].

Blurred vision associated with cystoid macular edema or other ocular effects has been reported with niacin doses of 3000 mg/day or higher [52]. New or aggravated peptic ulcer was described in the older niacin literature, but is rare today. Nausea and vomiting may occur with higher doses of niacin, but likewise are rare at doses up to 2000 mg/day [44]. Active gout can deter niacin use because nicotinic acid competes with uric acid for secretion by kidney tubules, raising serum uric acid by 5–15% [53,54]. Laboratory abnormalities include small reductions in platelet count (11% mean reduction with 2000 mg ER niacin) and serum phosphate (11% reduction similarly) [53]. The latter effect might eventually prove to be useful in patients with chronic kidney disease [55].

Atrial fibrillation occurred with higher frequency among patients assigned to the niacin group in the CDP [4]. However, atrial fibrillation thus far has not emerged as a problem in other randomized trials or in any case report. Established atrial fibrillation should not deter niacin use, because ventricular response rate is unaffected.

Drug Interactions and Compatibilities Niacin has minimal drug interactions, apart from its use with other agents that can potentially cause liver toxicity. Previous warnings about increased risk of myopathy when used with statins may apply only to SR niacin at higher doses [44].

Clinical Trials

Randomized clinical trials employing niacin with clinical and cardiovascular end points are summarized in Tables 26.2–26.4.

Randomized Trials with Clinical End Points (Table 26.2) The CDP randomized 1110 men with prior MI to niacin 1.0 g three times daily and 2789 to placebo. Subjects were followed for 6 years with average adherence of approximately 2000 mg niacin daily. The niacin group had 26%

Table 26.2 Randomized niacin trials with clinical cardiovascular outcomes

Study	Subjects	Treatments (duration)	Principal outcomes with niacin
Coronary Drug Project [4]	Men with previous MI, $N=3,899$	IR niacin vs. placebo (6 years)	MI ↓ 26% ($P<0.05$) and cerebrovascular events ↓ 24% ($P<0.05$) over 6 years. Total mortality ↓ 11% over 15 years ($P=0.0004$)
Stockholm Ischemic Heart Disease [57]	Recent MI, $N=555$	Niacin + clofibrate vs. no treatment (5 years)	Total mortality ↓ 26% ($P<0.05$) and CHD mortality ↓ 36% ($P<0.01$)
AIM-HIGH [58]	Established atherosclerotic disease and low HDL, $N=3414$	ER niacin vs. placebo added to baseline simvastatin ± ezetimibe (3 years)	No effect on primary endpoint of combined CV events
HPS-2 THRIVE [59, 60]	High-risk established atherosclerotic disease, $N=25,673$	ER niacin/laropiprant vs. placebo added to baseline simvastatin ± ezetimibe (3 years)	No effect on primary endpoint of combined CV events

IR immediate release, *MI* myocardial infarction, *ER* extended release, *HDL* high-density lipoprotein, *CV* cardiovascular, *CHD* coronary heart disease, *AIM-HIGH* Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes, *HPS2-THRIVE* Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events

fewer nonfatal MIs and 24% fewer cerebrovascular events ($P<0.05$ for each), but only a 4% nonsignificant reduction in total mortality, the primary end point [4]. A follow-up study after a total duration of 15 years demonstrated 11% total mortality reduction in the niacin group ($P=0.0004$) [56].

The Stockholm Ischemic Heart Disease study enrolled 555 MI survivors, who were randomized 4 months after hospital discharge either to no lipid therapy or to combined therapy with niacin and clofibrate. After 5 years, the primary outcome of total mortality was decreased by 26% ($P<0.05$) and ischemic heart disease mortality was decreased by 36% ($P<0.01$) in the treatment group [57].

The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial and the Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) were large randomized trials assessing the effect of adding ER niacin versus placebo to intensive LDL-lowering therapy with statin ± ezetimibe [58, 59]. Both studies revealed no impact on cardiovascular events [60]. In AIM-HIGH, a small subgroup with baseline HDL-C < 33 mg/dl and simultaneous triglyceride > 198 mg/dl showed a trend toward fewer cardiovascular events in the niacin group (hazard ratio 0.77, $P=0.074$) [61]. These trials will be discussed further after reviewing smaller, previous trials with niacin involving anatomic arterial end points quantified by imaging techniques.

Randomized Trials with Anatomic and Clinical End Points (Table 26.3) Two niacin trials with primary anatomic end points had sufficient clinical outcomes to examine as a secondary end point. The HDL-Atherosclerosis Treatment Study (HATS) was a 3-year angiographic trial that randomized 160 coronary heart disease (CHD) patients to simvastatin plus niacin or to placebo. Mean stenosis increased in the placebo group, but decreased in the simvastatin–niacin group. Cardiovascular events were decreased 70% in the simvastatin–niacin group ($P=0.03$) [62].

The Armed Forces Regression Study (AFREGS) randomized 143 CHD patients to combined therapy with gemfibrozil, niacin, and cholestyramine or to dietary advice only for 50 weeks. Angiographic stenosis increased in the placebo group and decreased in the treatment group ($P=0.04$). Hospitalized cardiovascular events or death occurred in 26% of the placebo group and 13% of the treatment group ($P=0.04$) [63].

Randomized Trials with Anatomic End Points (Table 26.4) Most randomized trials of niacin therapy with anatomic end points had too few

Table 26.3 Randomized niacin trials with arterial imaging end points and more than 15 CV events in the control group

Study	Subjects	Treatments (duration)	Principal outcomes with niacin
HATS [62]	CHD and low HDL, N = 160	Niacin (SR and IR) + simvastatin vs. placebo (3 years)	Coronary angiographic regression with niacin–statin combination (P < 0.001 vs. placebo); clinical events reduced by 70 % (P = 0.03)
AFREGS [63]	CHD and low HDL, N = 143	Niacin, gemfibrozil, and cholestyramine vs. limited use cholestyramine alone (2.5 years)	Coronary angiographic regression with intensive treatment vs. progression in controls (P < 0.05); clinical events reduced by 50 % (P < 0.05)

CV cardiovascular, CHD coronary heart disease, SR slow release, IR immediate release, HDL high-density lipoprotein, HATS HDL-Atherosclerosis Treatment Study, AFREGS Armed Forces Regression Study

Table 26.4 Randomized niacin trials with arterial imaging end points and less than 15 CV events in the control group

Study	Subjects	Treatments (duration)	Principal outcomes with niacin
CLAS [64]	Previous CABG and hypercholesterolemia. *N* = 162	IR niacin + colestipol vs. placebo (2–4 years)	Fewer progressing lesions ($P < 0.03$), less new atheroma formation ($P < 0.03$), more overall regression ($P = 0.002$)
UCSF-SCOR [69]	Heterozygous familial hypercholesterolemia, *N* = 72	Niacin + colestipol ± lovastatin vs. placebo ± low-dose colestipol (2 years)	Coronary angiographic regression with intensive treatment vs. progression in control patients ($P = 0.039$)
FATS [46]	Elevated apoB and family history of CHD, N = 146	Niacin (SR and IR) + colestipol vs. lovastatin + colestipol vs. placebo (2.5 years)	Coronary angiographic regression in intensively treated groups vs. progression in control group ($P < 0.003$); clinical events reduced by 73 % ($P < 0.05$)
HARP [71]	CHD and total cholesterol <6.5 mmol/L (252 mg/dl), *N* = 79	Stepwise pravastatin, SR niacin, cholestyramine, gemfibrozil vs. no lipid treatment	No change in coronary angiographic progression
ARBITER-2,3 [67,68]	Men with low HDL, *N* = 167	ER niacin added to simvastatin (1–2 years)	Mean regression of CIMT at 2 years ($P \leq 0.001$ vs. baseline)
Thoenes CIMT [70]	Metabolic syndrome, *N* = 50	ER niacin or placebo (12 months)	Mean regression of carotid intima-media thickness ($P = 0.021$)
ARBITER-6/HALTS [65]	CHD and LDL < 100 mg/dl and low HDL, *N* = 208	ER niacin and statin vs. ezetimibe and statin (14 months)	Reduction in CIMT (≤ 0.001)
Carotid MRI [66]	Low HDL, atherosclerotic disease, *N* = 71	ER niacin added to statin therapy (12 months)	Reduction in carotid wall area by MRI ($P = 0.03$)

CV cardiovascular, *CHD* coronary heart disease, *SR* slow release, *IR* immediate release, *ER* extended release, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *ARBITER-6/HALTS* Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6-HDL and LDL Treatment Strategies, *CLAS* Cholesterol-Lowering Atherosclerosis Study, *UCSF-SCOR* University of California, San Francisco- Specialized Center of Research, *FAT* Familial Atherosclerosis Treatment Study, *HARP* Harvard Atherosclerosis Reversibility Project, *CIMT* carotid intima-media thickness, *MRI* magnetic resonance imaging, *CABG* coronary artery bypass grafting

clinical events to gauge clinical outcomes. The Cholesterol-Lowering Atherosclerosis Study (CLAS) compared colestipol–niacin combination therapy versus placebo in 162 men with prior coronary bypass surgery [64]. Follow-up angiography at 4 years showed progression in native coronary arteries of 85 % of placebo-treated subjects versus 48 % of drug-treated subjects. The Familial Atherosclerosis Treatment Study (FATS), conducted over 2.5 years in 146 men with elevated apoB and family history of CHD, was notable for net regression of coronary lesions in two intensively treated groups—niacin–colestipol and lovastatin–colestipol—versus progression with conventional lipid treatment [41].

The Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6-HDL and LDL Treatment Strategies (ARBITER-6/HALTS) study compared ER niacin versus ezetimibe added to statin treatment in CHD patients over 14 months [65]. The niacin group showed reduction of carotid intima-media thickness (CIMT, $P \leq 0.001$ for all comparisons). Lee et al. in 2009 reported results of a trial with ER niacin 2000 mg versus placebo added to statin treatment in 71 high-risk patients with low HDL. After 1 year, niacin significantly reduced carotid wall area, assessed by magnetic resonance imaging (MRI) [66].

Three other niacin randomized trials gave similar results of improvement in atherosclerotic lesions—the University of California San Francisco Arteriosclerosis Specialized Center of Research (UCSF-SCOR) trial of combination treatment in patients with familial hypercholesterolemia, the ARBITER-2,3 trial and extension with addition of ER niacin to ongoing statin therapy, and a recent trial by Thoenes et al. of ER niacin in statin-averse or statin-intolerant patients [67–70]. The Harvard Atherosclerosis Reversibility Project (HARP) used SR niacin as part of four-drug step therapy targeting reduction of total cholesterol and ratio of LDL-C to HDL-C, resulting in no change in progression of coronary stenosis [71].

Role of Niacin in Atherosclerotic Risk Reduction

In the aftermath of AIM-HIGH and HPS2-THRIVE, which showed no impact of ER niacin on cardiovascular events [58,60], estimates for the value of niacin in atherosclerotic risk reduction range from very little to moderately high. The minimal estimates are based squarely on these two large randomized trials with little consideration of previous results. Those who would assign a larger role for niacin, including the authors of this chapter, point to the breadth of prior evidence and to the specific conditions under which negative results were obtained in AIM-HIGH and HPS2-THRIVE—that is, use of bedtime ER niacin in high-risk secondary prevention patients with low LDL-C on statin treatment.

Any kind of ancillary treatment in the setting of statin-induced low LDL-C may have difficulty showing additional benefit. If this is true, then niacin perhaps should be advised only for patients with insufficient LDL-C lowering. Optionally, one must choose niacin for those with very low HDL-C (< 33 mg/dl) accompanied by high triglyceride, based on nonsignificant subgroup analysis in AIM-HIGH [61].

However, we would raise the possibility of a serious pathophysiologic flaw in the bedtime niacin dosing strategy employed in both AIM-HIGH and HPS2-THRIVE, in contrast with mealtime dosing used in prior clinical trials. Niacin-induced antilipolysis in the absence of substantial food intake (i.e., niacin dosed apart from mealtime) creates impairment in fuel supply (sharply decreased plasma NEFA) for the heart and other muscles. The late overshoot in plasma NEFA, as previously discussed (Fig. 26.3), gives evidence for a counter-regulatory hormone response that supports fuel supply. Thus, bedtime niacin, but not mealtime niacin, may be expected to produce a daily unphysiologic surge of counter-regulatory (stress) hormones, which can promote thrombosis, arrhythmias, and perhaps plaque rupture with a result of increased cardiovascular events. This would cancel the benefit of niacin on

cardiovascular events. Prior niacin clinical trials with mealtime dosing (CDP, Stockholm, HATS, and AFREGS) showed reductions in events.

Antilipolysis is a nonlipoprotein action of niacin which according to this hypothesis may have adverse clinical consequences. On the other hand, GPR109A-dependent niacin effects on macrophages are nonlipoprotein actions that appear to be beneficial. The AIM-HIGH trial provides evidence that such nonlipoprotein effects may be clinically relevant. In-trial levels of LDL-C and non-HDL-C predicted cardiovascular events in the AIM-HIGH placebo group (hazard ratios of 1.39 and 1.31, respectively), but not in the ER niacin group (hazard ratios of 1.01 and 0.98, respectively, interaction *P* values both 0.01). This signifies that bedtime ER niacin changes the relationship between lipoproteins and events. The implication is that either ER niacin modifies LDL enough to make it nonatherogenic or ER niacin influences events independently of lipoproteins. Since the former mechanism is unlikely, the results are most consistent with the hypothesis that nonlipoprotein effects of niacin influenced events in AIM-HIGH [61].

The smaller randomized trials with niacin, regardless of dosing regimen, are remarkably consistent in showing decreased progression or actual regression of atherosclerotic lesions, either statistically significant or a substantial trend in nine of ten trials (Tables 26.3 and 26.4). Additional data from a carotid MRI substudy in AIM-HIGH are pending. At present, it appears that niacin generally improves atherosclerotic lesions, but bedtime niacin does not reduce events. The hypothesis of fuel supply impairment and counter-regulatory stress response might suggest a specific adverse effect of bedtime niacin on events, without impairing niacin's beneficial effect lesions. It is only a hypothesis at this point, however, and further research is needed to determine the implications of mealtime versus bedtime dosing of niacin. Overall, niacin is likely to remain a useful pharmacologic treatment for dyslipidemia, but whether the scope of its application should be limited or broad remains to be decided. A recent demonstration of upregulation of GPR109A in circulating white blood cells and in substantia nigra of patients with Parkinson's disease, as well as localization of GPR109A to microglial cells, raises the possibility that anti-inflammatory and other cellular effects of niacin might be useful in disorders other than atherosclerosis [72].

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Bile Acid Sequestrants: Risk–Benefits and Role in Treating Dyslipidemias

27

Om P. Ganda and Abhimanyu Garg

Introduction

Bile acid sequestrants (BAS) have proven to be an effective and relatively safe therapy in the management of hypercholesterolemia since the 1960s. These agents were developed as a therapeutic intervention to enhance cholesterol excretion via the gut by interrupting the enterohepatic circulation and recycling of bile acids. These agents were the mainstay of treatment prior to the discovery and availability of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) in the 1980s. BAS continue to be of value in the treatment of lipid disorders in combination therapy with statins and other lipid drugs, as well as in some situations as monotherapy. The recent resurgence of interest in BAS emanates from the recently discovered and ongoing advances in the understanding of the physiology of bile acids, their role in lipid as well as carbohydrate metabolism, and the potential implications of the current research in this area.

In optimal dosing, BAS can deplete the bile acid pool by up to 40 %, which in turn results in the upregulation of cholesterol 7 alpha-hydroxylase (CYP7A1) to enhance bile acid synthesis and also upregulation of low-density lipoprotein (LDL) receptors leading to reduction in plasma cholesterol [1, 2]. In the maximum approved dosage, these agents can reduce LDL-C by 15–30 %, along with a modest increase in high-density-lipoprotein-cholesterol (HDL-C) and apolipoprotein A1 (apo-A1) by up to 4–8 % [1–4]. Moreover, they have additive effects on the LDL-lowering effects of statins by virtue of complementary actions of statins in inhibiting the cholesterol synthesis otherwise augmented by a reduction in the intrahepatic pool of cholesterol by bile acid depletion caused by BAS [5] (Fig. 27.1). Thus, the combination of low to moderate statins with BAS can result in an impressive, ~40–60 % reduction in LDL-C, equivalent to or greater than the maximum dose of statin [2, 6, 7]. This is a distinct advantage considering that every doubling of the statin dose alone results in approximately a 6 % further decrement in LDL-C, while increasing the risk of certain adverse effects, e.g. myalgia in many patients [8]. Similarly, additive effects of BAS with other cholesterol-lowering agents, including ezetimibe, niacin, and fibrates, have been reported [9–12]. On the other hand, BAS generally result in a modest rise in circulating triglycerides via indirect effects. The bile acids are known to regulate the activity of farnesoid

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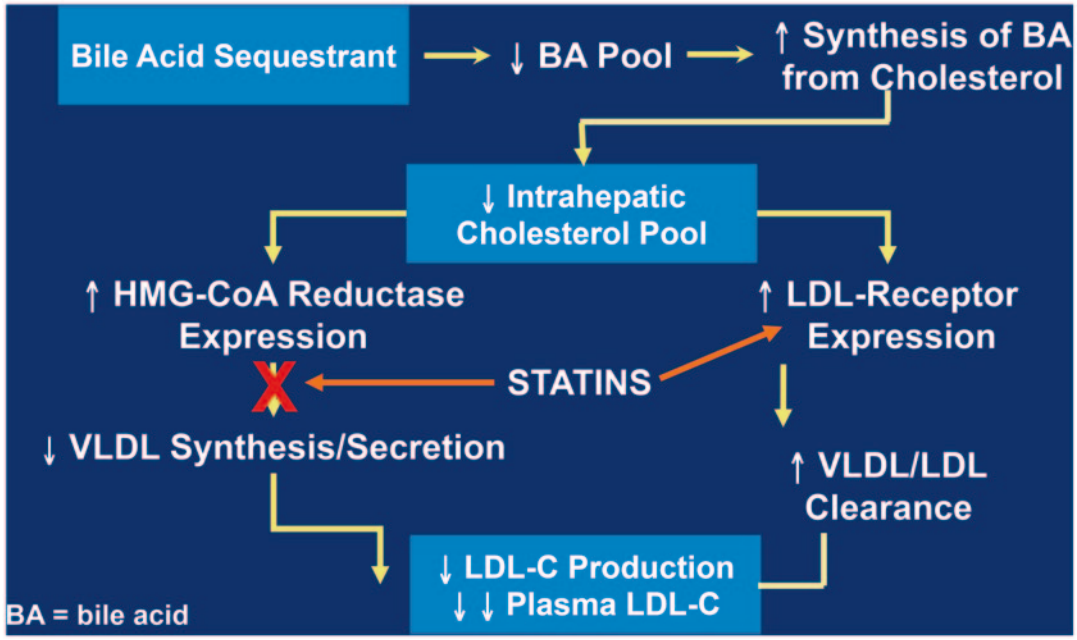

Fig. 27.1 Rationale for the combination therapy with statin and BAS therapy. BAS-induced reduction in hepatic cholesterol pool leads to augmentation of cholesterol synthesis via HMG-CoA reductase pathway, an effect inhibited by statins. *BAS* bile acid sequestrants, HMG-CoA 3-hydroxy-3-methylglutaryl-CoA. (Adapted from [5])

X receptor (FXR), and by changing the bile acid pool and composition, BAS act as FXR antagonists and likely impair the activity of apo-CII and activate liver X receptor (LXR), thus increasing triglyceride levels [1, 2].

Among the limited number of studies on the other anti-atherosclerosis effects, colesevelam was shown to reduce C-reactive protein (CRP) levels by 16–23 % in monotherapy [13], or in combination therapy with statin [14], and to reduce LDL particle number [15].

History of Bile Acid Sequestrants

Bile acid sequestrants (BAS) were developed after Siperstein et al. [16] reported that ferric chloride, which binds to bile acids, inhibited the rise in serum cholesterol upon cholesterol feeding in cockerels. This suggested that binding of bile acids in the intestine may serve as a means of controlling serum cholesterol. Soon thereafter, in 1960, Tennent et al. [17] from the Merck Sharp and Dohme Laboratories, reported blood cholesterol-lowering action of two bile-acid-binding polymeric organic bases, MK-325 and MK-135, in cholesterol-fed cockerels and normocholesterolemic cockerels and dogs. MK-135, a quaternary ammonium anion exchange resin in which the basic groups are attached to a styrene-divinyl benzene copolymer skeleton by carbon-to-carbon bonds, was later named cholestyramine. Bergen et al. [18] reported cholesterol-lowering effects of cholestyramine in humans, and Hashim et al. [19] reported the bile-acid-binding capacity of cholestyramine in humans by demonstrating steatorrhea upon feeding 30 g of cholestyramine to healthy subjects. The second BAS colestipol was developed by Upjohn in 1970 and was reported to lower serum cholesterol in men [20]. Colesevelam hydrochloride was discovered in Geltex Pharmaceuticals in 1995 and later developed by Daiichi Sankyo. Figure 27.2 depicts the chemical structure of the BAS.

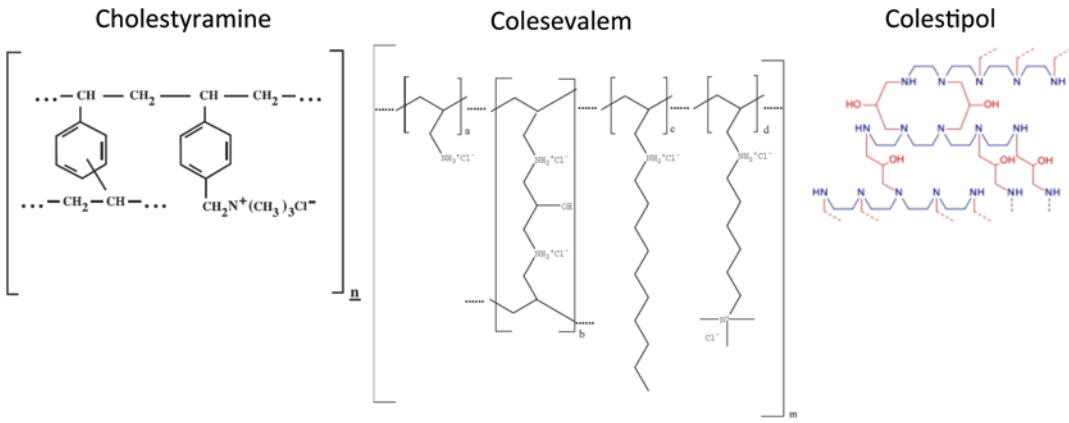

Fig. 27.2 The chemical structure of BAS available in the USA. *BAS* bile acid sequestrant

Characteristic Features of BAS

The two primary bile acids, cholic acid and chenodeoxycholic acid, are synthesized from cholesterol in the human liver, via a cascade of steps, starting with cholesterol-alpha-hydroxylase (CYP7A1) and cholesterol 27-hydroxylase (CYP27A1), respectively. These are conjugated and transported from the liver as anionic bile salts. In the gut, the bile acids undergo further modifications, and solubilize dietary fat and fat-soluble vitamins for their absorption. Most of the bile acid pool (~95%) undergoes extensive enterohepatic recycling [1, 2]. In addition to their role in fat absorption, recent work in progress is investigating their critical role in the transcriptional regulation of several nuclear hormone receptors and as signaling molecules in energy metabolism [2, 21, 22].

The BAS are cationic agents with a large polymeric, unabsorbable structure designed to bind the anionically charged bile acids. Currently available BAS include cholestyramine, colestipol, and colesevelam [1–4]. The first-generation agents cholestyramine and colestipol preferentially bind the dihydroxy bile acids (chenodeoxycholic acid and deoxycholic acid), compared to trihydroxycholic acid and cholic acid. This eventually leads to a limited binding capacity of these agents as the trihydroxy bile acid pool is increased. The newer BAS, colesevelam, has differential binding features, enabling it a greater affinity and specificity to bind to bile acids (Fig. 27.3). In the studies by Braulin et al. [23], colesevelam had a better binding affinity for cholylglycine than cholestyramine and colestipol but a similar binding affinity for cholyltaurine, chenodeoxycholylglycine, deoxycholylglycine, chenodeoxycholyltaurine, and deoxycholyltaurine. These physicochemical differences enhance the binding capacity of colesevelam at lower dosage, and reduce the drug interactions [4, 23]. BAS are also approved cholesterol-lowering agents in children [24] and belong to Food and Drug Administration (FDA) class B category for use in pregnancy. A fourth agent colestimide is available in some countries, but not yet approved by the US FDA [25].

The older BAS cholestyramine and colestipol are available in powder form and administered in suspension form with a dose–response relationship requiring 8–24 and 10–30 g dosing, respectively. Colestipol is also available in 1-g tablet form, requiring up to 16 pills daily. On the other hand, colesevelam is available in powder for suspension (3.75-g packet) or 625-mg pills, requiring six to seven pills daily for maximal response [3, 4].

Effects of BAS on Cardiovascular Events

Agents in the BAS family were the first effective LDL-C-lowering drugs to be studied in a number of angiographic or clinical trials, starting in

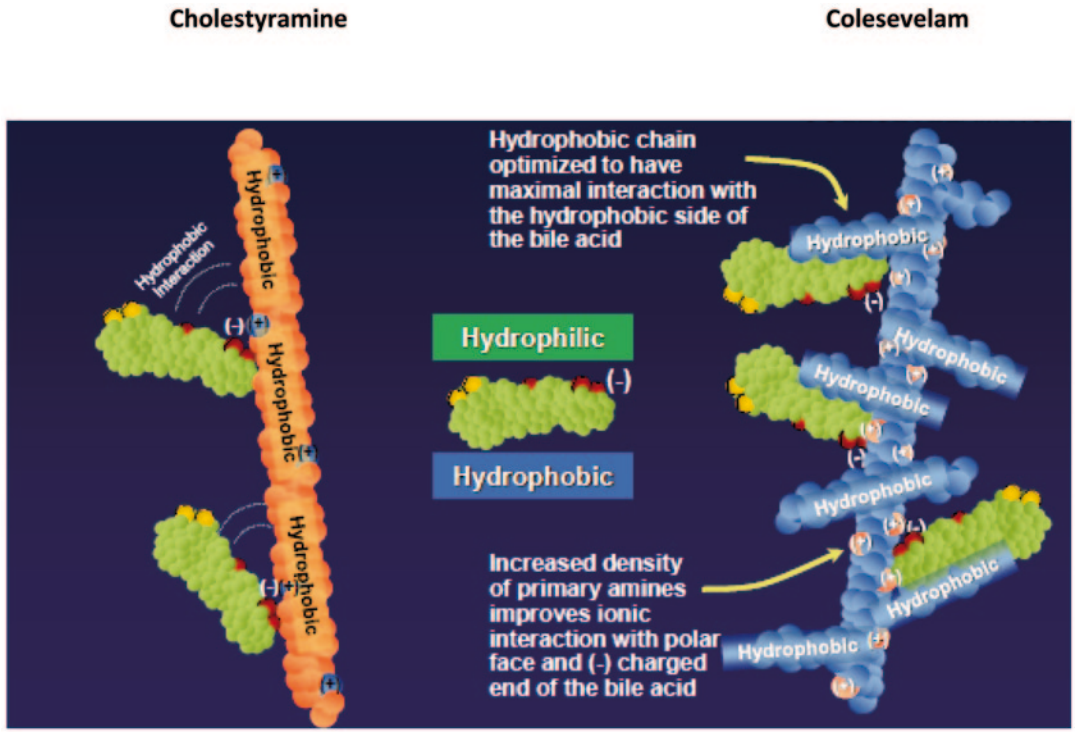

Fig. 27.3 Differential binding characteristics of older bile acid sequestrants (cholestyramine) and the newer agent colesevelam (see text for details)

the pre-statin era. Table 27.1 lists the noteworthy studies, which include BAS in monotherapy, compared to placebo or diet [26–30], or in combination with other lipid-lowering agents [9, 10, 31, 32]. The first major monotherapy study was the National Heart, Lung and Blood Institute (NHLBI) angiographic study which demonstrated atherosclerotic lesion progression in 12% of cholestyramine-treated patients, compared to 33% of placebo-treated patients over 5 years in those with >50% stenosis in a coronary vessel at baseline [29]. This was followed by the St Thomas' Atherosclerosis Regression Study (STARS), a much smaller study with similar results with cholestyramine or a low-saturated (or trans)-fat diet [30]. Other angiographic trials also showed the additional benefits of LDL-C reduction and possibly rise in HDL when BAS were used in combination with niacin [10, 11, 31, 32], statin [11, 31], or gemfibrozil [32]. In these multidrug trials, particularly when used in combination with niacin, some of the coronary lesions showed significant regression, in addition to less progression.

There have been two large-scale randomized clinical trials with BAS monotherapy to study the impact of these agents on coronary events. The first study, also known as the Upjohn colestipol trial [26], although associated with reduced events, had insufficient power due to short duration of 2 years. Subsequently, the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) was published in 1984, a truly landmark clinical trial which proved the cholesterol hypothesis in 3806 men, with a mean follow-up duration of 7.4 years [27, 28]. In this trial, the primary endpoint of definite coronary heart disease (CHD) death or nonfatal myocardial infarction (MI) was 7.0 and 8.6% in cholestyramine- and placebo-treated men, respectively, a reduction of 19% accompanied with a 13% reduction in LDL-C as compared to placebo treatment ($p<0.05$). In addition, the new positive exercise tests, angina, and coronary bypass surgery

Table 27.1 Angiographic or clinical trials with bile acid sequestrants in monotherapy or combination therapy

Trial (Ref.)	*N*, M/F (%)	Drug (dose)	Duration (year)	% LDL-C change	% HDL-C change	Comments
Dorr et al. [26])	2278, 48/52	Colestipol (15 g) versus placebo	2	Total-C −12 versus −2		Short duration
LRC-CPPT [27, 28]	3806 All male	Cholestyramine (24 g) versus placebo	7.4	−20 versus −8	+ 5	19 % CHD risk reduction ($P< 0.05$)
NHLBI Type II study [29]	116, 81 % male	Cholestyramine (24 g) versus placebo	5	−26 versus −5	+8	Angiography: 32 versus 49% of patients progressed
STARS [30]	94 All males	Cholestyramine (16 g) + diet versus diet versus usual care	3.3	−36 versus 16 versus 0	+4	Improved angiographic and clinical status
CLAS I and II [10]	265 All males	Niacin (3–12 g) + colestipol (30 g)	2 and 4	−40 %	+37%	Less progression and more regression
FATS [11]	120 All males	Colestipol (30 g) + niacin (4 g) versus Colestipol (30 g) + lovastatin (40 mg) versus placebo	2.5	−32 −46 −7	+43 +15 +5	CHD events 4 versus 7 versus 19% Less progression and more regression versus placebo
UCSF-SCOR [31]	72 45 % males	Colestipol (15–30 g) + niacin (1.5–7.5 g) + lovastatin (40–60 mg) versus usual care	2.2	−39	+26	Less progression and more regression
AFREGS [32]	143 92 % males	Cholestyramine (16 g) + niacin (3 g) + gemfibrozil (1.2 g) versus placebo	2.5	−22 versus +5	+ 36	13 versus 26% CHD events; less progression, more regression

AFREGS Armed Forces Regression Study, *CLAS* Cholesterol Lowering Atherosclerosis Study, *FATS* Familial Atherosclerosis Treatment Study, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *LRC-CPPT* Lipid Research Clinics Coronary Primary Prevention Trial, *NHLBI*, National Heart Lung Blood Institute, *STARS*, St. Thomas' Atherosclerosis Regression Study, *UCSF-SCOR*, University of California San Francisco-Specialized Center for Research in Arteriosclerosis

were reduced by 25, 20, and 21 %, respectively. However, the overall mortality was reduced by a nonsignificant 7 % in the cholestyramine group [27]. The mean LDL-C at baseline was 205 mg/dl, declining to 175 mg/dl at the end of trial. The mean triglyceride increased by 15 % and HDL-C rose by ~5 %.

The relationship between adherence to therapy and event rates was examined in detail in the LRC-CPPT trial. There was a remarkable relationship between adherence to regimen, the decline in LDL-C, and the effects on CHD incidence (Table 27.2). In fact, those 965 men who took the full dose of 20–24 g cholestyramine daily had up to 35 % decline in LDL-C and a 50 % decline in CHD incidence [28]. Moreover, after a 6-year posttrial follow-up of the participants, the reduction in CHD risk decreased after discontinuation of treatment, indicating the need for maintaining LDL-lowering therapy [33]. Thus, the LRC-CPPT trial was very successful as a first long-term primary prevention lipid trial that led

Table 27.2 Relationship between cholestyramine dose, lipid effects, and CHD event rate (LRC-CPPT study). (Adapted from [28])

Dose (g/day)	*n*	% Change in LDL-C	% Change in HDL-C	% Change in TG	% Change CHD events
0–4	294	−6.6	+5.2	+10.7	−10.9
4–8	145	−8.7	+2.3	+12.7	
8–12	135	−13.1	+5.5	+12.9	−26.1
12–16	156	−16.5	+6.0	+14.2	
16–20	205	−20.9	+3.8	+15.5	−39.3
20–24	965	−28.3	+4.3	+17.1	

The mean changes in patients on placebo were: LDL-C (−3.6 to −8.4% reduction), HDL-C (+1.2 to +5.4% increase), and triglycerides (+7.9 to +11.7% increase)

to launch of a highly effective National Cholesterol Education Program (NCEP).

Effects of BAS on Carbohydrate Metabolism

A study in 1994 investigating the effect of cholestyramine on dyslipidemia in type 2 diabetes led to a serendipitous observation of a glucose-lowering effect of this agent [34]. In this 8-week, randomized study of 21 patients, compared to placebo, there was a significant reduction of 13% in mean glucose level ($p=0.003$) and a nonsignificant 0.5% lowering in total glycosylated hemoglobin. Since then, a number of randomized controlled trials have explored this phenomenon in greater detail. In three pivotal trials encompassing 1074 patients with type 2 diabetes with ongoing treatment with predominantly metformin [35], sulfonylurea [36], or insulin [37], randomization to colesevelam or placebo, over 16–24 weeks, resulted in a significant 14–15 mg/dl reduction in fasting glucose and a 0.5–0.54% reduction in hemoglobin A1c levels (Fig. 27.4). In addition, there were expected changes in lipids, including a 13–17% decrease in LDL-C, 5–8% decrease in apo-B, 2–4% increase in Apo-A1, and a 5–21% increase in triglycerides [35–38]. These lipid changes were superimposed on the baseline statin therapy in 41–57% of patients. In another 16-week trial, drug-naïve patients ($n=245$) were randomized to metformin and colesevelam or active control group of metformin alone [39]. The results showed a mean decline in hemoglobin A1c of 1.1 and 0.8%, respectively ($p=0.0035$). The lipid and lipoprotein effects were similar to those reported in pivotal studies above (Fig. 27.5). Of note, only 7% of these patients were on statin therapy at baseline. In addition to the lipid effects, there was a significant 17% reduction in CRP level, compared to placebo, similar to that observed in other studies [13, 14]. Finally, in a 16-week, randomized study of colesevelam alone versus placebo in impaired fasting glucose ($n=216$), there was a small but significant 0.1% reduction in hemoglobin A1c [40]. In this and the previous study [39], there was also a significant 10–15% increase in apo-CIII levels, consistent with the increase in triglycerides. The glucose-lowering effects of another BAS, colestimide, have also been reported with a few publications from the Japanese population [2, 25]. In one of these publications, an appreciable and significant reduction in visceral abdominal fat was also observed [25].

The mechanism of the glucose-lowering effect of BAS remains under active investigation, and currently poorly understood [2, 21, 22, 41–45]. The bile acid pool is increased in patients with type 2 diabetes, and a number of hypotheses have been postulated (Table 27.3). A part of the inconsistent results relate to the difficulties in applying the data from the animal models to the in vivo situations in humans. Much of the recent investigations suggest an enhancement of incretin-mediated effect via increased luminal bile acid concentration leading to activation of TGR-5, a G-protein-coupled receptor [2, 42, 43]. Alternatively, increased delivery of unabsorbed fatty acids to distal parts of gut could enhance both glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) release via the GPR-40

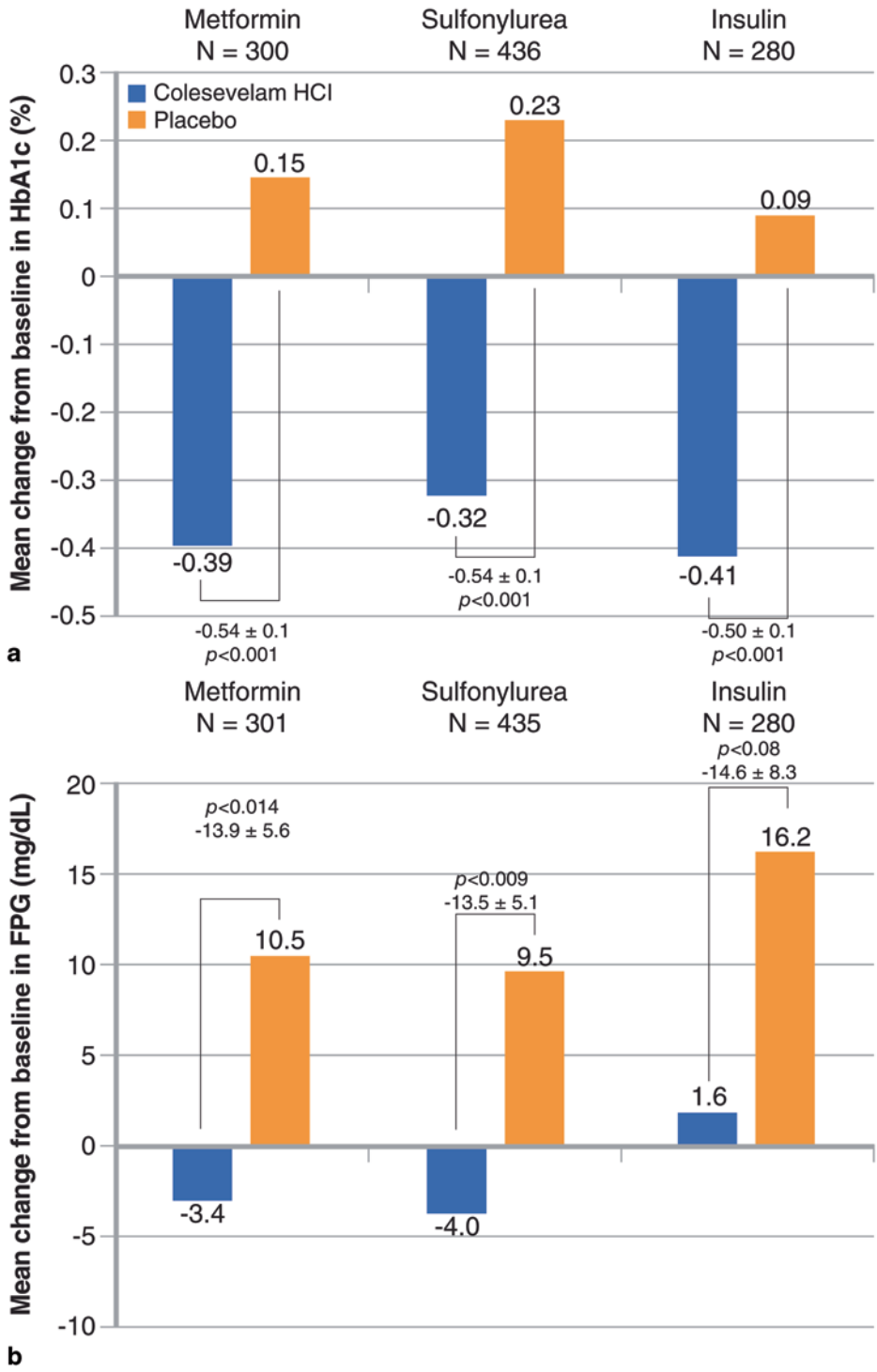

Fig. 27.4 Mean changes in hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) in pivotal trials of colesevelam versus placebo in type 2 diabetes patients with ongoing treatment regimens of conventional agents [38]

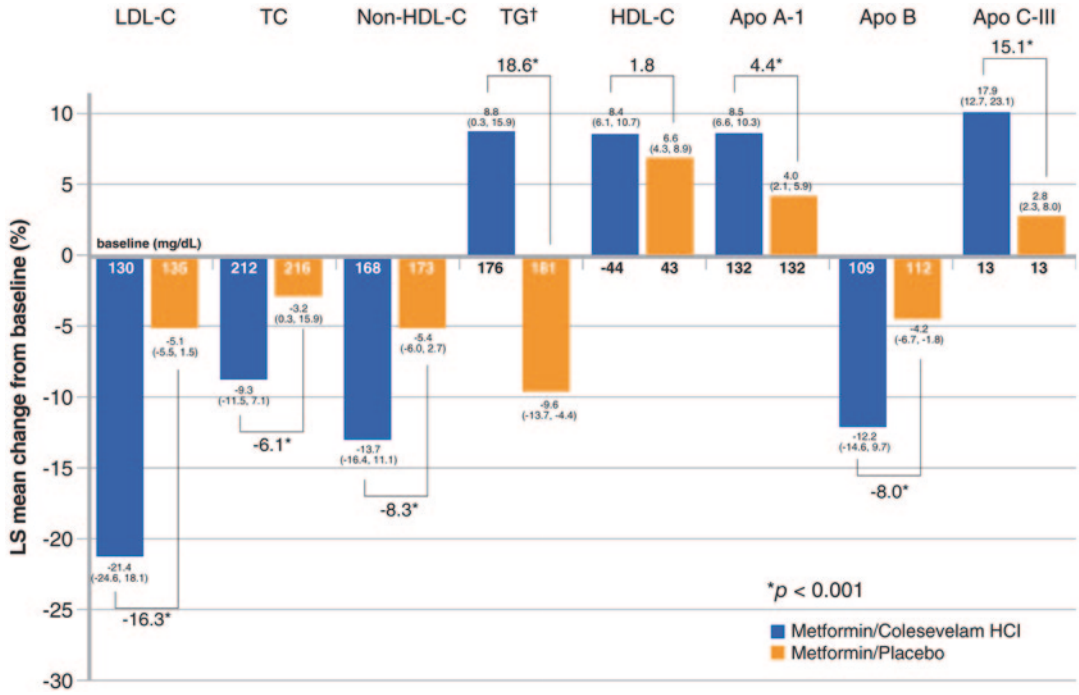

Fig. 27.5 Changes in lipids and apolipoproteins in a multicenter trial of patients with type 2 diabetes treated with colesevelam and metformin, compared to metformin alone [39]

Table 27.3 Postulated mechanisms for glucose-lowering effects of bile acid sequestrants

1	Changes in bile acid pool and composition, leading to reduced glucose absorption
2	Effects on nuclear hormone receptors, e.g., downregulation of FXR and small heterodimer protein (SHP) > upregulation of LXR and FGF 19 leading to reduced hepatic gluconeogenesis
3	Induction of GLP-1 secretion via activation of TGR-5, a G-protein-coupled receptor
4	Increased levels of unabsorbed fatty acids in gut lumen leading to enhanced GPR 40 activity > increased GLP1 and GIP

FGF19 fibroblast growth factor 19, *FXR* farnesoid X receptor, *GIP* gastric inhibitory polypeptide, *GLP-1* glucagon-like peptide-1, *LXR* liver X receptor

pathway [43]. In a recent mechanistic study with colesevelam in type 2 diabetes, employing a euglycemic hyperinsulinemic technique and glucose tolerance tests, while the overall glucose control improved, it was not explained by any significant effects on hepatic or peripheral insulin sensitivity [45]. On the other hand, the diurnal pattern of blood glucose excursions, in an ambulatory setting, studied by continuous glucose monitoring confirmed a rapid improvement in glucose levels, with colesevelam compared to placebo [46]. These studies underscore the complexity of the mechanism underlying glucose-lowering effects of BAS in the human model.

Role of BAS in the Context of Current Cholesterol Guidelines

The recently available American Heart Association/American College of Cardiology (AHA/ACC) cholesterol management guidelines emphasize the key role of statins as the preferred drug class in both primary and secondary

prevention of CVD [47]. While effective in LDL-C reduction in monotherapy and in combination therapy with statins, there are no randomized controlled trials of BAS in combination therapy. The AHA/ACC guidelines advise the consideration of BAS as adjunct therapy with statins in patients with familial hypercholesterolemia when adequate LDL-C reduction is not achieved with the maximum tolerated dose of statin, or in patients with statin intolerance [47]. The National Lipid Association (NLA) also recommends the use of BAS when statin therapy is inadequate in achieving cholesterol goals [48]. A fasting lipid panel should be obtained before starting BAS, and again at 3 months, and every 6–12 months thereafter.

BAS may be used with caution in patients with triglyceride level > 300 mg/dl or those with type III hyperlipoproteinemia. In patients with triglyceride level 250–299, BAS use should be followed by another triglyceride measurement after 4–6 weeks, and the drug discontinued if triglyceride level exceeds 400 mg/dl. BAS are contraindicated in patients with triglyceride > 500 mg/dl [47].

Adverse Effects Associated with BAS

Due to the lack of systemic absorption, BAS generally have a good safety record. The main adverse effects are gastrointestinal, particularly constipation [1, 4]. It seems to wane with time, as seen in the largest clinical trial, LRC-CPPT, where it was reported in 39% of patients in year 1 and only 8% by year 7 [27]. This side effect was noted in only 8–10% of patients in the colesevelam trials that lasted only up to 24 weeks [35–37]. Other less common gastrointestinal side effects include heartburn, bloating, nausea, and abdominal pain [47]. Patients should be encouraged to consume plenty of fluids and fiber, and may consider psyllium to minimize the gastrointestinal side effects. BAS are contraindicated in the setting of bowel obstruction.

An important consideration with BAS is the physical binding to anionically charged drugs administered concomitantly. The examples of such drugs include digoxin, thyroxine, warfarin, propranolol, estrogens, oral contraceptives, olmesartan, cyclosporine, sulfonylureas, and hydrochlorothiazide [1, 4, 47]. It is therefore recommended that other oral drugs be taken 1 h before or 4 h after taking the BAS. Generally, these drug interactions are less common with colesevelam [3, 4]. Finally, absorption of fat-soluble vitamins may be impaired with BAS; therefore, supplementation is recommended [4, 47].

One of the rare adverse effects reported with the use of BAS is the development of nonanion gap hyperchloremic acidosis, when consumed in high doses, e.g., children, or in the presence of advanced renal insufficiency, dehydration, or with concomitant administration of spironolactone [49].

In view of the triglyceride-raising effects of BAS, these agents are relatively contraindicated in patients with triglyceride levels > 300–500 mg/dl, to minimize the risk of pancreatitis. Rare case reports of precipitation of xanthoma have been reported in a susceptible individual with underlying genetic hypertriglyceridemic state [50].

Concluding Remarks

The launch of statin therapy in the 1980s and subsequent clinical trials paved the way for the drugs in the statin family as the initial choice for LDL-C lowering. However, BAS currently remain an important class as the second line of drugs with established value and a safe benefit to risk ratio in lipid management. Clinical trials with BAS in the pre-statin era documented their role in limiting atherosclerotic lesion progression and in reducing CVD events in both primary prevention and secondary intervention. In combination therapy with statins, BAS have additive effects on LDL-C reduction, when statin dosage cannot be escalated or LDL-C goals with statin therapy alone are not met. Unlike statins, niacin, ezetimibe, and fibrates, their demonstrable effect on a significant reduction in glucose levels in patients with diabetes offers a unique benefit, on top of the lipid effects. Caution should be exercised in their use in patients with high or very high triglycerides.

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Cholesterol Absorption Inhibitor Ezetimibe: Risk–Benefits and Role in Treating Dyslipidemias

28

Shizuya Yamashita, Daisaku Masuda and Akifumi Matsuyama

Introduction

Niemann-Pick C1-Like 1 (NPC1L1) is a 13-transmembrane domain cell surface cholesterol-sensing receptor. It is localized on the apical membrane or brush border of small intestines (especially jejunum) and has recently been reported to play an important role in dietary cholesterol absorption and biliary cholesterol reabsorption by enterocytes [1–2]. Genetic inactivation of *NPC1L1* gene decreases cholesterol levels and atherosclerotic lesions in mice with diet-induced hyperlipidemia [3] and in hyperlipidemic apolipoprotein (apo) E-knockout mice fed a Western diet [4]. Ezetimibe, a novel lipid-lowering compound, selectively inhibits intestinal cholesterol absorption by binding to NPC1L1 [5] and inhibiting the internalization of NPC1L1 [6]. NPC1L1 has three large loops that protrude into the extracellular space, several smaller cytoplasmic loops, and a C-terminal cytoplasmic tail [7]. Studies using in vitro ezetimibe-binding assays, demonstrated that ezetimibe directly binds to the second extracellular loop of NPC1L1 [8–9].

Ezetimibe reduces the hepatic influx of cholesterol via chylomicrons (CM) remnants, which enhances the hepatic expression of low-density lipoproteins (LDL) receptor, and thus reducing LDL-cholesterol (LDL-C) levels. Ezetimibe is also reported to reduce the development of atherosclerosis in apoE-knockout mice [10]. Clinically, the administration of ezetimibe has been shown to decrease the fasting levels of total cholesterol and LDL-C in patients with primary hypercholesterolemia [11] and plant sterols (sitosterol and campesterol) in patients with sitosterolemia [12–13]. A meta-analysis demonstrated that a significantly greater percentage reduction in LDL-C levels was achieved in patients treated with ezetimibe–statin combination compared with statin monotherapy [14]. Since ezetimibe is an inhibitor of intestinal cholesterol absorption, the pharmacological effects of ezetimibe have been focused primarily on the metabolism of sterols, including cholesterol, plant sterols, and oxidized cholesterol rather than triglycerides (TG) or TG-rich lipoproteins (TRL).

Ezetimibe has been reported to significantly decrease fasting TG levels in patients with combined hyperlipidemia [15] and those with hypertriglyceridemia (TG≥150 mg/dl); however, its underlying mechanism of action on TRL metabolism has not yet been elucidated. We have recently reported the effects of ezetimibe in patients with

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type IIb hyperlipidemia with a special reference to postprandial TRL and remnant metabolism. We reported that ezetimibe administration could attenuate postprandial hyperlipidemia in oral fat-loading tests [16]. We also evaluated the mechanisms for the attenuation of postprandial hyperlipidemia in mouse models and reported that ezetimibe can reduce the production of CM from the small intestines and decrease the absorption of free fatty acids (FFA) [17].

More recently, ezetimibe has been reported to attenuate nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). This chapter highlights the effects of ezetimibe on postprandial hyperlipidemia, hepatic lipid depositions in NAFLD or NASH, and insulin resistance. The potential and comprehensive mechanisms for the improvement by ezetimibe of these conditions usually associated with metabolic syndrome are presented.

History of Ezetimibe

Although cholesterol was supposed to be absorbed in the small intestine, especially the jejunum, the detailed mechanisms for cholesterol absorption were not understood. From the initial screening of an acyl-CoA to cholesterol acyltransferase 2 (ACAT2) inhibitor, ezetimibe was discovered by Davis HR and colleagues at the Research Institute of Schering-Plough as an inhibitor of cholesterol absorption in the small intestine [18–19]. It was demonstrated that ezetimibe is a potent and selective inhibitor of intestinal cholesterol uptake and absorption in animal models and humans. It was launched into the market before its molecular target was finally identified. Extensive studies were performed to identify the molecular target of ezetimibe. Since ezetimibe was shown localized in the brush border of enterocytes in the jejunum, it was speculated that the target of ezetimibe was localized there. Several candidate genes which are localized in the brush border of the small intestine were investigated, including scavenger receptor class B type I (SR-BI), ATP-binding cassette transporter A1 (ABCA1), and CD36, a transporter of long-chain fatty acids. However, the knockout mice of these genes did not show any changes in the absorption of radio-labeled cholesterol [20]. Thus, it had been very difficult to discover the molecular target of ezetimibe.

Genomic bioinformatics approach was then applied to identify genes involved in the absorption of cholesterol in the small intestine. Altmann et al. [1] hypothesized that intestinal cholesterol transporter should be localized mainly in the luminal surface and brush border of jejunum and possess sequence motifs known to interact with sterols. They generated a complementary deoxyribonucleic acid (cDNA) library of rat intestine, sequenced ~16,500 genes and examined these data in comparison with the database of mice and human genes. They analyzed the sequence database of all transcripts containing transmembrane domains, extracellular signal peptides, N-linked glycosylation sites, and sterol-sensing domain. They finally identified a candidate gene and it was a rat homologue of NPC1L1.

Structure, Function, and Regulation of NPC1L1

NPC1L1 possesses a secretion signal, 13 transmembrane domains, extensive N-linked glycosylation sites located in the extracellular loops, and a sterol-sensing domain. NPC1L1 was shown highly and exclusively expressed in the jejunum of mice. It was localized on the luminal surface of jejunal enterocytes. Altmann et al. [1] generated NPC1L1-knockout mice and showed that cholesterol absorption in these mice was reduced by more than 70%. Furthermore, the low levels of cholesterol absorption in NPC1L1-knockout mice were not affected by administration of ezetimibe. Acute cholesterol absorption was decreased by ~90% in the NPC1L1-knockout mice, which was similar to the inhibition of cholesterol absorption in mice, hamsters, and rats treated with ezetimibe. Thus, NPC1L1 is involved in the uptake and absorption of cholesterol from the lumen of jejunum at the brush border membrane of the enterocytes [21]. The uptake of TG by the intestine and its absorption were not altered in the NPC1L1-knockout mice and animals treated with ezetimibe.

NPC1L1 messenger ribonucleic acid (mRNA) levels in the liver and small intestines are upregulated in animals deprived of cholesterol [22, 23]. Intestinal NPC1L1 mRNA levels are downregulated in cholesterol/cholate-fed mice [24] or ACAT2-deficient and phospholipid transfer protein (PLTP)-deficient mice in which free cholesterol is accumulated [25, 26]. The regulation of NPC1L1 expression by sterol is mediated by the binding of sterol regulatory element-binding protein (SREBP)-2 to 2 sterol regulatory elements within the promoter region of *NPC1L1* gene. Statins are known to increase the expression of intestinal NPC1L1 mRNA [27], leading to an increase in cholesterol absorption. Atorvastatin increased intestinal NPC1L1 mRNA levels by 19%, while it decreased mRNA levels of both ATP-binding cassette transporter G5 (ABCG5) and ATP-binding cassette transporter G8 (ABCG8) by 14% in hyperlipidemic men [27]. These effects were most likely mediated by upregulation of the transcription factors SREBP-2 and hepatocyte nuclear factor-4α (HNF-4α) [27]. Statins that are more potent in lowering LDL-C levels increase NPC1L1 expression in the small intestine more than regular statins [28]. In streptozotocin-induced diabetic rats, and in Zucker diabetic fatty *fa/fa* rats the expression of NPC1L1 in the small intestine and thus cholesterol absorption are enhanced [29]. In mice, the expression of NPC1L1 increases with aging [30]. In humans, 45 single nucleotide polymorphisms (SNPs) of nonsynonymous sequence variants in the NPC1L1 gene have been reported [31, 32]. Some of these SNPs influence the sterol absorption and plasma LDL-C levels [33, 34].

NPC1L1 is abundantly expressed in the small intestine of all species, but not expressed in the liver of mice [1]. In contrast to mice, the expression level of NPC1L1 mRNA is similarly high in the liver of humans, monkeys, pigs, and dogs. NPC1L1 is localized in the bile canalicular membrane in the human liver [35, 36]. Therefore, its function may be the reabsorption of cholesterol excreted into the bile, while ABCG5/G8 excretes cholesterol and phytosterol into bile. Overexpression of NPC1L1 in the transgenic mice liver reduced biliary cholesterol and increased plasma cholesterol level, suggesting that bile canalicular NPC1L1 is involved in the absorption of cholesterol from bile and its reuptake into the hepatocyte [36]. Ezetimibe may also inhibit reabsorption of cholesterol from bile.

Mechanisms of Intestinal Cholesterol Absorption and Chylomicron Synthesis

Plasma TG is mainly found in TRL, including CM, very low density lipoproteins (VLDL), and their remnants. TRL constitute a population of particles of heterogeneous size, origin, and apolipoprotein and lipid content. The cholesterol and plant sterols absorbed from the intestinal lumen via NPC1L1 are esterified by ACAT2, forming cholesteryl esters or plant sterol esters (Fig. 28.1) [37]. These cholesteryl esters are assembled with TG, phospholipids, and apoB-48 by microsomal TG transfer protein (MTP) to form CM, which are secreted into the intestinal lymph [37]. CM enter thoracic lymph, from which they flow into the systemic circulation [37]. CM particles undergo partial hydrolysis predominantly by lipoprotein lipase (LPL) into smaller and denser particles known as CM remnants, which are believed to be more atherogenic than the larger CM [38]. LPL hydrolyses the TG moiety of CM to FFA, and residual particles become CM remnants which are taken up by the liver via remnant receptors.

After the uptake of CM remnants by hepatocytes, VLDL are assembled from endogenous hepatic TG, cholesterol, and apoB-100 and are secreted directly into the blood stream. Thereafter, the TG moiety of VLDL is hydrolyzed to FFA by LPL, becoming VLDL remnants, intermediate-density lipoproteins (IDL). The liver takes up VLDL remnants and LDL via LDL receptors, while these particles are supplying energy and lipids to peripheral tissues. In the postprandial state, the serum levels of CM and CM remnants rise quickly to reflect the increased exogenous lipid supply [39]. The increased hepatic lipid inflow leads to an augmented hepatic production of VLDL.

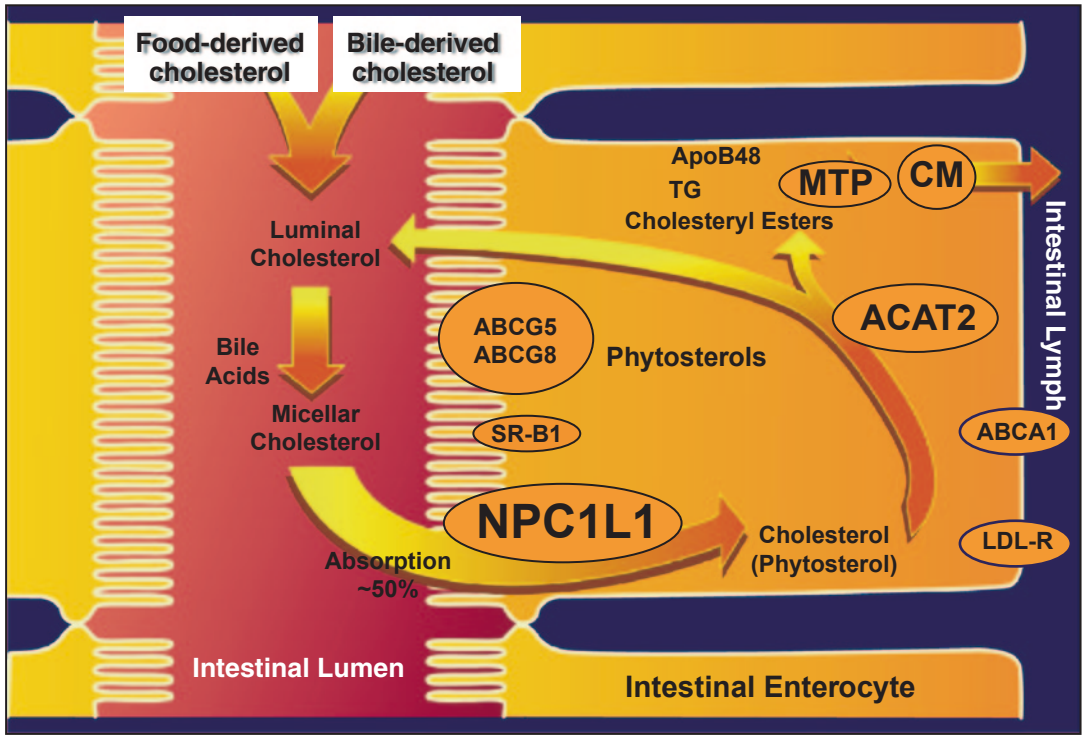

Fig. 28.1 Molecular mechanisms of cholesterol absorption, chylomicron synthesis, and secretion in the small intestines

Absorption, Metabolism, and Pharmacodynamics of Ezetimibe

Ezetimibe (SCH58235; 1-(fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azetidinone) was first discovered by utilizing in vivo models of cholesterol absorption [18]. Its chemical structure is illustrated in Fig. 28.2. It was found by the characterization of the active biliary metabolites of its predecessor, SCH48461, and analysis of structure–activity relationship based upon cholesterol feeding of hamsters. Ezetimibe inhibited diet-induced hypercholesterolemia in hamsters and its ED_{50} was 0.04 mg/kg. In rats, ezetimibe inhibited the absorption and appearance of radiolabeled cholesterol into plasma with an ED_{50} of 0.0015 mg/kg [40]. Ezetimibe was also effective in cholesterol-fed rhesus monkeys with an ED_{50} of 0.0005 mg/kg/day [41].

The cholesterol in the lumen of small intestines derives from bile as well as foods. The cholesterol synthesized in the liver is approximately 400 mg/day; however, the food-derived cholesterol intake is 300–500 mg/day and reabsorption of bile-derived cholesterol is two- to fourfold (800–2000 mg/day) more than that from foods. Ezetimibe is a selective inhibitor of cholesterol absorption in the small intestines and does not influence the esterification of ACAT2, hydrolysis of cholesteryl ester by cholesterol esterase (CEase), and the absorption of fatty acids. Ezetimibe does not affect the activity of pancreatic lipase nor the absorption of TG, vitamins A and D, and taurocholic acid in rats. Most importantly, ezetimibe is completely different from resins such as cholestyramine, colestipol, or colestimide since it does not bind bile acids nor inhibit their absorption. Ezetimibe has no significant effect on fat-soluble vitamin levels.

Ezetimibe is rapidly glucuronidated by uridine 5-diphosphate (UDP)-glucuronosyl-transferase in the intestine, after which the glucuronidated ezetimibe is excreted into the bile. Gluc-

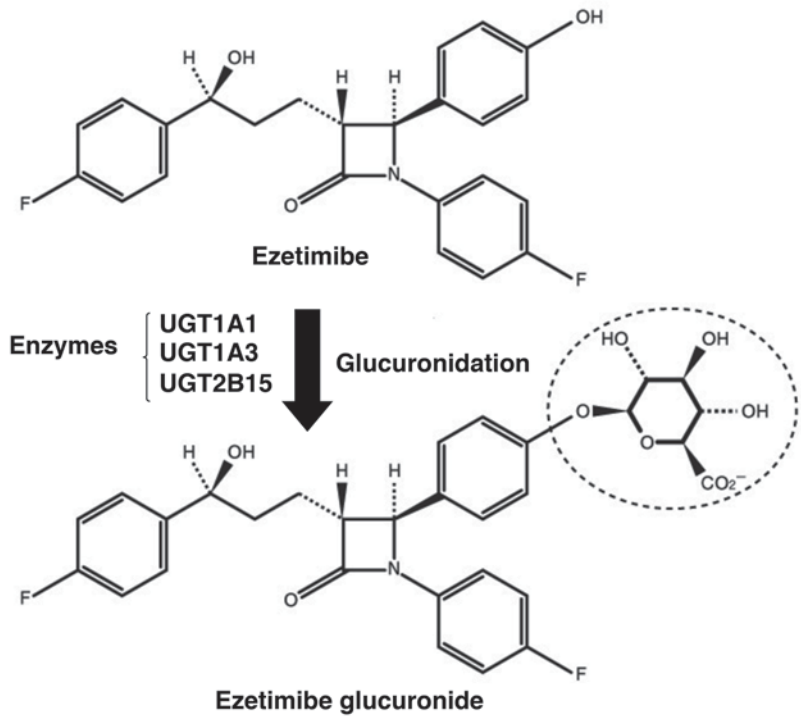

Fig. 28.2 Chemical structure of ezetimibe

uronidated ezetimibe is more potent than original ezetimibe and it is localized in the brush border of enterocytes [40, 42]. In humans, ezetimibe is glucuronidated in the small intestine and the liver and is not metabolized via P450.

Effects of Ezetimibe on Lipid Metabolism and Mechanisms for Its Action

Molecular Mechanisms for Ezetimibe-Induced Inhibition of Cholesterol Absorption

Although ezetimibe was reported to bind NPC1L1 at loop C composed of 61 amino acid residues [43] and block cholesterol absorption, the molecular mechanism of NPC1L1-mediated cholesterol uptake and how ezetimibe inhibits this process were poorly understood. Ge et al. [6] found that cholesterol specifically promotes the internalization of NPC1L1 and that this process requires microfilaments and the clathrin/activator protein (AP2) complex. If the endocytosis of NPC1L1 was blocked, it dramatically decreased cholesterol internalization, suggesting that NPC1L1 may mediate cholesterol uptake via its vesicular endocytosis. Ezetimibe prevents NPC1L1 from incorporating into clathrin-coated vesicles, attenuates cholesterol uptake, and impairs cholesterol influx. Thus, cholesterol is internalized into cells with NPC1L1 through clathrin/AP2-mediated endocytosis and ezetimibe was shown to inhibit cholesterol absorption by blocking the internalization of NPC1L1.

Effects of Ezetimibe on Lipoprotein Metabolism

Ezetimibe Monotherapy

Sudhop et al. [44] examined the effects of placebo or ezetimibe (10 mg/day) for 2 weeks on ra-

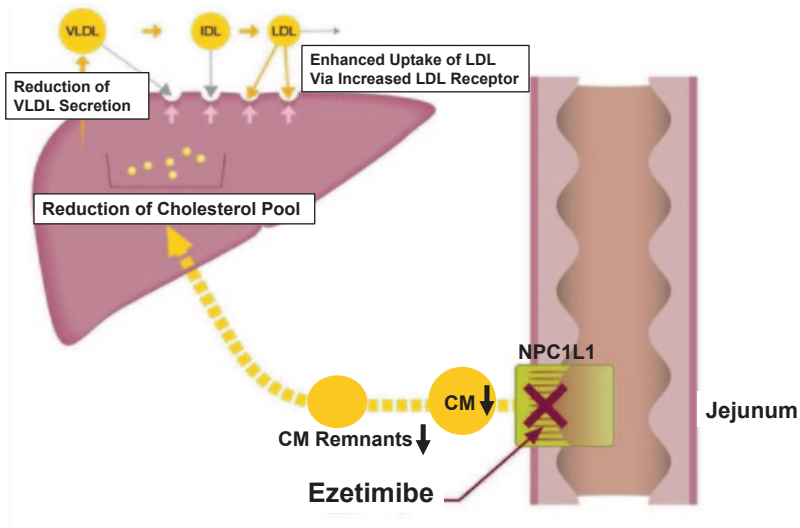

Fig. 28.3 Mechanisms for reduction of serum low-density lipoproteins-cholesterol (LDL-C) levels

diolabeled cholesterol absorption in patients with mild-to-moderate hypercholesterolemia. Ezetimibe reduced total cholesterol by 15%, LDL-C by 20%, campesterol by 48%, and sitosterol by 41%, respectively. Fractional cholesterol absorption rate was reduced by 54% by ezetimibe treatment. Thus, ezetimibe was demonstrated to reduce cholesterol as well as plant sterols in humans. Even in vegetarians, the fractional cholesterol absorption rate was reduced by 58% by ezetimibe treatment and LDL-C levels were also decreased by 17%, although dietary cholesterol intake was less than 30 mg/day, suggesting that ezetimibe inhibits the absorption of both bile-derived and food-derived cholesterol.

In other clinical trials, compared to placebo, ezetimibe (10 mg/day) monotherapy reduced LDL-C levels by 17–22% in hypercholesterolemic patients [45, 46]. In a pooled analysis of 1719 patients with primary hypercholesterolemia, ezetimibe significantly reduced the mean LDL-C levels by 18% (versus a 0.9% increase with placebo) [47]. It also significantly reduced the TG and apoB levels, while it increased HDL-C levels. For Japanese patients, ezetimibe (10 mg/day) was shown to reduce the mean LDL-C level by 18%, but the effect was not enhanced by increasing the dose more than 10 mg/day. Ezetimibe was reported to reduce remnants such as remnant-like particles cholesterol (RLP-C) [16]. It also reduced small dense LDL.

Mechanisms for Reduction of Serum LDL-C Levels (Fig. 28.3)

Ezetimibe inhibits the absorption of cholesterol by inhibiting the internalization of NPC1L1. Thus, the cholesterol content in the CM synthesized in the intestinal epithelium is decreased. The TG moiety of CM is hydrolyzed by the action of LPL, and CM become CM remnants which contain less cholesterol than those without ezetimibe treatment. CM remnants are subsequently taken up by the liver via remnant receptors including LDL receptor-related protein (LRP) and LDL receptor. The flux of exogenous cholesterol into the liver is reduced and the cholesterol pool in the liver is decreased, which causes the upregulation of hepatic LDL receptor. Thus, the uptake and catabolism of LDL by the liver are enhanced, leading to the reduction of serum LDL-C levels. Furthermore, the reduced pool of exogenous cholesterol may attenuate the synthesis and secretion of VLDL from the liver, and thus the metabolized

products of LDL such as IDL and LDL are also reduced. The reduction of hepatic VLDL synthesis and secretion may reduce serum TG levels. Ezetimibe was demonstrated to reduce serum LDL-C levels in patients with homozygous familial hypercholesterolemia (FH) who are deficient in LDL receptors [48, 49]. Therefore, the mechanism of LDL-C reduction in homozygous FH may be partly attributed to the reduction of VLDL synthesis which may lead to the production of IDL and LDL.

Ezetimibe in Combination with Statins and Other Lipid-Lowering Drugs

In clinical practice, doubling the dose of statins reduces LDL-C levels by an additional 6% (6% rule) and may increase side effects such as hepatic dysfunction, myalgia, and rhabdomyolysis. Ezetimibe added to ongoing statin therapy or coadministered with low-dose statins in statin-naive patients was shown to further reduce levels of LDL-C by 5–27%. This combination therapy resulted in favorable effects on several other lipid parameters as well as high-sensitivity C-reactive protein (hsCRP), compared with statins alone in patients with hypercholesterolemia, mixed hyperlipidemia, type 2 diabetes, metabolic syndrome, and older patients during 6–12 weeks of therapy [50]. Moreover, ezetimibe combined with statins increased the attainment of recommended levels of LDL-C, non-HDL-C, and apoB in these patients [51].

When combined with bile acid sequestrants (resins), fenofibrate or niacin, ezetimibe provided significant improvements in LDL-C, TG, total cholesterol, non-HDL-C and apoB, and (variably) HDL-C, compared with the individual component agents alone in patients with hypercholesterolemia and mixed hyperlipidemia (reviewed in ref [52]). Long-term administration of statins may enhance the expression of NPC1L1 in the small intestines and cholesterol absorption, leading to the attenuation of the effects of statins on LDL-C levels. More potent statins are also known to increase the cholesterol absorption more than less potent statins [28]. Therefore, the combination of statins and ezetimibe may be a reasonable strategy [14].

Reduction of Serum TG Levels and Increase of Serum HDL-C Levels by Ezetimibe

Ezetimibe reduces serum TG levels more markedly in hypertriglyceridemic (TG≥150 mg/dl) patients compared with normotriglyceridemic subjects [53]. Moreover, the addition of ezetimibe to statin-treated patients can further reduce serum TG levels. Ezetimibe increases serum HDL-C levels by several percent, and the addition of ezetimibe to statin-treated patients can further increase serum HDL-C levels.

Inhibition of Absorption of Plant Sterols

Ezetimibe was demonstrated to reduce the plasma levels of plant sterols such as sitosterol and campesterol in patients with hypercholesterolemia. It was also shown to reduce the plasma levels of plant sterols as well as serum cholesterol levels in patients with sitosterolemia caused by mutations in ABCG5 or ABCG8 [12, 13]. Both ABCG5 and ABCG8 form a heterodimer and are expressed in the luminal and apical surface of enterocytes and hepatocytes. The function of ABCG5 and ABCG8 is to export plant sterols into the intestinal lumen and the bile. Plant sterol levels were almost undetectable in NPC1L1-knockout mice. The absorption of radiolabeled sitosterol was so much reduced in NPC1L1-knockout mice and wild-type mice treated with ezetimibe. Therefore, NPC1L1 plays an important role as an intestinal transporter for the uptake of both cholesterol and structurally related plant sterols and ezetimibe inhibits the absorption of both cholesterol and plant sterols. Thus, ezetimibe is known as the most appropriate drug for the treatment of sitosterolemia. The long-term administration of ezetimibe was shown to attenuate xanthomas in some patients [54].

Inhibition of Absorption of Oxidized Cholesterol

Staprans et al. [55] examined the effects of ezetimibe in blocking intestinal absorption of oxidized cholesterol in a pilot study. Seven adult subjects were fed a diet containing oxidized cholesterol, α-epoxycholesterol, and 7-keto cholesterol, before and after ezetimibe (10 mg daily for 30 days). Ezetimibe was shown to reduce the serum levels of both cholesterol oxidation products, and this was attributed to the significantly reduced incorporation of oxidized cholesterol into CM and LDL.

Ezetimibe Attenuates Postprandial Hyperlipidemia

Postprandial hyperlipidemia is a very atherogenic state in which CM remnants accumulate in plasma [56]. We investigated the effects of ezetimibe on fasting lipid and lipoprotein profiles as well as postprandial hyperlipidemia [16]. Ezetimibe (10 mg/day) was administered for 2 months in patients with type IIb hyperlipidemia, and it significantly decreased not only fasting serum total cholesterol, LDL-C, and apoB-100 levels but also TG, apoB-48, and remnant lipoprotein cholesterol (RemL-C) levels. High-performance liquid chromatography (HPLC) analysis of serum at fasting state showed that ezetimibe decreased cholesterol and TG levels in the VLDL and LDL size ranges as well as apoB-100 levels, suggesting a decrease in the number of VLDL and LDL particles. After oral fat-loading test, ezetimibe decreased the area under the curve for TG, apoB-48, and RemL-C. Ezetimibe decreased postprandial elevations of cholesterol and TG levels in the CM size range, suggesting that the postprandial production of CM particles was suppressed by ezetimibe. Taken together, ezetimibe improved fasting lipoprotein profiles and postprandial hyperlipidemia by suppressing intestinal CM production in patients with type IIb hyperlipidemia and such treatment may prove to be effective in reducing atherosclerosis. Hiramitsu et al. [57] and Kikuchi et al. [58] also reported the similar effects of ezetimibe on the attenuation of postprandial hyperlipidemia. Yunoki et al. [59] demonstrated that ezetimibe improves postprandial hyperlipidemia and endothelial dysfunction induced postprandially.

Ezetimibe treatment for 3 weeks dramatically reduced postprandial hyperlipidemia in both wild-type mice on a Western diet and CD36-knockout mice, a model of postprandial hyperlipidemia on a normal chow diet [17]. HPLC analysis indicated that the decrease in TG content in CM and CM remnants-sized particles contributed to this suppression. Both TG content and apoB-48 mass in intestinal lymph after oral fat loading were decreased in ezetimibe-treated mice. The mRNA expression of fatty acid transport protein 4 (FATP4), apoB fatty-acid-binding proteins (FABP2), diacylglycerol O-acyltransferase (DGAT) 1, DGAT2, and stearoyl-coA desaturase-1 (SCD1) were reduced after ezetimibe treatment. Intestinal absorption of radiolabeled oleate was significantly reduced by ezetimibe in both animal models, suggesting that the absorption of FFA may be downregulated by the decrease in FATP4 and possibly FABP2 (Fig. 28.4). Taken together, ezetimibe reduces postprandial hyperlipidemia by blocking both the absorption of cholesterol and the intracellular trafficking and metabolism of fatty acids in enterocytes, resulting in the reduction of the formation of apoB-48 necessary for the CM production in the small intestines.

Effects of Ezetimibe on Atherosclerosis in a Variety of Animal Models

The effect of ezetimibe on atherosclerosis was examined in several animal models and reviewed [60]. ApoE-knockout mice develop severe hypercholesterolemia and premature atherosclerosis with features similar to those observed in humans. Techniques ranging from gross visualization of plaques to high-resolution magnetic resonance imaging (MRI) have demonstrated that ezetimibe inhibits atherosclerosis significantly [61]. Scavenger receptor class B type I (SR-BI)/apoE double knockout mice show additional characteristics similar to human coronary heart disease

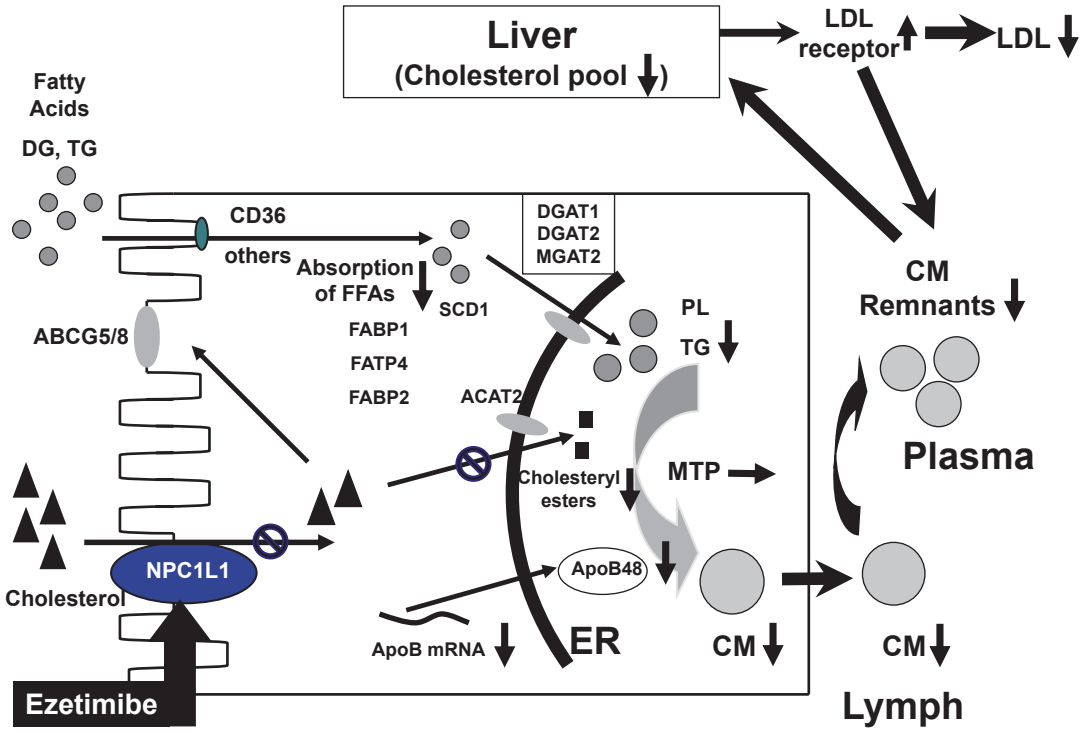

Fig. 28.4 Molecular mechanisms for attenuation of postprandial hyperlipidemia by ezetimibe

(CHD). One study in SR-BI(−/−)/apoE(−/−) mice showed that ezetimibe significantly reduced aortic sinus plaque (57%), coronary arterial occlusion (68%), myocardial fibrosis (57%), and cardiomegaly (12%) compared with untreated controls [62]. Intestinal SR-BI does not impact cholesterol absorption or transintestinal cholesterol efflux in mice [63], and these favorable effects of ezetimibe on atherosclerosis may be attributed to the reduction of cholesterol in the IDL/LDL-size range. The effects of ezetimibe was also evaluated in LDL receptor(−/−)/apoE(−/−) mice [64]. It was demonstrated that functional LDL receptors were not necessary for ezetimibe-mediated reduction of plasma cholesterol or atherosclerosis. In rabbits, ezetimibe was shown to significantly inhibit diet and vascular injury-induced atherosclerosis as measured by intima/media thickness, atherosclerotic lesion composition, and thrombosis. The current preclinical evidence consistently demonstrated that ezetimibe reduces atherosclerosis in animals due primarily to the decrease in atherogenic lipoproteins.

Effect of Ezetimibe on NAFLD in Human Studies

Park et al. [65] treated 45 patients with liver biopsy-proven NAFLD for 24 months with ezetimibe (10 mg/day) in an open-labeled trial and reported reductions in body weight, visceral fat area, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-R), serum TG, total cholesterol, LDL-C, and serum alanine aminotransferase and hsCRP levels. Histological features of steatosis grade, necroinflammatory grade, ballooning score, and NAFLD activity score (NAS) were significantly improved from baseline, whereas the fibrosis stage was not significantly changed.

In another open-labeled pilot study, Yoneda et al. [66] reported improvement of liver histology (NAS and steatosis) in ten NAFLD patients treated with ezetimibe 10 mg daily for 6 months. Chan et al. [67] reported that compared to a hypocaloric, low-fat diet, a combination of hypocaloric, low-fat diet and ezetimibe for 10 weeks decreased intrahepatic TG content (measured by

magnetic resonance imaging) by 18 %. However, rigorous, double-blind, placebo-controlled trials are needed to ascertain beneficial effects of ezetimibe in improving NASH/NAFLD.

Potential Therapeutic Targets of Ezetimibe

Potential therapeutic applications of ezetimibe may be patients with type IIa and IIb hyperlipidemia and those with homozygous and heterozygous FH who are very resistant to statin treatment. In patients for secondary prevention of CHD under strong statin treatment, ezetimibe add-on therapy may further lower the levels of serum LDL-C. The first-line therapy of ezetimibe may be targeted to patients with sitosterolemia and those patients who are supposed to have an increased rate of cholesterol absorption, including those with type 2 diabetes, obesity, metabolic syndrome, and CHD. From the point of drug safety, ezetimibe may be used for aged patients and those with chronic kidney disease (CKD). For patients with type IIb, the combination of statins and fibrates may increase the risk of rhabdomyolysis, muscle symptoms, and liver dysfunction. Thus, the combination of ezetimibe with fibrates may be a better tolerated.

Dosing Regimen

During the clinical development of ezetimibe, a wide range of doses of ezetimibe were evaluated, from 0.625 to 40 mg. Five milligram per day of ezetimibe lowered LDL-C significantly, although to a slightly lesser degree than 10 mg/day in some trials [68, 69]. The majority of these trials were done in patients not receiving statins and looked at LDL-C lowering, as opposed to the achievement of National Cholesterol Education Program (NCEP) goals. The usual dose of ezetimibe was set at 10 mg/day, but 5 mg/day dose was reported to significantly reduce LDL-C levels [70]. The effect of ezetimibe on LDL-C levels reaches the maximum with 10 mg/day dose and no more additional LDL-C lowering effect is observed.

Risks and Precaution

Prescribing Information

Ezetimibe (ZETIA) is prescribed as one 10-mg tablet once daily, with or without food. Dosing should occur either ≥2 h before or ≥4 h after administration of a bile acid sequestrant.

The data of clinical trials of 6–48 week durations showed that ezetimibe administered alone or in combination with statins was generally well tolerated with safety profiles similar to those of placebo or statins in patients with hypercholesterolemia and in high-risk populations [71]. Adverse events were not observed with combination ezetimibe + statins compared with statin monotherapy in a meta-analysis of 18 randomized clinical trials (n=14,497) [72], and in a pooled analysis of 16 studies (n=14,471) in patients aged 65–74 and 75 years or older [73].

In studies with longer durations of 2 years or more (n=12,313), the incidence of adverse events was similar for ezetimibe + statins compared with placebo or statin alone [74]. There were no significant differences in adverse events for gastrointestinal symptoms, hepatic dysfunction, and gall-bladder-related diseases, allergic reactions, or creatine kinase elevations. In the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) Trial, a significant increase in the rate of liver enzyme elevations was reported in the ezetimibe + simvastatin group compared with placebo, however, this was within the range of adverse event rates reported for the combination [75].

In the SEAS Trial, increased numbers of incident and fatal cancers were noted in the ezetimibe + simvastatin group compared with the placebo group. However, an independent meta-analysis of interim safety data from Study of Heart and Renal Protection (SHARP) Trial and IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT; ~20,000 patients) showed that ezetimibe+simvastatin treatment did not increase the risk of cancer [76]. Furthermore, the SHARP study showed an identical incidence of cancers in the ezetimibe + simvastatin and placebo groups. Taken together, ezetimibe alone or in combination with statins is well tolerated and safe.

Drug Interactions and Compatibilities

Some medications for cholesterol lowering should not be taken at the same time. These drugs include cholestyramine, colestipol, colesevelam, or colestimide. Before taking ezetimibe, the patients have to wait at least 4 h after taking any of these medicines. Patients may also take ezetimibe 2 h before taking any of these other medicines. In contrast, ezetimibe may be taken at the same time with fenofibrate or with any of statins.

If patients are allergic to ezetimibe, if they have liver disease, or if they have any of these other conditions, they may need a dose adjustment or special tests to safely use ezetimibe (kidney disease, a thyroid disorder, or when using corticosteroids or hormones including birth control pills). Rhabdomyolysis or muscle symptoms are reportedly very rare for ezetimibe. Food and Drug Administration (FDA) pregnancy category for ezetimibe is C. It is not known whether ezetimibe is harmful to an unborn baby, therefore administration of ezetimibe to pregnant women should be avoided. It is not known whether ezetimibe passes into breast milk or if it could harm a nursing baby. Administration of ezetimibe to breast-feeding women should be avoided. Older adults may be more likely to have side effects from this medicine.

Clinical Trials for Prevention of Atherosclerotic Cardiovascular Disease (CVD)

Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) Trial (ClinicalTrials.gov, NCT00552097)

ENHANCE Trial [77] is the first clinical trial of ezetimibe. It is a double-blind, randomized, 24-month trial comparing the effects of daily therapy with 80 mg of simvastatin with placebo or 10 mg of ezetimibe in 720 patients with heterozygous FH. The intima-media thickness (IMT) of walls of carotid and femoral arteries of the patients were assessed by B-mode ultrasonography. The primary outcome measure was the change in the mean carotid-artery IMT, which was defined as the average of the means of the far-wall IMT of right and left common carotid arteries, carotid bulbs, and internal carotid arteries. At the end of the study, the mean LDL-C level was 192.7 mg/dl in the simvastatin group and 141.3 mg/dl in the combined-therapy group with a statistically significant 16.5% reduction. The differences between the two groups in reductions in levels of TG and hsCRP were 6.6 and 25.7%, respectively, with significant greater reductions in the combined-therapy group. However, the primary outcome, the mean change in the carotid-artery IMT, was not significantly different between the simvastatin group and simvastatin + ezetimibe group. Secondary outcomes, consisting of other variables regarding the IMT of carotid and femoral arteries, also did not differ significantly between the two groups. Thus, in FH heterozygotes, combined therapy with ezetimibe and simvastatin did not reduce IMT compared with simvastatin alone, despite decreases in LDL-C and hsCRP. The results of this study were disappointing, however the mean IMT of carotid arteries before treatment was very thin (mean 0.69 mm) compared with that usually seen in FH heterozygotes of this age. Therefore, similar studies are strongly recommended for FH heterozygotes with thicker IMT.

Simvastatin and Ezetimibe in Aortic Stenosis Trial (ClinicalTrials.gov, NCT00092677)

SEAS Trial [75] is a randomized, double-blind trial involving 1873 patients with mild-to-moderate, asymptomatic aortic stenosis who received 40 mg/day of simvastatin plus either 10 mg/day of ezetimibe or placebo daily. The primary outcome was a composite of major cardiovascular events, including death from cardiovascular causes, aortic valve replacement, nonfatal myocardial infarction, hospitalization for unstable angina pectoris, heart failure, coronary artery bypass grafting, percutaneous coronary intervention, and nonhemorrhagic stroke. Secondary

outcomes were events related to aortic valve stenosis and ischemic cardiovascular events. During a median follow-up of 52.2 months, the primary outcome occurred in 35.3% of patients in the simvastatin-ezetimibe group and in 38.2% of patients in the placebo group (not significant), respectively. Aortic valve replacement was performed in 28.3% of patients in the simvastatin-ezetimibe group and in 29.9% of patients in the placebo group (not significant), respectively. Fewer patients had ischemic cardiovascular events in the simvastatin-ezetimibe group than in the placebo group (hazard ratio, 0.78; 95% CI, 0.63 to 0.97; $P=0.02$), mainly because of smaller number of patients who underwent coronary artery bypass grafting. Cancer occurred more frequently in the simvastatin-ezetimibe group, although later meta-analysis revealed that ezetimibe did not increase the risk of cancer [76]. Thus, simvastatin and ezetimibe did not reduce the composite outcome of combined aortic valve events and ischemic events in patients with aortic stenosis. However, it was indicated that such therapy may reduce the incidence of ischemic cardiovascular events but not events related to aortic valve stenosis.

Stop Atherosclerosis in Native Diabetics Study (SANDS) Trial (ClinicalTrials.gov, NCT00047424)

It has not been clarified whether the addition of ezetimibe to statin therapy affects subclinical atherosclerosis. The secondary analysis from the SANDS Trial examined the effects of lowering LDL-C with statins alone versus statins plus ezetimibe on common carotid artery intima-media thickness (CIMT) in patients with type 2 diabetes and no prior cardiovascular event [78]. Within an aggressive group (target LDL-C≤70 mg/dl; non-HDL-C≤100 mg/dl; systolic blood pressure ≤115 mmHg), change in CIMT over 36 months was compared in diabetic individuals >40 years of age receiving statins plus ezetimibe versus statins alone. The CIMT changes in both aggressive subgroups were compared with changes in the standard subgroups (target LDL-C≤100 mg/dl; non-HDL-C≤130 mg/dl; systolic blood pressure ≤130 mm Hg). Mean LDL-C was reduced by 31 and 32 mg/dl in the aggressive group receiving statins plus ezetimibe and statins alone, respectively, compared with changes of 1 mg/dl in the standard group ($p<0.0001$) versus both aggressive subgroups. Within the aggressive group, mean CIMT at 36 months regressed from baseline similarly in the ezetimibe (−0.025 [−0.05–0.003] mm) and nonezetimibe subgroups (−0.012 [−0.03–0.008] mm), but progressed in the standard treatment arm (0.039 [0.02–0.06] mm), intergroup $p<0.0001$). Thus, reducing LDL-C to aggressive targets resulted in similar regression of CIMT in patients who attained equivalent LDL-C reductions from a statin alone or statin plus ezetimibe. CIMT increased in those achieving standard targets.

The Study of Heart and Renal Protection Trial (ClinicalTrials.gov, NCT00125593, and ISRCTN54137607)

Although lowering LDL-C with statin therapy has been shown to reduce the incidence of myocardial infarction, ischemic stroke, and the need for coronary revascularization in people without kidney disease, it remains uncertain whether it is beneficial among people with CKD. The SHARP Trial [74] assessed the efficacy and safety of the combination of simvastatin plus ezetimibe in patients with CKD. This is a randomized double-blind trial, including 9270 patients with CKD (3023 on dialysis and 6247 not) without known history of myocardial infarction or coronary revascularization. Patients were randomly assigned to simvastatin 20 mg plus ezetimibe 10 mg daily versus placebo. The primary outcome was the first major atherosclerotic event (nonfatal myocardial infarction or coronary death, nonhemorrhagic stroke, or any arterial revascularization procedure). Four-thousand six-hundred fifty patients were assigned to receive simvastatin + ezetimibe and 4620 to placebo. Allocation to simvastatin + ezetimibe yielded an average LDL-C difference of 0.85 mmol/L (with about two-thirds compliance) during a median follow-up of 4.9 years and

produced a 17% proportional reduction in major atherosclerotic events with simvastatin + ezetimibe versus placebo. Nonsignificantly fewer patients allocated to simvastatin+ezetimibe had a nonfatal myocardial infarction or died from CHD. There were significant 25% reductions in nonhemorrhagic stroke and 21% decrease in arterial revascularization procedures, respectively. These effects were consistent among subgroups of patients evaluated including dialysis and nondialysis patients. The reduction of cardiovascular events was proportional to the observed degree of LDL-C lowering, consistent with expectations from the Cholesterol Treatment Trialists' meta-analysis of statin trials in patients without CKD [79]. Thus, reduction of LDL-C with simvastatin 20 mg+ ezetimibe 10 mg daily safely reduced the incidence of major atherosclerotic events in a wide range of patients with advanced CKD.

Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6-HDL and LDL Treatment Strategies in Atherosclerosis (ARBITER6-HALTS) Trial (ClinicalTrials.gov, NCT00397657)

The ARBITER 6-HALTS Trial [80] was terminated early on the basis of a prespecified interim analysis showing superiority of niacin over ezetimibe on change in CIMT. Patients with CHD or CHD equivalent with LDL-C < 100 mg/dl and HDL-C < 50 mg/dl for men or 55 mg/dl for women while receiving stable statin treatment were randomly assigned to ezetimibe (10 mg/day) or extended-release niacin (target dose, 2000 mg/day). The primary end point was change in mean CIMT. Three-hundred and fifteen patients (208 with 14-month follow-up and 107 after mean treatment of 7 ± 3 months) were included. Niacin ($n = 154$) resulted in significant reduction (regression) in mean CIMT (-0.0102 ± 0.0026 mm; $p < 0.001$) and maximal CIMT (-0.0124 ± 0.0036 mm; $p = 0.001$), whereas ezetimibe ($n = 161$) did not reduce mean CIMT (-0.0016 ± 0.0024 mm; $p = 0.88$) or maximal CIMT (-0.0005 ± 0.0029 mm; $p = 0.88$) compared with baseline. There was a significant difference between ezetimibe and niacin treatment groups on mean changes in CIMT, favoring niacin, for both mean CIMT and maximal CIMT. Increased cumulative drug exposure was related to regression of CIMT with niacin, and progression of CIMT with ezetimibe. In this trial, niacin-induced regression of CIMT and was superior to ezetimibe for patients taking statins.

Ongoing Clinical Trials

Recently, the results of IMPROVE-IT (The IMProved Reduction of Outcomes: Vytorin Efficacy International Trial) [81] have been reported at the annual meeting of the American Heart Association in Chicago (November 2014). Although the final paper has not been published yet, the results are available at the AHA website. IMPROVE-IT is a multicenter, randomized, double blind trial to evaluate the potential benefit for reduction in major cardiovascular (CV) events from the addition of 10 mg/day ezetimibe versus placebo to 40 mg/day of simvastatin therapy in 18,144 patients who present with acute coronary syndromes (ACS). Their LDL-C levels were 50-125 mg/dL (statin naïve) or 50-100 mg/dL if they have been treated with prior lipid lowering therapy. The simvastatin dose was uptitrated to 80 mg if LDL-C were more than 79 mg/dL in a double-blind fashion in both treatment groups. The primary endpoint was first occurrence of CV death, nonfatal myocardial infarction (MI), rehospitalization for unstable angina, coronary revascularization (≥30 days following randomization) or stroke. Patients were followed for minimum 2.5 years and until ≥5250 patients experienced a primary endpoint.

The mean LDL-C was significantly lower in patients treated with simvastatin and ezetimibe relative to those treated with simvastatin and a placebo (53.2 mg/dL vs. 69.9 mg/dL at one year, median time average 53.7 mg/dL vs 69.5 mg/dL). Relative to simvastatin with a placebo, simvastatin with 10 mg/d of ezetimibe reduced ischemic stroke by 21% and MI by 13%, respectively, and resulted in a significantly lower incidence of the primary combined endpoint (34.7% vs. 32.7%,

P=0.016, NNT=50). On-treatment analysis also confirmed the effects of ezetimibe on CV events. The safety of ezetimibe on simvastatin was also established. IMPROVE-IT is the first trial demonstrating an incremental clinical benefit by adding a non-statin agent to statin therapy and reaffirming the LDL hypothesis stating that reduction of LDL-C prevents CV events and even lower LDL-C is better.

In Japan, Ezetimibe Lipid Lowering Trial on Prevention of Atherosclerosis in 75 or Older (EWTOPIA75) is now ongoing. This study includes 6000 high LDL-C (≧140 mg/dl) patients aged 75 years or older who do not have prior CHD but have coronary risks such as diabetes mellitus and hypertension. The patients will be treated either with diet therapy alone or with diet therapy plus ezetimibe (10 mg daily). The primary endpoint is a composite of cardiovascular events and stroke. This study will reveal for the first time the significance of ezetimibe alone in aged patients with high risk.

Conclusion

Ezetimibe is a specific inhibitor of NPC1L1, inhibits the absorption of cholesterol, plant sterols, and oxidized cholesterol. This chapter summarized the most recent information on the pleiotropic effects of ezetimibe in addition to the reduction of LDL-C. Especially, ezetimibe exerts additional effects on TRL and postprandial hyperlipidemia, absorption of FFA from the small intestines, and thereby reduce hepatic steatosis. It should be clarified in future studies whether these actions translate into clinical benefit in prevention of atherosclerotic CVD.

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Low-Density Lipoprotein (LDL) Apheresis

29

P. Barton Duell

Introduction

Familial hypercholesterolemia (FH) is an autosomal codominant disorder that is associated with severe hypercholesterolemia and early onset of atherosclerosis and cardiovascular death, particularly in the homozygous or compound heterozygous state [1]. Although low-density lipoprotein (LDL)-lowering drug therapy reduces the risk of cardiovascular complications in this population, most individuals with homozygous FH and some heterozygotes have persistent severe hypercholesterolemia and rapidly progressive atherosclerotic vascular disease despite drug therapy and lifestyle modification. Patients with untreated heterozygous FH have a lifetime risk of cardiovascular events of about 85 %, with an approximately 50 % risk of myocardial infarction by the age of 50 in men and the age of 60–65 in women [2, 3]. Compared to the general population, the risk of cardiovascular disease is increased 10–20-fold in patients with FH [1, 4]. Patients with homozygous FH have greatly accelerated development of atherosclerosis and often experience myocardial infarction prior to the age of 20, but sometimes as early as age 2–5 years [4]. The development of LDL apheresis was stimulated by the quest to achieve further reductions in LDL cholesterol concentrations in these very-high-risk patient groups. In the context of other secondary strategies for lowering LDL cholesterol in homozygous FH, such as liver transplantation, ileal bypass, and portal-caval shunting, LDL apheresis is a comparatively lower-risk intervention despite the invasive nature of the procedure.

History of LDL Apheresis

The initial experience with extracorporeal removal of LDL from plasma involved plasmapheresis (plasma exchange) in studies beginning more than 40 years ago [5, 6]. A number of subsequent case reports and case series supported the concept that plasmapheresis reduced atherosclerosis and improved survival in patients with homozygous FH by markedly lowering LDL levels in plasma [7, 8].

Disadvantages of plasmapheresis, which included nonselectivity for LDL removal and a need for administration of human albumin, led to subsequent efforts to develop procedures to selectively remove LDL from plasma. In 1976, a novel LDL-lowering technique was demonstrated in two FH patients and a normal control that involved manual withdrawal of one unit of blood, mixing the blood with heparin-agarose beads in a transfusion bag, and subsequent reinfusion of the filtered blood [9]. In 1981, the initial use of a column containing anti-LDL antibodies for selective removal of LDL from plasma was reported first

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Table 29.1 Techniques for LDL apheresis

Selective	Dextran sulfate adsorption (Liposorber)
	Heparin precipitation (HELP)
	Immunoadsorption (TheraSorb, LDL-Excorim; not available in USA)
	Polyacrylamide adsorption (DALI; not available in USA)
Semiselective	Cascade filtration (e.g., double-filtration plasmapheresis)
Nonselective	Plasmapheresis (plasma exchange)

HELP heparin-induced extracorporeal LDL precipitation, *LDL* low-density lipoprotein, *DALI* direct adsorption of lipoproteins

Table 29.2 Advantages of selective LDL apheresis procedures in comparison to plasmapheresis

Greater reduction in LDL cholesterol
Minimal HDL cholesterol reduction
Preservation of plasma albumin, immunoglobulins, and clotting factors
Avoidance of human blood products

LDL low-density lipoprotein, *HDL* high-density lipoprotein

in pigs [10] and subsequently in humans with FH in a procedure that was coined LDL apheresis [11]. The term apheresis is derived from the Greek words *από* (apo), which means "from," and *αφαιρώ* (aphairo), which means "remove or subtract." Although these early techniques were supplanted by other technologies, the discoveries from these early studies led to the advent of clinical use of LDL apheresis.

The subsequent development of the techniques for LDL apheresis that are commercially available in the USA were based on adsorption of apolipoprotein (apo) B-containing lipoprotein particles by dextran sulfate or precipitation of apo B-containing lipoprotein particles with heparin. The clinical development of these procedures led to the two primary LDL apheresis systems that are currently in use in the USA. These are the Liposorber system developed by Kaneka in Japan [12, 13] and the heparin-induced extracorporeal LDL precipitation (HELP) system developed by B. Braun in Germany [14, 15], both of which were created in the mid-1980s. Both techniques involve extracorporeal treatment of plasma to remove apo B-containing lipoproteins followed by reinfusion of the processed blood, as described below. An additional commercially produced selective LDL apheresis system, TheraSorb, utilizes immunoadsorption of apo B-100-containing lipoprotein particles. It is available in Germany and elsewhere in the European Economic Area (EEA), but this system is not available in the USA. Another system from Germany, direct adsorption of lipoproteins (DALI), involves adsorption of apo B-containing lipoproteins from whole blood using polyacrylate-coated polyacrylamide beads, but this system is also not available in the USA. Since selective LDL apheresis is not available in every country or in every community in countries in which it is available, alternative nonselective techniques are sometimes utilized such as plasmapheresis (Table 29.1), but selective LDL apheresis is the preferred technique when it is available. Advantages of selective LDL apheresis over alternative techniques are shown in Table 29.2.

LDL Apheresis Availability

With an estimated frequency of 1:500 or higher in the general population, it is predicted that there may be about 14 million individuals with heterozygous FH in the world. Homozygous FH is considerably less common, with a predicted prevalence of $1:10^6$ or higher, occurring in an estimated 7000 or more individuals worldwide. Recent genetic studies have suggested that the prevalence of homozygous and compound heterozygous FH may be as much as an order of magnitude more common that previously predicted. On the basis of our patient population, it is estimated that about 2–4 % of heterozygous FH patients and the majority of homozygous FH patients cannot be

Table 29.3 Guidelines for initiating LDL apheresis (after maximal tolerated LDL-lowering therapy)

	Without CHD	With CHD
USA (FDA)	LDL-C > 300 mg/dl in heterozygous FH	LDL-C > 200 mg/dl
	LDL-C > 500 mg/dl in homozygous FH	
Japan	Total cholesterol > 250 mg/dl	
Germany	Homozygous FH	LDL-C > 130 mg/dl
		Lp(a) > 60 mg/dl (with progressive disease)
NLA guidelines [17]	LDL-C > 200 mg/dl in high-risk heterozygous FH patients	LDL-C > 160 mg/dl
	LDL-C > 300 mg/dl in homozygous FH and low-risk heterozygous FH	
European guidelines [18]		LDL-C > 4.2 mmol/L (161.5 mg/dl)

FDA Food and Drug Administration, *CHD* coronary heart disease, *LDL-C* low-density lipoprotein cholesterol, *FH* familial hypercholesterolemia, *Lp(a)* lipoprotein(a), *NLA* National Lipid Association

adequately treated with standard pharmacologic therapy and may require additional interventions such as LDL apheresis. This means that there may be more than 560,000 patients with FH in the world who could benefit from treatment with LDL apheresis, but less than 1% are actually receiving this treatment (it is estimated that < 3500 patients are being treated with LDL apheresis worldwide [16]). LDL apheresis is not available in most of Africa, most of Asia (except Japan), and has very limited availability in South America. Europe, North America, Japan, and Russia are the areas where the majority of LDL apheresis treatments are being administered. Out of an estimated 12,000–24,000 patients with refractory FH in the USA who may benefit from LDL apheresis (out of an estimated 600,000 with FH), only about 500 patients are currently being treated with LDL apheresis in North America [16]. The reasons for this disparity include lack of access to LDL apheresis centers, the high cost of treatment, barriers from insurance providers, intolerance of the procedure, and patient preference. Here on the West Coast of the USA, the closest LDL apheresis center for some patients may be more than 600 miles from home.

FDA Approved Indications for LDL Apheresis

LDL apheresis has been Food and Drug Administration (FDA) approved in the USA for more than 18 years. The Liposorber dextran sulfate adsorption system was FDA approved in 1996 and the HELP heparin precipitation system was FDA approved in 1997. The current FDA approved indications for LDL apheresis, which are more than a decade out of date, are a treated LDL cholesterol concentration > 500 mg/dl in patients with homozygous FH, > 300 mg/dl in patients with heterozygous FH without coronary artery disease, or LDL cholesterol > 200 mg/dl with coronary artery disease in patients receiving maximal tolerated LDL-lowering therapy. Different thresholds are used in other countries, as shown in Table 29.3. Since the FDA approved guidelines were formulated 18 years ago and have not been updated on the basis of data from numerous clinical trials documenting the clinical efficacy of aggressive LDL cholesterol lowering in high-risk patients, revised guidelines are needed. An expert panel of the National Lipid Association recently proposed lowering the LDL cholesterol threshold for initiating LDL apheresis to 160 mg/dl in patients with coronary artery disease or a very high risk of coronary heart disease (CHD) events [17]. The same LDL cholesterol threshold was previously promulgated by a European-based international panel [18].

In addition to approval for treatment of severe hypercholesterolemia, LDL apheresis with the Liposorber LA-15 system recently was FDA approved for treatment of pediatric patients with nephrotic syndrome associated with primary focal segmental glomerulosclerosis (FSGS), when either (a) standard treatment options,

including corticosteroid and/or calcineurin inhibitor treatments, are unsuccessful or not well tolerated and the patient has a glomerular filtration rate (GFR)≥60 mL/min/1.73m^2 or (b) the patient is post-renal transplantation. The Liposorber LA-15 system is indicated for up to 12 procedures in 3 months for treatment of FSGS on a schedule of twice weekly for 3 weeks followed by weekly procedures for 6 weeks [19].

Non-FDA-Approved Indications for LDL Apheresis

Lipoprotein(a)

It is also reasonable to consider using LDL apheresis for off-label treatment of patients with severely elevated plasma concentrations of lipoprotein(a) (Lp(a)) and progressive atherosclerotic vascular disease despite maximal tolerated therapy, as reflected by the guidelines in Germany (Table 29.3), but a Lp(a) threshold of >90–100 mg/dl or more may be preferable. Data from small clinical trials and anecdotal reports suggest that LDL apheresis may reduce progression of atherosclerosis and reduce events among patients who are receiving the treatment for elevated plasma Lp(a) concentrations [20]. Use of LDL apheresis specifically for Lp(a) lowering is not FDA approved, but the procedure is used extensively in Germany in patients with plasma Lp(a) concentrations>60 mg/dl and progressive atherosclerosis. The Lp(a) Lipopak system is available in Russia and specifically removes Lp(a) from plasma (Pocard Ltd., Moscow, Russia) [21].

Lipoprotein X

Other off-label uses of LDL apheresis (or in this case, plasmapheresis) include treatment for lowering lipoprotein X, an unusual lipoprotein composed predominantly of free cholesterol and phospholipids (almost 90% of the mass) with a small amount of albumin and apo C (I, II, and III) and other constituents that are formed in the setting of obstructive liver disease and may contribute to formation of xanthomas. LDL apheresis is ineffective for removing lipoprotein X from plasma because the particle does not contain apo B, but plasmapheresis is efficacious. Lipoprotein X also accumulates in the setting of the rare condition, lecithin–cholesterol acyltransferase (LCAT) deficiency, but the composition of the lipoprotein is somewhat different from the particles observed in obstructive liver disease. It is believed that lipoprotein X itself is not atherogenic, but other forms of dyslipidemia in obstructive liver disease may contribute to the development of atherosclerosis in some patients [22]. The indications for LDL apheresis treatment and the potential clinical benefits in patients with lipoprotein X accumulation are unclear, but semi-selective double-filtration plasmapheresis may be the preferred technique because lipoprotein X does not contain apo B and is not readily cleared by selective LDL apheresis. In one case report, double-filtration plasmapheresis removed both LDL and lipoprotein X, reducing the total plasma cholesterol concentration by 48% [23]. In contrast, dextran sulfate adsorption LDL apheresis removed only LDL from plasma and reduced the total plasma cholesterol concentration by only 30%. Ongoing treatment with double-filtration plasmapheresis was associated with complete regression of the patient's xanthomas, which raised the possibility that the treatment could have attenuated arterial plaque formation, but this effect could be mediated by reductions in apo B-containing atherogenic lipoproteins and not lipoprotein X. Further studies are needed to clarify whether there is any clinical benefit of performing plasmapheresis as an adjunct to standard pharmacologic treatment for routine dyslipidemia in patients who have lipoprotein X accumulation in plasma.

Type III Hyperlipidemia

Although selective LDL apheresis effectively removes remnant lipoproteins from plasma [24], the potential role of LDL apheresis in the

treatment of severe refractory type III hyperlipidemia remains uncertain and is off label. Type III hyperlipidemia is characterized by defective apo E (typically E2 homozygosity) in combination with overproduction of very low-density lipoprotein (VLDL), resulting in the accumulation of atherogenic remnant lipoproteins and reduced levels of true LDL. In one case report of a patient with type III hyperlipidemia and lipoprotein glomerulopathy (with baseline urine protein:creatinine ratio 3.3), initiation of selective LDL apheresis was associated with complete remission of proteinuria after about 7 months, but it is unclear whether the LDL apheresis procedure produced the remission [25]. Type III hyperlipidemia is an atherogenic condition that warrants aggressive lipid lowering, but most patients can be well controlled with lifestyle modification, correction of underlying causes of VLDL overproduction, and the use of statins, fibrates, niacin, and possible use of estrogen replacement in postmenopausal women. LDL apheresis might be a consideration for treatment of patients with refractory dyslipidemia due to heterozygous FH and type III hyperlipidemia or other forms of refractory type III hyperlipidemia and progressive atherosclerosis. The clinical utility and appropriate lipoprotein thresholds for possible consideration of LDL apheresis in refractory type III hyperlipidemia need to be ascertained.

Focal Segmental Glomerulosclerosis and Minimal Change Nephrotic Syndrome

The results from case reports, case series, and small clinical trials have suggested that LDL apheresis may provide nephro-protective benefit in patients with FSGS and minimal change nephrotic syndrome. The rationale behind this application of LDL apheresis is the notion that atherogenic apo B-containing lipoproteins may have toxic effects on renal tubules and nephrons that aggravate progression or perpetuation of these disorders [26]. Possible mechanisms of nephrotoxicity include tubular injury and fibrosis induced by lipiduria, stimulation of mesangial cell proliferation and matrix deposition, accumulation of lipid-laden macrophages in nephrons resulting in aggravation of glomerulosclerosis, and vascular endothelial injury. In one early study, patients with FSGS and minimal change nephrotic syndrome (MCNS) were treated with steroids in combination with LDL apheresis treatments twice a week for 3 weeks and weekly for 6 weeks, which produced a 71% rate of remission or partial remission compared to steroid therapy alone [27, 28]. Urinary thromboxane excretion was also significantly attenuated in these studies. Reductions in serum interleukin (IL)-8 concentrations and restoration of IL-12-induced interferon-gamma production by peripheral blood cells have also been reported after a series of LDL apheresis treatments [26]. In Japan, LDL apheresis to control hyperlipidemia in patients with refractory nephrotic syndrome associated with FSGS is covered by the national health insurance for up to 12 treatments over 3 months. As previously noted, in the USA, the FDA recently approved LDL apheresis with the Liposorber LA-15 system for treatment of pediatric patients with nephrotic syndrome associated with primary FSGS, when either (a) standard treatment options, including corticosteroid and/or calcineurin inhibitor treatments, are unsuccessful or not well tolerated and the patient has a GFR $\geq$ 60 ml/min/1.73 m^2 or (b) the patient is post-renal transplantation [19].

Sudden Hearing Loss

Sudden sensorineural hearing loss is a multifactorial disorder that may be related to alterations in microcirculation, autoimmunity, and viral infection. It was theorized that the subgroup with sudden hearing loss due to abnormal microcirculation and possible hyperviscosity may achieve clinical improvement in response to treatment with LDL apheresis [29]. The results of an early prospective randomized study in 27 patients treated with LDL apheresis ($n = 18$) or standard treatment with prednisolone, dextranes, and pentoxifylline ($n = 9$) suggested that a single LDL apheresis treatment was superior or at least equal to standard

therapy for improving hearing in patients with sudden hearing loss presumed to be due to vascular dysfunction [29]. Decreases in plasma levels of LDL cholesterol, Lp(a), and fibrinogen in association with decreased plasma viscosity, decreased erythrocyte aggregation, and increased resistance to oxidative modification of LDL particles were hypothesized to mediate the clinical effects. The results of a much larger randomized trial involving 201 patients with sudden hearing loss did not demonstrate significant improvement in the primary outcome of recovery of hearing measured by pure-tone audiometry 48 h after starting treatment, but the mean sound level at which 50 % of recorded digits were recognized was significantly lower at 48 h in patients treated with LDL apheresis ($P = 0.034$) [30]. Among patients with plasma fibrinogen concentrations > 295 mg/dL, speech perception was improved much more at 48 h in patients treated with LDL apheresis compared to standard treatment ($P = 0{\cdot}005$). A more recent nonrandomized retrospective study of LDL apheresis was conducted in 217 subjects with sudden hearing loss who failed to improve after treatment with steroids and plasma expanders [31, 32]. A single LDL apheresis treatment produced complete or partial remission in 61 % of these subjects, but the absence of a placebo control and the retrospective analysis makes the data less conclusive. Another randomized trial included 132 patients with sudden hearing loss and plasma LDL cholesterol > 120 mg/dL and/or fibrinogen > 320 mg/dL [33]. Sixty were given standard treatment with dexamethasone and glycerol for 10 days and 72 were treated with a single HELP-apheresis followed by 10 days of standard treatment. Rates of hearing recovery were 75 % at 24 h and 76 % at 10 days among patients treated with LDL apheresis compared to 42 % at 24 h and 45 % at 10 days in the group that received standard treatment without LDL apheresis [33]. The aggregate results suggest that LDL apheresis might provide therapeutic benefit in some patients with sudden hearing loss, but proper role of LDL apheresis in treatment of sudden hearing loss is currently unclear.

Refsum Disease

Refsum disease is a rare autosomal recessive disorder that is caused by mutations in the genes for either phytanoyl-CoA hydroxylase (PHYH) or peroxin-7 (PEX7;the receptor for the type 2 peroxisomal targeting signal (PTS-2)) that disrupt peroxisomal breakdown of an unusual branched-chain fatty acid, phytanic acid [34]. The disorder is characterized by accumulation of phytanic acid in plasma, fatty tissues, myelin sheaths, the heart, kidneys, and retina, resulting in retinitis pigmentosa, peripheral polyneuropathy, anosmia, deafness, cerebellar ataxia, icthiosis, and cardiac abnormalities [35–38]. Although restriction of dietary intake of chlorophyll and foods containing phytol, phytanic acid, or their precursors can lower plasma phytanic acid concentrations and may produce some clinical benefit [39], the implementation of plasmapheresis or LDL apheresis performed once or twice monthly can more effectively lower phytanic acid levels, allow liberalization of the diet, and prevent progression of the disease. Since phytanic acid is carried in plasma by LDL, VLDL, and to a lesser extent in HDL, the levels of phytanic acid are substantially lowered by LDL apheresis as a consequence of removal of LDL, VLDL, and VLDL remnants [40].

LDL Apheresis Procedures

Selective LDL apheresis is typically performed using one of four techniques, two of which are available in the USA (Table 29.1). When selective LDL apheresis is not available, plasmapheresis or cascade filtration are sometimes used as alternative procedures in lieu of the preferred selective LDL apheresis treatment. All techniques require fairly high-flow venous access for blood removal and lower-flow venous access for blood return, commonly achieved through the antecubital veins of both arms. If venous access is inadequate to provide sufficient extracorporeal blood flow and blood return, surgical creation of an arteriovenous (AV) fistula is necessitated, which is commonly placed in the

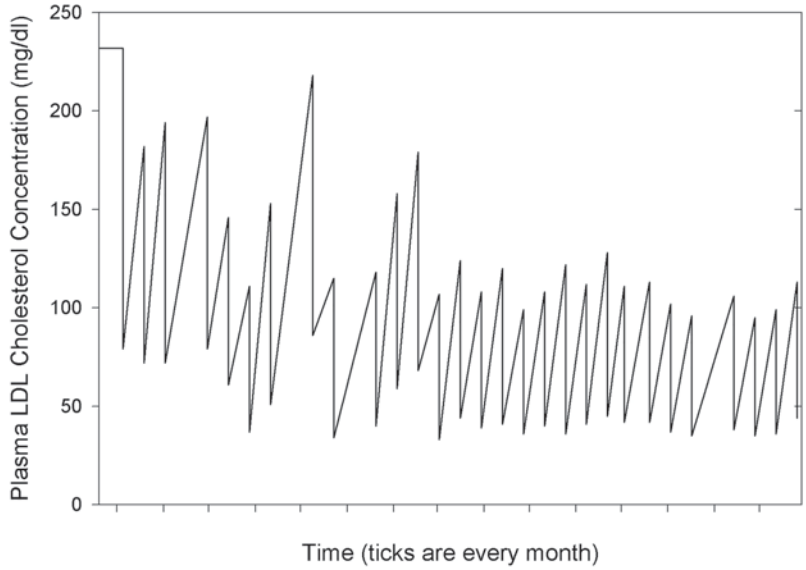

Fig. 29.1 Cyclical changes in plasma LDL cholesterol during ongoing biweekly LDL apheresis treatment

forearm. During the typical 6–8-week period of maturation of the AV fistula, LDL apheresis can temporarily be performed with the use of a dual-port tunneled central venous catheter. A plasma volume of 2.5–5 L is typically processed during a single LDL apheresis procedure, varying with the technique and patient size, and requiring 2–4 h to complete.

Although the LDL cholesterol concentration can be acutely lowered 70–80 % during the 2–4-h LDL apheresis treatment, LDL particles quickly re-accumulate in plasma, reaching pretreatment levels after 2–3 weeks in patients with heterozygous FH. This necessitates LDL treatments every 2 weeks in heterozygotes, whereas treatments are done weekly in homozygotes because of the higher baseline plasma LDL cholesterol concentrations. Over time, regular LDL apheresis treatments produce gradual reductions in both the pretreatment and posttreatment plasma LDL cholesterol concentrations. The typical cyclical changes in LDL cholesterol levels are shown in Fig. 29.1. The time-averaged reduction in LDL cholesterol in response to treatment with LDL apheresis is calculated as the sum of post-apheresis LDL-cholesterol + K (acute change in LDL cholesterol during apheresis procedure), where K is approximately 0.68–0.7 among patients with heterozygous FH, as demonstrated in studies of LDL turnover after LDL apheresis [41].

In addition to lowering plasma concentrations of LDL cholesterol, LDL apheresis also reduces other apo B-containing lipoproteins, such as VLDL, remnant lipoproteins, and Lp(a). This has led to the concept that LDL apheresis may be more correctly referred to as lipid apheresis or lipoprotein apheresis. The hepatitis C viral load is also reduced 37–90 % by LDL apheresis as a consequence of the association of hepatitis C with apo B-containing lipoproteins [42]. In other studies, LDL apheresis with the DALI system altered hepatitis C RNA levels by −35 to + 72 % (no consistent decrease or increase), whereas plasmapheresis consistently lowered hepatitis C RNA levels by 35–43 % [43]. The results of more recent studies have demonstrated that plasma levels of proprotein convertase subtilisin/kexin 9 (PCSK9) are also reduced by LDL apheresis, presumably as a consequence of clearance of active PCSK9 that is bound to LDL [44, 45]. Variable decreases in fibrinogen occur with the various LDL apheresis procedures, which may or may not have therapeutic benefit. Unwanted acute decreases in plasma HDL cholesterol can occur, most severely with plasmapheresis which can decrease the level

Table 29.4 Effects of various LDL apheresis procedures on plasma lipoproteins, triglycerides, and fibrinogen. (Adapted from [46])

Mean percent reductions					
Procedure	LDL-C	HDL-C	Lp(a)	Triglycerides	Fibrinogen
Dextran sulfate adsorption	49–75	4–17	19–70	26–60	17–40
Heparin precipitation	55–61	5–17	55–68	20–53	51–58
Immunoadsorption	62–69	9–27	51–71	34–49	15–21
Polyacrylamide adsorption	53–76	5–29	28–74	29–40	13–16
Cascade filtration	56–62	25–42	53–59	37–49	52–59
Plasmapheresis	53–63	40–60	43–50	50–60	68–76

LDL-C low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *Lp*(*a*) lipoprotein(a)

40–60 %, but generally 4–17 % with the Liposorber and HELP systems that are available in the USA. With selective LDL apheresis, the HDL cholesterol concentration often returns to baseline within 2 days. The effects of various LDL apheresis procedures on plasma lipoproteins, triglycerides, and fibrinogen are shown in Table 29.4.

Side effects of LDL apheresis can include hypotension, nausea, aggravation of angina, bleeding related to anticoagulation and venous access, problems with inadequate venous access, and fatigue that may persist for hours after the procedure. In one series of 5576 treatments using three different LDL apheresis techniques, side effects were generally minor and were reported in <5 % of patients [47]. The only serious side effect was anaphylactoid reactions in patients taking angiotensin-converting enzyme (ACE) inhibitors during dextran sulfate adsorption procedures. These serious reactions are a consequence of procedure-induced bradykinin release. Polyacrylamide adsorption LDL apheresis procedures also may induce bradykinin release.

LDL apheresis is typically initiated in adulthood in patients with heterozygous FH, but treatment should be initiated in childhood in patients with homozygous FH or patients with heterozygous FH and a family history of severely accelerated progression of atherosclerosis and very early onset of CHD events (e.g., prior to age 20–30). The timing of initiation of LDL apheresis in children is based on the magnitude of residual LDL cholesterol elevation after maximal tolerated drug therapy, family dynamics and preferences, accessibility of LDL apheresis, and the clinical status of the child. Delaying initiation of treatment allows progression of atherosclerosis in proportion to the magnitude of LDL elevation, so the most severely affected patients should generally start LDL apheresis treatment as early as possible. Treatment with LDL apheresis has been initiated as early as the age of 2 years in children with early evidence of atherosclerosis. LDL apheresis is not FDA approved for use during pregnancy and lactation, but continuation of LDL apheresis is justifiable in pregnant women with homozygous FH and extant CHD who may experience CHD events or death during pregnancy if the apheresis treatment is discontinued.

Plasmapheresis

Plasmapheresis or plasma exchange involves the separation of plasma from whole blood by membranes or centrifugation, which allows the plasma to be removed from the blood and discarded [6–8]. The removed plasma is replaced with human albumin and saline, combined with the remaining blood cells and reinfused. This procedure effectively removes LDL particles from the blood, but it also removes HDL particles, clotting factors, fibrinolytic factors, immunoglobulins, and other plasma proteins. Since only albumin is typically replaced, deficiency of other plasma proteins can occur. Plasmapheresis does not require anticoagulation because clotting factors are removed from the blood and the patient's plasma is not reinfused.

Cascade Filtration

Cascade filtration or double-filtration plasmapheresis is a modification of standard plasmapheresis that involves separation of cells from plasma by a hollow-fiber first filter followed by a second semi-selective filter that retains HDL particles and other desirable plasma factors in the effluent, but excludes larger particles such as LDL, Lp(a), VLDL, chylomicrons (if present), and some lipoprotein X [48]. Selective LDL apheresis procedures do not remove lipoprotein X because it does not contain apo B. Thermofiltration is a variant of double-filtration plasmapheresis that increases LDL removal and decreases HDL removal by warming the plasma to 38 °C prior to the double-filtration plasmapheresis. Anticoagulation is required for cascade filtration, but albumin infusion is obviated.

Dextran Sulfate Adsorption LDL Apheresis (Liposorber)

Dextran sulfate adsorption using the Liposorber (Kaneka, Japan) system is an automated multistep procedure that involves the separation of plasma from heparinized blood (heparin bolus and infusion are administered) followed by passing the plasma over dual columns of dextran sulfate bound to cellulose beads [49]. The dextran sulfate selectively binds apo B in LDL, VLDL, and Lp(a) through electrostatic interactions between the positively charged apo B and pseudoreceptor-like sites on dextran sulfate. Plasma is alternately passed through one column while the other column is regenerated. The apo B depleted plasma is combined with blood cells and reinfused into the patient. This procedure can induce bradykinin release that can create adverse effects (including anaphylactoid reactions) in patients taking (ACE) inhibitors, so it is recommended to change the patient to an angiotensin-receptor blocker or hold ACE inhibitors for 2–3 days prior to the apheresis treatment. The Liposorber D system (Kaneka, Japan) is a whole blood perfusion device that is not available in the USA.

Heparin Extracorporeal LDL Precipitation Apheresis

The HELP (B Braun, Germany) system is a procedure that involves extracorporeal precipitation of LDL and other apo B-containing lipoproteins by exposure of acidified plasma to high concentrations of heparin [50]. Blood is initially passed through a filter to separate plasma from blood cells. The plasma is heparinized and acidified to pH 5.2, resulting in electrostatic interactions between negatively charged heparin and positively charged apo B, which induces precipitation of apo B-containing lipoproteins, but also can remove approximately 50 % of complement C3, complement C4, plasminogen, and fibrinogen. Plasma volumes larger than 3 L cannot be processed without increasing the risk of bleeding complications. The plasma is subsequently passed through an adsorption column to remove heparin and dialyzed against a bicarbonate buffer to normalize the pH prior to being mixed with blood cells and reinfused into the patient.

Immunoadsorption LDL Apheresis (Therasorb; LDL-Excorim)

The Therasorb immunoadsorption apheresis system (Miltenyi Biotec, Germany) and LDL-Excorim immunoadsorption apheresis system (Fresenius, Germany) are available only outside the USA. In this procedure, plasma is separated from blood and passed over a sepharose column that is coated with covalently bound sheep antihuman apo B-100 antibodies [51]. Each of the two columns can adsorb about 3 g of LDL cholesterol. The automated system alternately changes from one column to the other so the column almost saturated with LDL particles can be regenerated. The treated plasma is combined with blood cells and reinfused into the patient. The columns can be sterilized and reused up to 40 times or more, a strategy that is motivated by the high cost of the columns.

Table 29.5 Cardiovascular benefits from LDL apheresis (mostly uncontrolled studies)

Acute reduction in hsCRP (after 24 h)
Decreased endothelium-derived leukocyte adhesion molecules
Decreased blood viscosity
Decreased fibrinogen
Increased endothelium-dependent vasodilation
Increased coronary flow reserve
Increased coronary and carotid artery plaque regression/decreased progression
Decreased coronary calcium score (observational study)
Decreased angina
Improved exercise-induced ST segment depression
72 % reduction in CHD events in non-randomized trial compared to medical therapy alone

hsCRP high-sensitivity C-reactive protein, *CHD* coronary heart disease

Immunoadsorption Lp(a) Apheresis (Lp(a)-Excorim; Lp(a) Lipopak)

In addition to selective immunoadsorption of LDL particles, Fresenius in Germany has also produced a Lp(a)-Excorim apheresis system that selectively binds Lp(a) in sepharose columns that are coated with sheep antihuman Lp(a) antibodies. Pocard in Moscow also produced a polyclonal Lp(a) immunoadsorption system. Both Lp(a) immunoadsorption columns can be reused. Two personal columns are assigned to each patient [52].

Polyacrylamide Adsorption (DALI)

Direct adsorption of lipoproteins (DALI; Fresenius, Germany), involves adsorption of apo B-containing lipoproteins from whole blood using polyacrylate-coated polyacrylamide beads [53]. Unlike the other LDL apheresis techniques, this is a hemoperfusion system that is compatible with whole blood and does not require separation of plasma from blood cells. A single standard column of 480 ml of polyacrylate-coated polyacrylamide beads is typically used for the procedure without regeneration, which is sufficient to process about 1.6 blood volumes. Adult patients with homozygous FH or severe heterozygous FH may exceed the absorptive capacity of the standard 480 ml column (DALI-500), which may necessitate the use of a larger column (DALI-750 or DALI-1000). The pore size of the beads allows entry of lipoproteins, but is small enough to exclude red blood cells and platelets, which allows heparinized whole blood to be infused through the column without inducing hemolysis [54]. Negatively charged polyacrylate molecules are covalently bound to the porous polyacrylate beads and bind to positively charged apo B in LDL, VLDL, and Lp(a), thereby immobilizing the lipoproteins. The majority of the adsorptive surface of the column is located within the beads, which minimizes interactions between bound lipoproteins and blood cells.

Benefits of LDL Apheresis

There are no placebo-controlled trials of LDL apheresis to assess the efficacy of this treatment in reducing cardiovascular events or mortality, which is due in part to the unwillingness of patients and investigators to submit high-risk patients to placebo therapy. Despite the lack of placebo-controlled clinical trials, the results from a variety of studies lend strong support to the belief that LDL apheresis produces significant clinical benefit (Table 29.5). LDL apheresis has been shown to lower plasma levels of high-sensitivity C-reactive protein (hsCRP), a cardiovascular risk marker, and endothelial cell derived leucocyte adhesion molecules within 24 h [55, 56]. In other studies, a single LDL apheresis treatment

was shown to improve acetylcholine-induced and endothelium-dependent vasodilation measured by forearm strain gauge plethysmography or brachial artery ultrasonography [57, 58]. In addition, a 30% increase in coronary flow reserve (a measure of coronary vasodilatory capacity) assessed by positron-emission tomography (PET) imaging was demonstrated 24 h after a single apheresis treatment [59].

There are many anecdotal reports of resolution of angina and increased exercise capacity in patients after initiation of LDL apheresis, including many of our own patients [60]. This subjective improvement would be expected to occur in response to LDL apheresis-mediated augmentation of endothelium-dependent vasodilation and plaque stabilization. These anecdotal findings are substantiated by two observational studies that reported 21 and 87% reductions in symptoms of angina after 5 years of treatment with LDL apheresis in combination with LDL-lowering medications in patients with severe heterozygous FH [60, 61]. These findings are bolstered by the results of another study that demonstrated a reduction in exercise-induced ST segment depression in patients treated with LDL apheresis and LDL-lowering medications compared to patients who received standard LDL-lowering therapy without LDL apheresis [62]. Another randomized study showed increased regional myocardial perfusion in patients receiving LDL apheresis + simvastatin compared to simvastatin alone [63].

Decreased progression and increased regression of coronary atherosclerosis was demonstrated in three studies using digital subtraction angiography, intravascular ultrasound (IVUS), and quantitative coronary angiography, respectively [63–65]. In an observational study, LDL apheresis in combination with statin therapy was associated with decreased coronary calcium assessed by electron beam computed tomography [66]. In another study involving serial carotid artery ultrasonography in patients with homozygous and heterozygous FH, the mean maximal carotid artery intima-medial thickness (IMT) progressed by 0.002 mm/year in the group treated with LDL apheresis + drug therapy for a mean duration of 7.8 years, which was 1/10 of the 0.025 mm/year rate of progression in the group receiving the same medical therapy without apheresis for a mean of 5.5 years of follow-up ($P < 0.005$) [67].

Since improvements in surrogate endpoints do not always predict commensurate reductions in CHD events, the results from cardiovascular outcome trials are needed. Unfortunately, most studies have been nonrandomized and none have used a placebo-control design for LDL apheresis. In one study, the risk of myocardial infarction and cardiac death was reduced 45% compared to comparable patients not receiving LDL apheresis [68]. A small retrospective analysis of 42 patients with multivessel CHD and severe hypercholesterolemia was performed to compare cardiovascular outcomes 2 years after initiation of one of three treatments: LDL apheresis, coronary artery bypass graft (CABG) surgery, or percutaneous transluminal coronary angioplasty (PTCA). Patients treated with LDL apheresis had 14% fewer CHD events than patients who underwent CABG and 44% fewer events than patients who underwent PTCA [69]. In a more compelling but nonrandomized study of patients with heterozygous FH, 43 subjects treated with LDL apheresis and drug therapy for 6 years were compared to 87 subjects who received drug therapy without LDL apheresis. LDL apheresis was associated with a 72% reduction ($P < 0.01$) in the composite cardiovascular endpoint that included death from CHD, nonfatal myocardial infarction, and revascularization with stents or CABG surgery [49] (Fig. 29.2). Despite the lack of randomization and absence of a placebo control, one can surmise that the LDL apheresis group was most likely biased toward sicker patients, which would have diminished the observed clinical benefit. Hence, the 72% reduction in CHD events is notable in this high-risk population, particularly in comparison to the 25–40% reduction in CHD events typically observed in clinical trials of statin therapy.

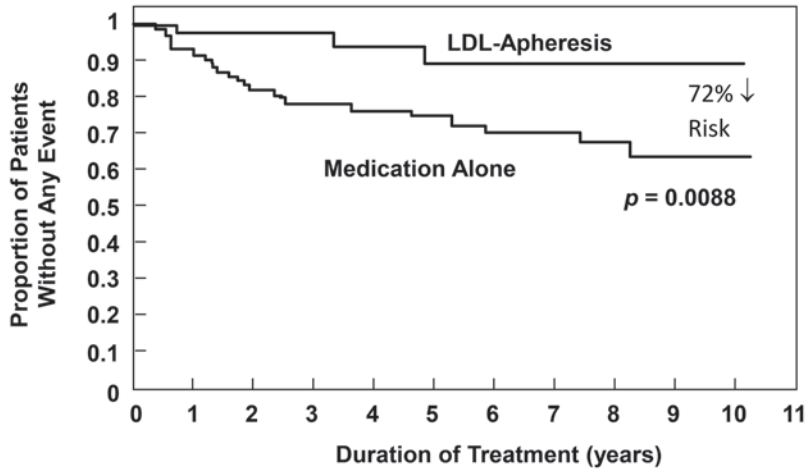

Fig. 29.2 Reduction in CHD events in heterozygous FH patients treated by LDL apheresis treatment (n = 43) compared to those treated with medications alone (n = 87). Graph shows Kaplan–Meier outcome curves for all cardiovascular events. (Adapted from [49]) *CHD* coronary heart disease, *LDL* low-density lipoprotein

Conclusion

Treatment of hypercholesterolemia is the cornerstone of cardiovascular prevention in all patients, but is especially important in patients with FH, a group that has greatly accelerated development of coronary atherosclerosis. Despite the advances in drug treatment during the past 25 years, most patients with homozygous FH and an estimated 2–4 % of patients with heterozygous FH are unable to achieve adequate LDL cholesterol lowering in response to standard medical therapy that includes high-dose high-potency statins in combination with other LDL-lowering medications and lifestyle modification. For refractory patients, LDL apheresis is a potentially lifesaving intervention that is likely to reduce cardiovascular events and have other cardiovascular benefits. The invasive nature of LDL apheresis and the high cost of the procedure (>US $ 2000 per treatment) need to be weighed against the clinical benefits, but LDL apheresis is nonetheless warranted in the highest risk patients. Increases in the number of LDL apheresis treatment centers and decreases in the cost of the procedure will help improve the availability of LDL apheresis for the estimated hundreds of thousands of patients with refractory FH who could benefit from LDL apheresis.

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Novel Lipid-Lowering Agents

30

Zahid Ahmad and Abhimanyu Garg

Introduction

Lipid-lowering drugs are the second most commonly prescribed medication class in the USA [1] with statins being the most commonly prescribed agent within this class. The widespread use of statins owes to overwhelming evidence of benefit and safety as well as awareness campaigns directed at both the physicians and consumers.

The current lipid-lowering agents effectively treat the vast majority of dyslipidemias. Still, a few challenges remain. First, 5–10% of statin users develop intolerance [2]. According to the Centers for Disease Control and Prevention (CDC) data from 2003, 37% of the US population has at least two risk factors for coronary heart disease (CHD), making them potential candidates for statin use. If 5–10% of these individuals experience statin-induced muscular symptoms, then the estimated potential prevalence of statin myopathy is five to ten million in the USA [3].

Second, some subgroups at high risk for CHD—such as patients with severe elevations in cholesterol due to heterozygous familial hypercholesterolemia (FH), nephrotic syndrome, severe polygenic hypercholesterolemia, familial combined hyperlipidemia, and metabolic syndrome—present treatment challenges and often fail to reach their low-density lipoprotein cholesterol (LDL-C) goals [4–9]. For example, in countries with nationwide screening programs to identify FH patients, only 15–33% of treated individuals achieve their LDL-C goal despite being treated with statins [4–6]. Such patients require high doses of statins placing them at increased risk for adverse events, or they require supplemental lipid-lowering agents such as bile-acid-binding sequestrants, ezetimibe, or niacin—but we lack clinical trial evidence for the efficacy of these regimens in the prevention of CHD [10].

Nephrotic syndrome patients present several additional challenges. Kidney dysfunction predisposes them to myopathy from both statins and fibrates [11, 12]. Also, analyses by the US Food and Drug Administration (FDA) revealed that some statins, especially rosuvastatin, can increase protein excretion [13], and we lack data on whether statins will exacerbate the progression of nephrotic syndrome.

Third, rare lipid disorders—such as homozygous FH and familial chylomicronemia syndrome—respond minimally or not at all to lipid-lowering agents and historically required surgical interventions: partial ileal bypass, liver transplantation, and pancreaticobiliary diversion (the latter to prevent episodes of pancreatitis in chylomicronemia).

Currently, homozygous FH patients typically need LDL apheresis, which has been shown to improve survival in these patients [14]. Still, several issues limit LDL apheresis therapy: given its invasive nature, vascular access problems occur

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Table 30.1 Potential new therapies to modify lipids and lipoproteins

Gene therapy
Lipoprotein lipase
Drugs that reduce synthesis or secretion of lipids and lipoproteins
Apolipoprotein B synthesis inhibitors
Microsomal triglyceride transfer protein inhibitors
Diacylglycerol O-acyltransferase 1 inhibitors
ETC-1002
Drugs that increase clearance of lipoproteins
Liver-specific thyroid agonists
Proprotein convertase subtilisin-like kexin-type 9 inhibitors
Therapies that affect HDL-C
Augmentation of lipid-poor apolipoprotein A1
Cholesteryl ester transfer protein inhibitors

HDL-C high-density lipoprotein cholesterol

and may require an arteriovenous shunt; adverse reactions include hypotension, angina, hemolysis, and allergic or anaphylactic reactions; and cost is high and availability of centers is low. At our center, each apheresis treatment costs roughly US $ 4000. Since homozygous FH patients typically require LDL apheresis every 1–2 weeks, total costs may exceed US $ 100,000–200,000 per year. This cost resembles the most expensive drugs in the world and exceeds hemodialysis (a similar invasive procedure) for end-stage-renal disease (US $ 70,000/year). The availability of LDL apheresis centers is limited; roughly 35 centers—mostly at academic institutes in major cities—exist in the USA, making it difficult for rural-dwelling patients to get access.

No drug therapy for familial chylomicronemia or type I hyperlipoproteinemia is available. Current triglyceride (TG)-lowering agents (niacin, fibrates, and fish oil) fail to reduce chylomicron formation in these patients and the only therapeutic option for them is to modify their diets to consume only 10–15 % of total energy as fat. This diet typically reduces TGs to less than 2000 mg/dL, greatly reducing the risk of pancreatitis. But diet therapy has limited durability, especially given the widespread availability of inexpensive energy-dense processed foods. Physicians desperately need new therapeutic options to treat patients with familial chylomicronemia syndrome.

Future Therapies

Novel therapies can be broadly divided into 1.) gene therapies (for familial chylomicronemia syndrome), 2.) drugs that inhibit lipoprotein synthesis and secretion, 3.) drugs that increase lipoprotein clearance, and 4.) drugs that affect high density lipoprotein (Table 30.1)

Gene Therapy

Lipoprotein Lipase Gene Therapy

The European Commission approved lipoprotein lipase (LPL) gene therapy in 2012, making it the first gene therapy to receive approval in the Western world. Gene therapy targets a gene delivery system to the desired cell type using physical means such as tissue injection. Once inside the body and in contact with the specifically targeted cells, inserted DNA incorporates into the tissue's cells where it encodes the production of the needed protein. Since LPL is expressed in skeletal muscles, direct muscular injection may be adequate, and Amsterdam Molecular Therapeutics (AMT) developed such an agent called alipogene tiparvovec (Glybera®). Alipogene tiparvovec is composed of the LPL gene along with an adeno-associated virus subtype 1(AAV1) vector (AAV1-LPL^{S447X}). This LPL gene contains a naturally occurring gain-of-function human variant

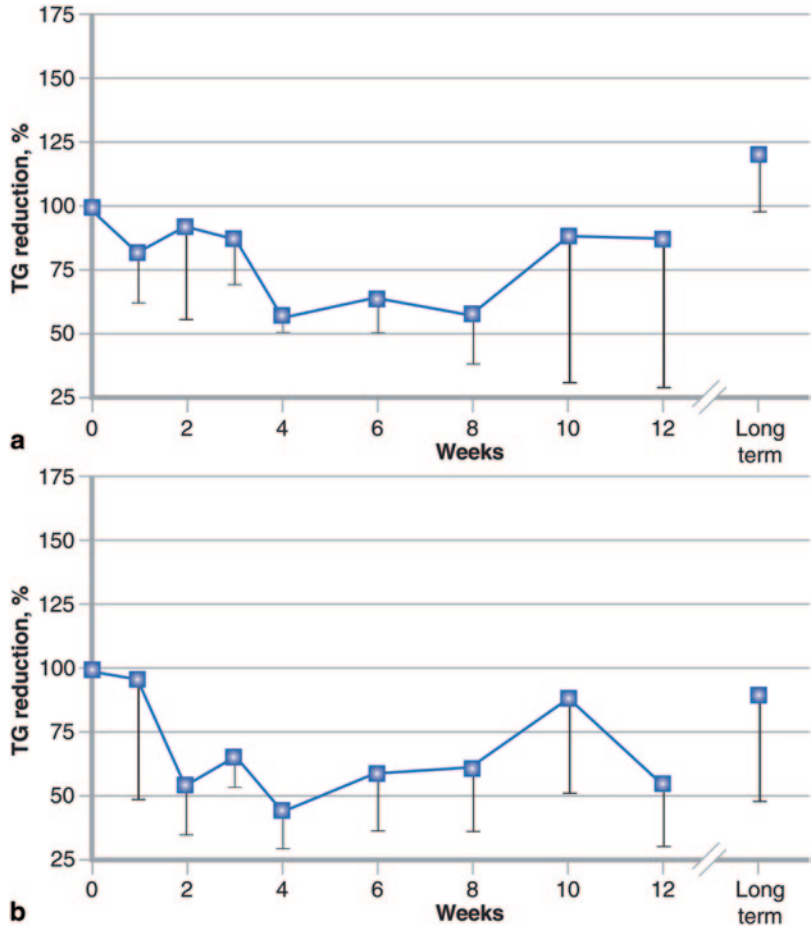

Fig. 30.1 Intramuscular administration of AAV1-Lipoprotein Lipase^{S447X} lowers triglycerides in 8 lipoprotein lipase-deficient patients. Mean % fasting triglyceride reduction compared to baseline following 1×10^{11} gc/kg (**a**) or 3×10^{11} gc/kg (**b**). Long-term: follow-up between 18 and 31 months. *AAV1* adeno-associated virus subtype 1

p.S447X associated with 8% lower serum TGs in the Copenhagen City Heart Study cohort [15].

The safety and efficacy of alipogene tiparvovec was best studied in an open-label dose escalation clinical trial of 14 LPL-deficient patients with a past history of acute pancreatitis [16, 17]. Circulating TGs dropped by 40% in the first 4 months after administration (with the 3×10^{11} gc/kg dose) but returned to baseline by month 6 despite clear long-term transgene expression (Fig. 30.1). Still, patients reported improvement in the quality of life, and the investigators demonstrated a reduction in the incidence of acute pancreatitis and/or intensity of the crisis up to 2 years post-alipogene tiparvovec injection. To explain this finding, they raise the possibility that chylomicron size, lipid content, and kinetics, rather than plasma TG concentration per se, are the best surrogate markers of acute pancreatitis risk in LPL deficiency.

No serious adverse events were attributed to the study drug. Common adverse reactions included local injections site responses, with the most serious being muscle-fiber degeneration and regeneration and neutral lipid accumulation within muscle fibers. No patients developed detectable antibodies towards LPL, but antibodies towards AAV1 developed in half of the participants.

Overall, alipogene tiparvovec is a promising therapy for patients with familial chylomicronemia syndrome due to LPL deficiency. It may

reduce acute pancreatitis episodes despite having only a transient effect on circulating TG levels. Of note, the benefit remains unclear for patients with familial chylomicronemia syndrome due to mutations in other genes such as apolipoprotein CII (*APOC2*), lipase maturation factor 1 (*LMF1*), glycosyl-phosphatidylinositol anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*), and apolipoprotein AV (*APOA5*).

Drugs That Reduce Synthesis or Secretion of Lipids and Lipoproteins

Apolipoprotein B Synthesis Inhibitors

Inhibition of apolipoprotein B (APOB)—an essential structural and receptor-binding component of all atherogenic lipoproteins—decreases serum cholesterol without involvement of the low-density lipoprotein receptor (LDLR), making it a potential therapeutic option for individuals lacking functional LDLRs (i.e., homozygous FH patients). The first APOB inhibitor, mipomersen (Kynamro™), obtained approval by the FDA in January 2013 for use in patients with homozygous FH. Mipomersen is an APOB antisense oligonucleotide: a synthetic phosphorothioate oligonucleotide sodium salt, 20 nucleotides in length, and complementary to APOB100, with the following sequence:

$$5' - \underline{G^{Me}C^{Me}C^{Me}U^{Me}C}\ AGT^{Me}CTG^{Me}CTT^{Me}C$$
$$\underline{G^{Me}CA^{Me}C^{Me}C} - 3'$$

where the underlined residues are 2′-O-(2-methoxyethyl) nucleosides; all other residues are 2′-deoxynucleosides. Substitution at the 5-position of the cytosine (C) and uracil (U) bases with a methyl group is indicated by ^{Me}. See structural formula in Fig. 30.2. The drug accumulates in hepatocytes and targets APOB100 messenger RNA (mRNA) for degradation rather than translation to the protein product (Fig. 30.3).

Mipomersen is best studied in a 26-week phase III clinical trial that enrolled 51 homozygous FH patients. Mipomersen 200 mg SQ weekly demonstrated a 25% LDL-C reduction compared to 3% by placebo (Fig. 30.4a) [18]. Of practical importance, mipomersen requires 15–20 weeks of administration before achieving the full effect. Variability in the response was noted for mipomersen, ranging from a 2% increase in LDL-C to an 82% decrease in LDL-C (Fig. 30.4b). High-density lipoprotein cholesterol (HDL-C) and serum TGs did not significantly change with mipomersen administration.

Mipomersen 200 mg SQ weekly has also been studied in patients with heterozygous FH, statin-intolerant patients, high-risk hypercholesterolemic patients, and patients with severe hypercholesterolemia on maximally tolerated lipid-lowering therapy [19–21]. These studies demonstrated LDL-C reductions between 28 and 48%.

The most common adverse events included injection site reactions, flu-like symptoms, and elevated hepatic transaminases and these events led to the discontinuation of mipomersen in 12–18% of participants [21]. Injection site reactions occurred in 84% of patients and typically consisted of one or more of the following: erythema, pain, tenderness, pruritus, and local swelling [22]. Flu-like symptoms occurred in 30% of patients within 2 days of injection and included one or more of the following: influenza-like illness, pyrexia, chills, myalgia, arthralgia, malaise, or fatigue [22]. According to the FDA documents [21], mipomersen's risk profile includes several findings of unclear significance: transient increases in high-sensitivity C-reactive protein (hsCRP)—16% in mipomersen group versus 0% in placebo group had shifts in hsCRP levels from <3 mg/L pre-dose to ≥ 3 mg/L post-dose; development of anti-mipomersen antibodies in 60% of homozygous FH patients; and increased rates of proteinuria (9% with mipomersen vs. 3% on placebo) by dipstick measurement.

Two major issues may limit the use of mipomersen. First, LDL-C reduction resembles that of high-dose statins in homozygous FH patients: an unimpressive 25% [21, 23, 24]. So the cost–benefit ratio should be carefully assessed, especially since statins and LDL-apheresis delay cardiovascular events and prolong survival—a benefit that has not yet been demonstrated for mipomersen [24].

Second, inhibition of hepatic VLDL production and secretion leads to hepatic TG accumulation, similar to what occurs in humans with

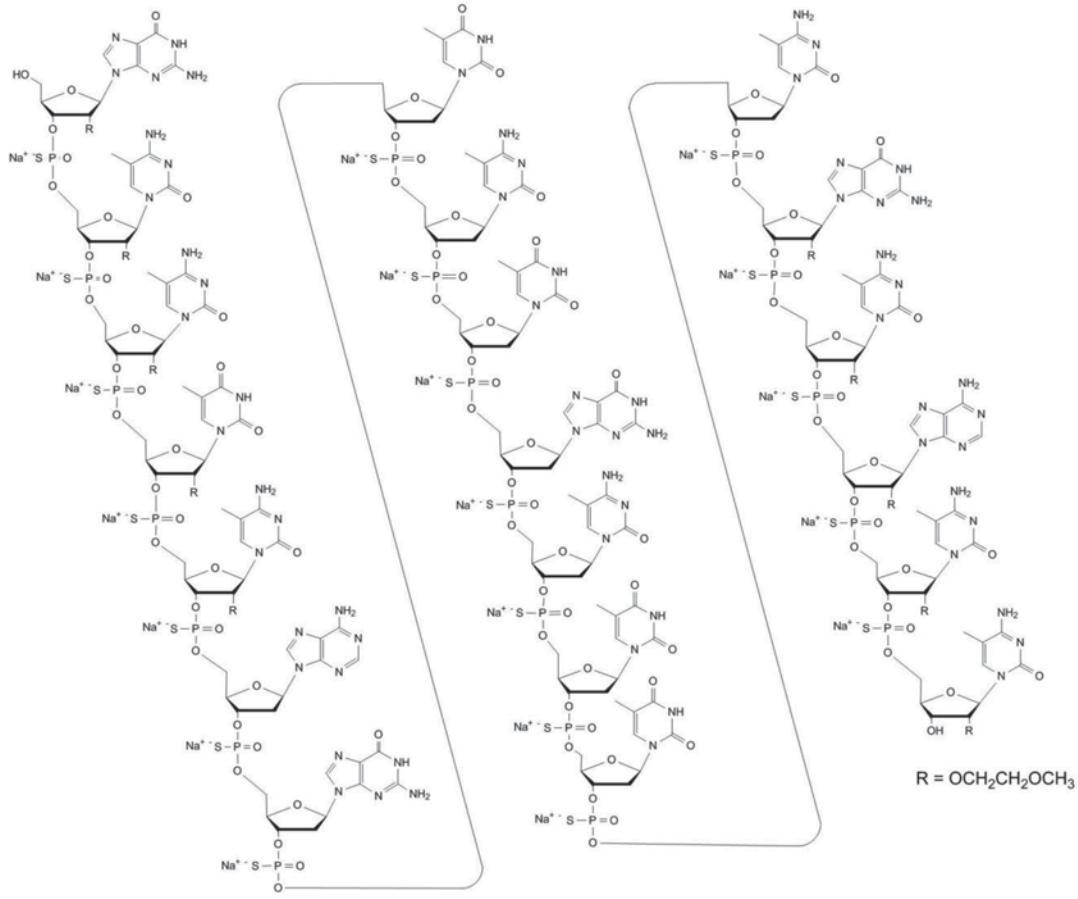

Fig. 30.2 Mipomersen sodium is represented by the following structural formula (from mipomersen prescribing information)

hypobetalipoproteinemia due to *APOB*-truncating mutations. Although an early study failed to find an elevation in intrahepatic triglyceride (IHTG) content (measured by proton magnetic resonance spectroscopy) [25], two phase 3 studies found a median increase of 9.6% in mipomersen-treated versus 0.02% in placebo-treated individuals [19, 21].

It remains unclear if long-term exposure results in irreversible liver injury. Of note, serum transaminases remained normal in most of the participants with increased IHTG, raising concerns about how to monitor for increased IHTG and progression to nonalcoholic fatty liver disease (NAFLD). Drug-induced NAFLD is not well studied with regard to long-term consequences, but nondrug-induced NAFLD can progress to cirrhosis and is associated with insulin resistance and increased cardiovascular risk [26].

Pooled results from all mipomersen trials reveal that "cardiac disorders"—specifically angina pectoris and palpitations—occurred in 3.8% of mipomersen-treated individuals and 3.1% of placebo-treated individuals [21]. These data and the liver toxicity data led the Committee for Medicinal Products for Human Use (European Medicines Agency) to recommend against approval in the European Union. Further data are needed regarding the drug's long-term safety and cardiovascular benefit [21] prior to widespread use in non-FH populations—such as statin-intolerant patients.

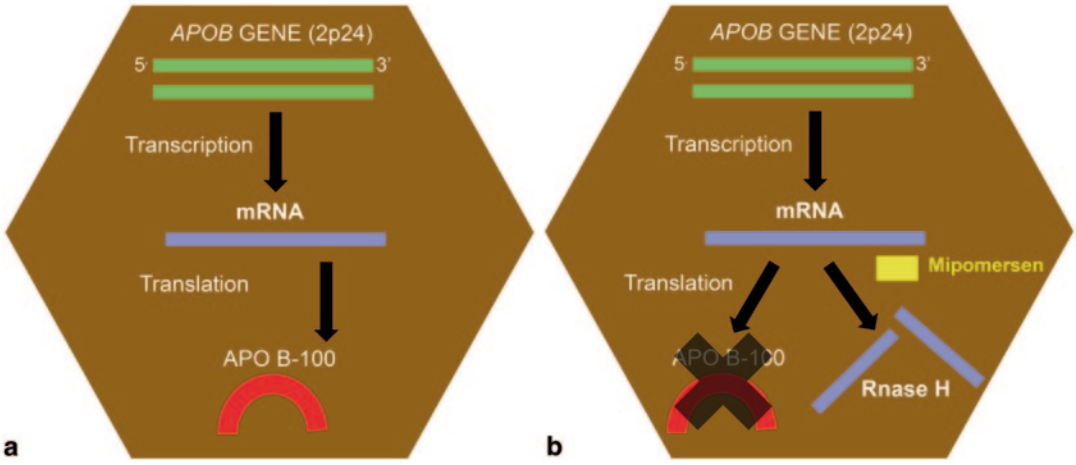

Fig. 30.3 Mechanism of action of an apolipoprotein B (APOB) antisense inhibitor. Within hepatocytes, the APOB gene is transcribed then translated (**a**) to APOB100—the major protein component of all atherogenic lipoproteins. Mipomersen, an antisense inhibitor, induces the degradation of APOB mRNA via the RNase H enzyme (**b**). *mRNA* messenger RNA, *RNase* ribonuclease

Microsomal Triglyceride Transfer Protein Inhibitors

The microsomal triglyceride transfer protein (MTP) inhibitor, lomitapide (Juxtapid™), obtained FDA approval for use in homozygous FH patients in December 2012. MTP assists in the initial packaging of cholesterol esters and TGs into both chylomicrons and VLDL in intestinal cells and hepatocytes, respectively.

Several pharmaceutical companies abandoned the production of MTP inhibitors in the early 2000s due to poor gastrointestinal tolerability, elevations in hepatic transaminases, and a high rate of hepatic steatosis [27]. Lomitapide eventually found its way to individual academic investigators: Rader et al. [28] administered the drug to six homozygous FH patients in an open-label dose escalation study as a proof-of-concept phase 2 study. After 4 weeks of therapy, TG decreased by 65% and LDL-C decreased by 51% (1 mg dose). However, serum alanine aminotransferase and IHTG (as measured by proton magnetic resonance spectroscopy) rose in four out of six patients.

Despite these setbacks, Aegerion Pharmaceuticals pursued approval from the FDA for use in patients with homozygous FH. The FDA based its approval on a 78-week phase III open-label unblinded forced-titration study evaluating the safety and effectiveness of lomitapide to reduce LDL-C levels in 29 adult patients with homozygous FH [29]. When added to existing lipid-lowering therapy—including LDL apheresis in 18 patients—lomitapide reduced LDL-C from a baseline average of 336 to 190 mg/dL (40% reduction) at 26 weeks (efficacy phase of study; Fig. 30.5). Six patients either stopped or increased the interval between LDL apheresis treatments, but data are lacking regarding lomitapide's superiority to LDL apheresis for cardiovascular outcomes [27]. It should also be noted that lomitapide is not studied in randomized, placebo-controlled trials beyond short-term phase 2 trials.

The most common adverse events were gastrointestinal related: 93% of subjects experienced at least one such event with the most common being diarrhea, nausea, vomiting, dyspepsia, and abdominal pain. These occur due to MTP inhibition in intestinal cells that result in sloughing of lipid-filled enterocytes. In order to minimize the gastrointestinal-related adverse events, patients must modify their diet to include <20% energy from fat.

Similar to mipomersen, lomitapide use results in accumulation of IHTG leading to drug-induced NAFLD. Eleven of the 29 patients in the homozygous FH study had at least one elevation in liver enzymes greater than or equal to three times the upper limit of normal. Four of those patients experienced liver enzymes greater than

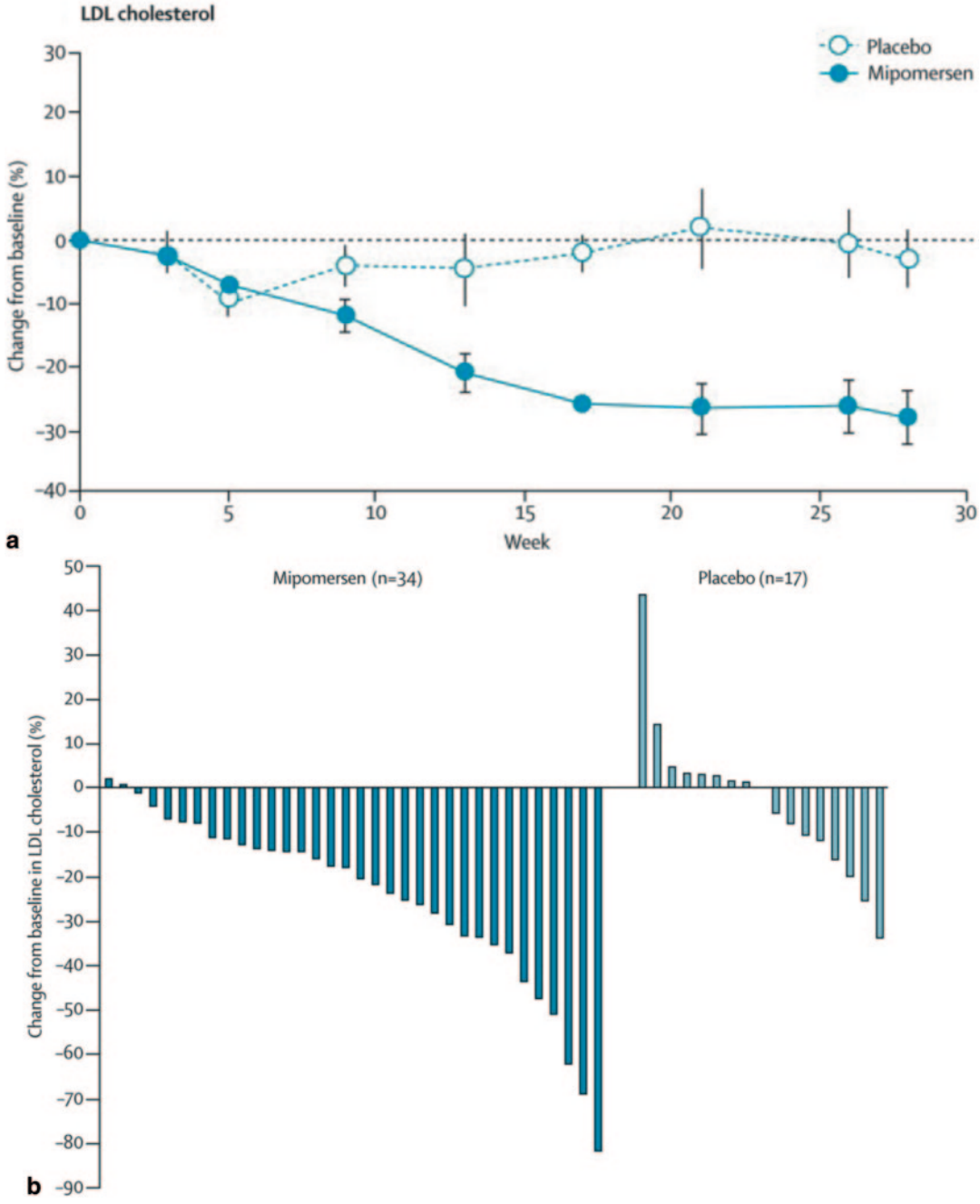

Fig. 30.4 LDL-C reduction with mipomersen
2a: Mean percentage change from baseline (week 0) to primary efficacy timepoint for LDL-cholesterol
2b: Percentage change in LDL-cholesterol from baseline for individual patients. Response is shown graphically for individual patients in the mipomersen and placebo treatment groups. Each vertical bar represents one patient, arranged in order of response. (Modified from: [18])

or equal to five times the upper limit of normal. IHTG increased from a baseline of 1% to a median absolute increase of 6% at 78 weeks. Eighteen (78%) of 23 subjects demonstrated a maximum absolute increase in hepatic fat >5%, and three (13%) had an absolute increase >20%.

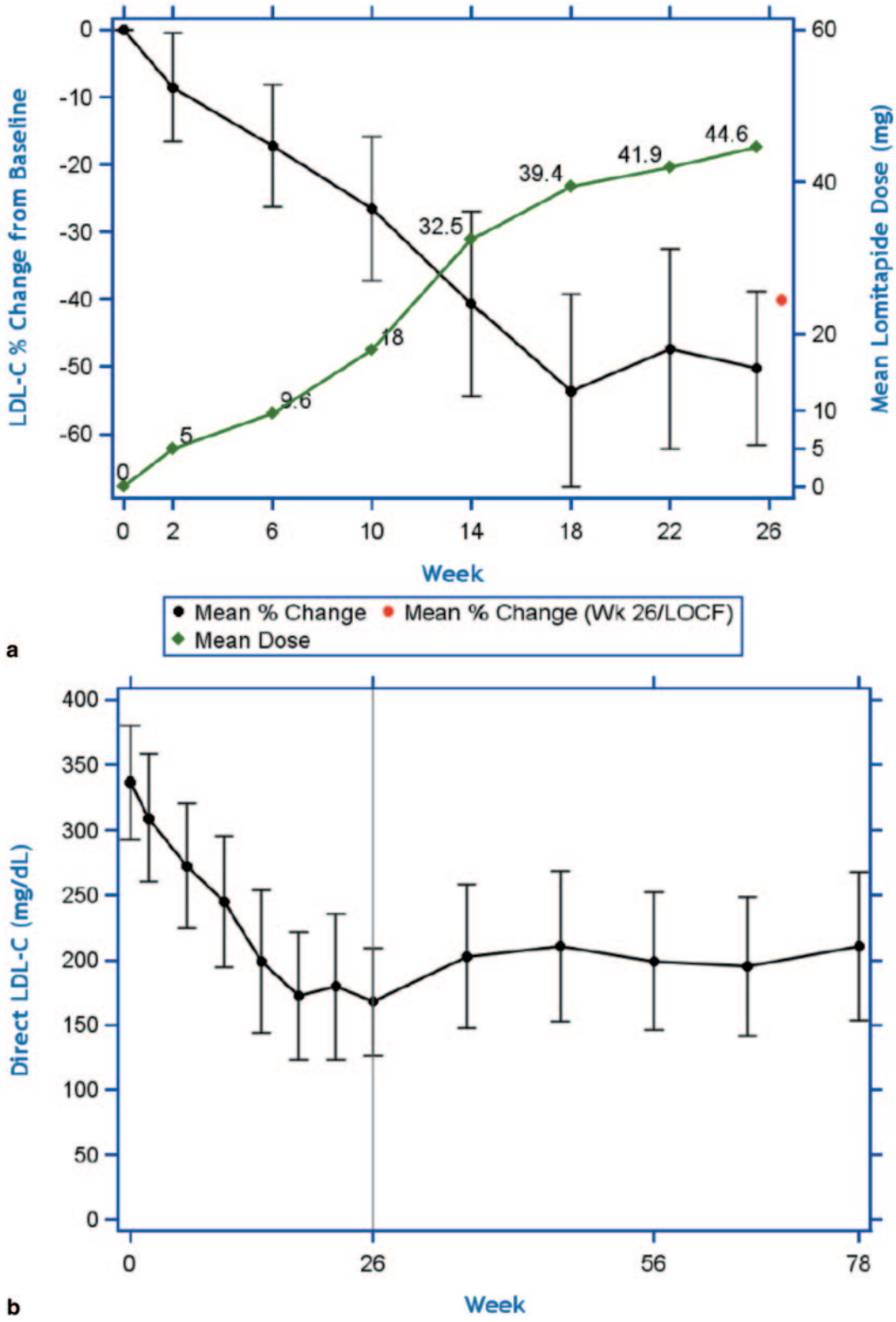

Fig. 30.5 Effect of lomitapide on LDL-C

Similar to mipomersen, transaminases remained within normal limits in many of the participants with increased IHTG—a phenomenon of unclear significance that makes transaminase measurements an insensitive method to screen for hepatic fat accumulation [27]. It remains unclear as to whether lomitapide-induced NAFLD and nondrug-induced NAFLD have similar long-term hepatic and cardiovascular consequences.

Lomitapide leads to deficiencies in fat-soluble nutrients by inducing intestinal fat malabsorption [27]. In the phase 2 study by Rader et al. [28], serum levels of several fatty acids declined including alpha-linolenic acid, gamma-linolenic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid. In the larger phase 3 trial, subjects consumed dietary supplements containing vitamin E, linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. Levels of vitamins A and D increased while vitamin K was unchanged. Total vitamin E decreased by a median of −43.3% at week 26 and −40.7% at week 78—an expected finding since APOB-containing lipoproteins are required for vitamin E absorption and transport. The ratio of serum vitamin E to lipid remained stable, suggesting that decreases in vitamin E occur due to lomitapide's effect on serum lipoproteins [27]. In clinical practice, patients taking lomitapide require dietary supplements that provide approximately 400 IU vitamin E, 200 mg linoleic acid, 110 mg eicosapentaenoic acid, 220 mg alpha-linolenic acid, and 80 mg docosahexaenoic acid per day.

Two additional practical issues complicate lomitapide use: slow titration and drug interactions. Titration to full dose takes 4–5 months with frequent clinic visits and careful monitoring for gastrointestinal-related adverse events and transaminitis. Only 40% of patients in the phase 3 tolerated the full dose of 60 mg [27]. Lomitapide is a CYP3A4 substrate, and it increases simvastatin exposure [27]—perhaps raising the risk of myopathy. Concomitant use with other moderate or strong CYP3A4 inhibitors has not been evaluated in a randomized, placebo-controlled trial.

Similar to mipomersen, data are lacking regarding lomitade's long-term safety and cardiovascular benefit. Pooling data from all phase 2 and 3 lomitapide trials, 3 (1.2%) of 255 subjects treated with lomitapide monotherapy had at least one cardiovascular event recorded compared with none of the 191 subjects treated with lomitapide combination therapy (e.g., lomitapide + lipid-lowering therapy), none of the 98 treated with placebo, and none of the 78 subjects treated with an active control [27]. Also of concern, lomitapide transiently reduces HDL-C by 12% [29]. Given the paucity of events in the lomitapide development program, none of which were adjudicated, it is premature to make conclusions regarding the effect of lomitapide on cardiovascular events [27].

In contrast to systemically inhibiting MTP, an alternative approach involves designing an inhibitor only for MTP localized to intestinal cells. An intestinal-specific MTP inhibitor would reduce chylomicron formation—offering a therapy for patients with familial chylomicronemia syndrome—and provide a key advantage over systemic MTP inhibitors: a better safety profile with regard to accumulation of IHTG. Two such drugs are under development. Surface Logix developed SLx-4090 [30], and Japanese Tobacco Inc. developed JTT-130 [31]. In early trials, SLx-4090 decreased postprandial TGs up to 50% in healthy subjects while transaminases remained normal. The only side effects noted were headache, flatulence, and diarrhea. Such a drug may provide a novel mechanism for TG lowering that targets chylomicron formation.

Diacylglycerol O-acyltransferase 1 Inhibitors

Diacylglycerol O-acyltransferase 1 (DGAT1) enzyme catalyzes the final step in TG synthesis in both intestinal cells and hepatocytes (Fig. 30.6).

a. Mean lomitapide dose and mean (SD) percent changes in LDL-C levels from baseline to week 26 (end of efficacy phase). Data available at each time points are expressed as mean (SD).
b. Mean LDL-C levels during efficacy (baseline to week 26) and safety (week 26 to 78) phases. (From [27] and [29])

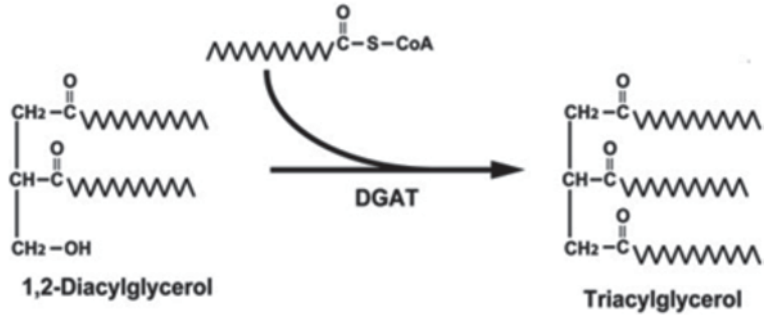

Fig. 30.6 Acyl coenzyme-A:1,2-diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent synthesis of triacylglycerol.

DGAT1 knockout mice display leanness, resistance to diet-induced obesity, increased insulin sensitivity, and reduced postprandial TG excursion [32, 33].

Recently, Farese and coworkers reported the first human cases of DGAT1 deficiency in two children (ages 16 months and 30 months) [34]. They presented with a congenital diarrheal disorder, and oddly, exhibited mild elevations in fasting TG.

Several companies are developing DGAT1 inhibitors: Novartis—LCQ908, Astra-Zeneca—AZD7687, Abbott—ABT-046 [35], Pfizer—PF 04620110 [36], and others. LCQ908 failed to reduce fasting TGs in diabetic patients but may still possess potential for reduction of postprandial TG [37]. The efficacy, safety, and tolerability in patients with familial chylomicronemia syndrome—who suffer from severe postprandial hypertriglyceridemia—are being assessed in an ongoing randomized, double-blind, placebo-controlled study (clinicaltrials.gov #: NCT01514461).

In phase 1 studies, AZD7687 decreased postprandial TG excursions by as much as 85 % [38]. No major adverse events occurred, but 43 % of AZD7687-treated patients experienced gastrointestinal intolerability: nausea, vomiting, and diarrhea. Two other phase 1 studies ended but data are not yet published (ClinicalTrials.gov identifiers: NCT01217905 and NCT01119352).

DGAT1 is most highly expressed in the small intestine and adipose tissue and also present in other organs such as the liver, pancreas, skeletal muscle, and heart; the effect of DGAT1 inhibition in these organs remains unknown.

ETC-1002

Esperion Pharmaceuticals developed an orally available small molecule that inhibits hepatic adenosine triphosphate-citrate lyase (ACL) and activates hepatic 5′-adenosine monophosphate-activated protein kinase (AMPK) [39], resulting in dual inhibition of sterol and fatty acid synthesis as well as enhanced mitochondrial long-chain fatty acid oxidation. The drug, named ETC-1002, has been studied thus far in phases I and II human trials and reported to lower LDL-C by 27–42 % [40]. In the only study with published data (a randomized, placebo-controlled, dose-finding phase II study of 177 patients with elevated LDL-C), ETC-1002 lowered LDL-C by 27 % at the highest studied dose, 120 mg daily [41]. TG decreased by 25–30 % irrespective of baseline TGs (eligibility criteria included TG <400 mg/dL) with no significant effect on HDL-C. With regard to safety, seven patients received the active drug and developed myalgia while no patients receiving placebo developed myalgia. A few laboratory abnormalities were also noted in patients who received ETC-1002: uric acid levels increased 7–16 %, homocysteine levels increased by up to 2.4 μmol/l, and hemoglobin decreased by roughly 0.5 g/dl. Larger, phases 2 and 3 studies will help elucidate the benefits and risks of ETC-1002

and whether the drug has metabolic benefits beyond lipid lowering.

Drugs That Increase Clearance of Lipoproteins

Liver-Specific Thyroid Agonists

The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), control a variety of physiologic functions including growth, development, and metabolism, and they reduce cholesterol as noted in humans with hyperthyroidism. Thyroid hormones exert tissue-specific effects via different thyroid receptor (TR) subtypes. Hepatocytes express TRβ1, which mediates LDL-C and TG lowering via several pathways: upregulation of hepatic *LDLR* expression, reduction of APOB levels by inhibition of sterol regulatory element binding protein-1 (SREBP-1) transcription, increased hepatic uptake of cholesterol from HDL through increased activity of scavenger receptor class B type I (SR-BI), and increased activity of cholesterol 7α hydroxylase—an enzyme involved in the rate-limiting step in the conversion of cholesterol into bile acids [42] (Fig. 30.7). TRβ1 activation may also increase the expression of adenosine triphosphate (ATP)-binding cassette transporter B11 (ABCB11), the major determinant of bile flow, as well as the expression of the transporters ABCG5 and ABCG8 (ABCG5/G8). Thus, a β1 selective thyroid hormone agonist has potential to be a novel lipid-lowering agent without affecting TRs in the heart or pituitary.

Karo Bio studied one such agent—eprotirome—in two randomized, double-blind, placebo-controlled human trials and found an LDL-C reduction of 30–40% [43, 44]. Weight and heart rate remained normal, but they observed a dose-dependent reduction in total and free T4. Regardless, in February 2012, Karo Bio discontinued the development program after long-term animal studies demonstrated cartilage damage [45].

The future of contemporary TRβ1 agonists remains unclear, but next-generation TRβ1 agonists—with additional tissue-specific effects with improved safety and efficacy—are under development [42]. Madrigal Pharmaceuticals (Fort Washington, PA, USA) recently reported results of a phase 1 study in which LDL-C and TG decreased by 30 and 60%, respectively, with their novel TRβ1 agonist [46].

Any novel thyroid agonist will have to be systematically studied for cardiovascular endpoints, especially in light of the findings from the Coronary Drug Project. The dextrothyroxine arm of that study terminated early due to excessive arrhythmias and a lack of mortality benefit after 3 years of follow-up [47].

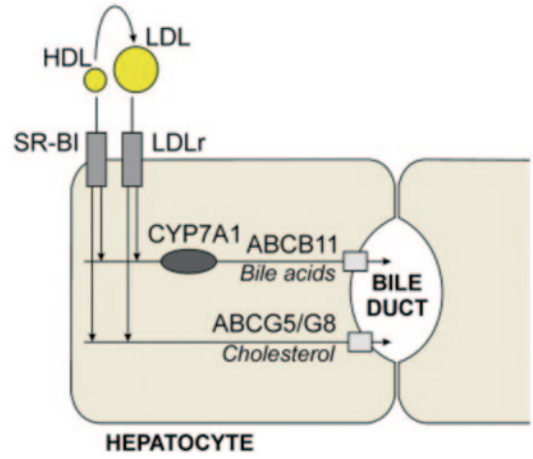

Fig. 30.7 Thyromimetics increase the hepatic expression of the LDL receptor (LDLR), leading to enhanced plasma clearance of LDL cholesterol. In addition, thyromimetics increase the hepatic expression of the HDL-cholesterol receptor (SR-BI), promoting hepatic delivery of cholesterol brought back to the liver from peripheral tissues by HDL particles. HDL-cholesterol can alternatively be transferred to LDL particles and taken up by the liver via the LDLR. Furthermore, thyromimetics induce hepatic cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol into bile acids, and increase the expression of ATP-binding cassette transporter B11 (ABCB11), the major determinant of bile flow, as well as the expression of the transporters ABCG5 and ABCG8 (ABCG5/G8), which secrete neutral sterols into bile upon dimerization. Thus, thyromimetics not only promote the hepatic uptake of excessive cholesterol from plasma into the liver but at the same time also promote its disposal into bile and ultimately the feces, either directly as cholesterol or after conversion into bile acids. *ATP* adenosine triphosphate, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *SR-B1* scavenger receptor class B type I, *ER* endoplasmic reticulum. (Figure and legend from [74])

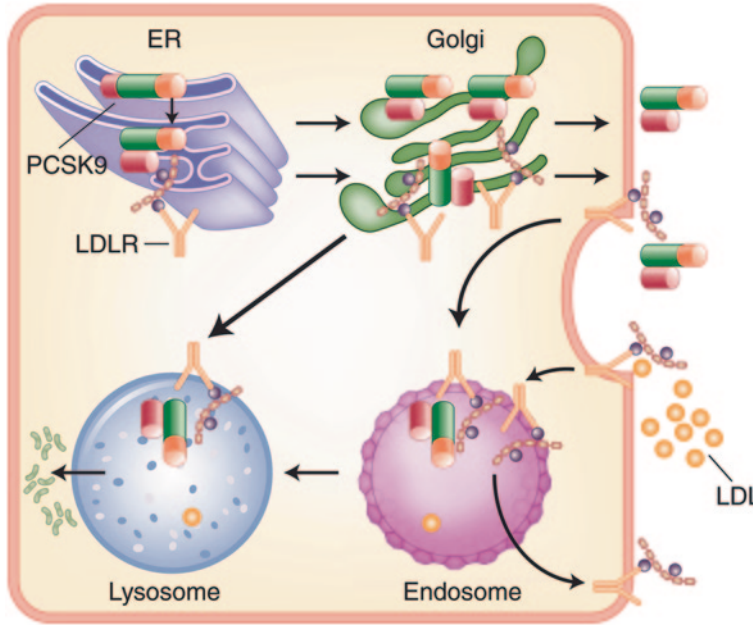

Fig. 30.8 Cellular trafficking and sites of PCSK9 action. After undergoing autocatalytic cleavage in the ER, the prodomain (*purple*) remains associated with the catalytic fragment (*green*) and the complex is secreted into the plasma. Secreted PCSK9 binds to LDLRs on the cell surface, and the LDLR/PCSK9 complex is internalized via the adaptor protein ARH (LDLRAP). PCSK9 prevents the recycling of LDLRs from endosomes to the cell surface or direct LDLRs to lysosomes for degradation. A second intracellular pathway has been proposed in which PCSK9 binds the LDLR in a post-ER compartment and targets LDLR for degradation in lysosomes. *ER* endoplasmic reticulum, *PCSK9* proprotein convertase subtilisin-like kexin- type 9, *LDLRs* low-density lipoprotein receptors, *ARH* autosomal recessive hypercholesterolemia. (Modified from [75]).

PCSK9 Inhibition

Based on the phenotype of humans with proprotein convertase subtilisin-like kexin-type 9 (PCSK9) deficiency—hypocholesterolemia without any apparent health consequences [48]—several pharmaceutical companies established development programs for PCSK9 inhibitors. PCSK9 induces the LDLR for degradation by interacting with its epidermal growth factor-like repeat A (EGF-A; Fig. 30.8). Blocking this interaction increases active LDL uptake and reduces circulating cholesterol [49, 50].

Several types of PCSK9-targeted agents are in development [51]: monoclonal antibodies (Sanofi/Regeneron, Amgen, Pfizer/Rinat Neuroscience, Merck), adnectins-specific inhibitors (Bristol-Myers Squibb/Adnexus), RNA interference (Alnylam/Novartis), locked nucleic acids (Santaris/Novartis), and small molecule inhibitors (Shifa Biomedical Corporation, Cadila Healthcare, Serometrix). Of these, monoclonal antibodies are the most advanced in development.

Several short-term trials studied the effect of monoclonal antibodies to PCSK9 in patients with heterozygous FH. Regeneron Pharmaceuticals' product, REGN727, reduced LDL-C by 40–50% [52, 53]—a reduction comparable to statins and superior to currently available non-statin lipid-lowering medications. Participants tolerated the study drug well, although the study involved only three administrations. Amgen's product, AMG145, reduced LDL-C up to 55%—with 89% of patients on the highest dose (420 mg) reaching a goal LDL-C of 100 mg/dl [54] (Fig. 30.9). Muscle-related adverse events compared favorably between AMG145 and placebo groups, but a few subjects on the study drug developed asymptomatic elevations in creatine kinase (CK) related to strenuous exercise. Other than muscle side effects, no notable differences

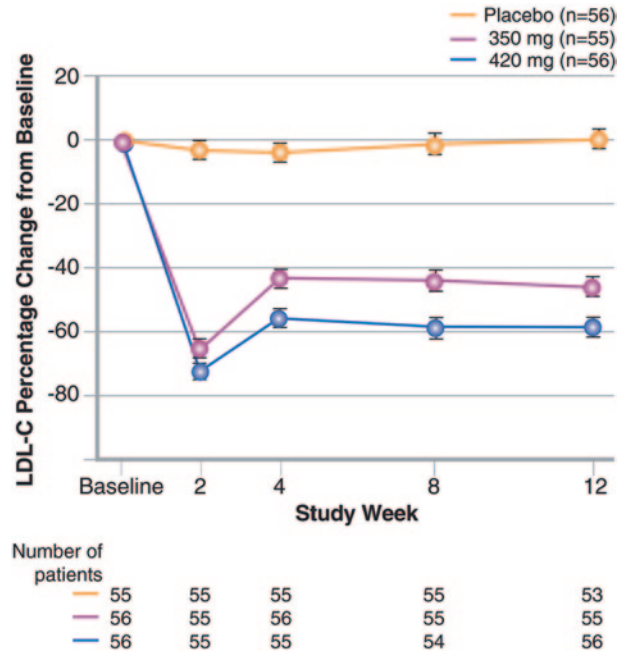

Fig. 30.9 LDL-C reduction with AMG-145 (PCSK9 monoclonal antibody) administered every 4 weeks to patients with heterozygous familial hypercholesterolemia (RUTHERFORD trial). LDL-C low-density lipoprotein cholesterol. (Modified from [54].

occurred in the safety profile relative to placebo. Based on these studies, PCSK9 inhibition may offer a new treatment option to further reduce LDL-C in heterozygous FH patients unable to achieve optimal LDL-C targets on current medications.

The Goal Achievement After Utilizing an Anti-PCSK9 Antibody in Statin-Intolerant Subjects (GAUSS) study assessed the efficacy and safety of AMG145 in patients ($n=160$) with a history of muscle-related adverse effects with statins [55]. After 12 weeks of treatment, LDL-C decreased by 50% with the highest dose of study drug (420 mg), and among the patients who got both AMG145 and ezetimibe, 90% achieved their goal LDL-C. Overall, the participants tolerated the study drug well, but myalgia did occur in six patients. Two patients in the AMG145-only group developed CK levels greater than ten times the upper limit of normal. Both cases occurred after exercise.

Future studies of longer duration and larger sample size will establish the safety and cardiovascular benefits of PCSK9 inhibition. Thus far, mild injection-site reactions were the most common adverse events, and one subject experienced a serious adverse event of leukocytoclastic vasculitis [56], which required steroid therapy; follow-up assessment found minimally detectable antidrug antibodies in this subject.

The *PCSK9* gene expresses at the highest levels in the liver, intestine, kidney, and brain [57], raising concern that pharmacologic PCSK9 inhibition may affect these organs. For example, PCSK9 enhances the degradation of the major hepatitis C virus receptors [51], so PCSK9 inhibition may potentiate hepatitis C infection.

Based on the available data, human PCSK9 deficiency lacks any apparent adverse consequences. However, we lack data regarding the phenotype of *PCSK9* heterozygotes other than their reduced risk of CHD. Furthermore, two patients with bi-allelic loss-of-function mutations have been discovered [58, 59], but only a limited phenotypic description of these patients is available. Future studies of human PCSK9 deficiency should help elucidate any potential adverse effects of pharmacological PCSK9 inhibition.

Nevertheless, PCSK9 inhibition is perhaps the most promising LDL-C-lowering therapy for patients with statin intolerance and heterozygous FH.

Drugs That Affect LPL Activity

Several cofactors, enzymes, and proteins affect LPL enzyme activity. Both apolipoprotein C3 (APOC3) and angiopoietin-like protein 3 (ANGPTL3) inhibit LPL activity and represent promising therapeutic targets based on human genetic studies [60–62]. In general-population studies, loss-of-function *APOC3* mutations are associated with lower circulating TG levels and reduced CHD risk [61, 62], and nonsense mutations in *ANGPTL3* have been found in one family with LDL-C levels as low as 28 mg/dL and TG levels as low as 17 mg/dL. Several pharmaceutical companies are focused on developing agents that affect one or both of these targets. Thus far, little clinical trial data are published although ISIS Pharmaceutical has started phase 3 clinical trials in patients with familial chylomicronemia syndrome (ClinicalTrials.gov identifier: NCT02211209). Both APOC3 and ANGPTL3 inhibition represent promising novel therapeutic targets for reducing LDL-C, TGs, and CHD events.

Therapies That Affect HDL-C

Low HDL-C predisposes to CHD, but augmenting circulating HDL-C levels lacks evidence as a therapeutic target. A recent meta-analysis investigated the association between treatment-induced changes in HDL-C and total death, CHD death, and CHD events adjusted for changes in LDL-C and drug class in randomized trials of lipid modifying interventions [60]. The analysis included 108 trials with nearly 300,000 participants and did not show any benefit of raising HDL-C. These findings suggest that simply increasing the amount of circulating HDL-C does not necessarily confer cardiovascular benefits. Regardless, several HDL modifying therapies are under investigation and perhaps the most promising involve augmentation of lipid-poor HDL particles that can participate in cholesterol efflux.

Cholesteryl Ester Transfer Protein Inhibitors

Cholesteryl ester transfer protein (CETP) mediates the exchange of cholesteryl ester from HDL for TG in VLDL. CETP inhibition raises HDL-C by blocking this exchange of lipids during reverse cholesterol transport.

The first CETP inhibitor was developed by a Japanese group in 2000 [61]. They developed a drug that formed a disulfide bond with CETP and increased HDL-C, decreased non-HDL cholesterol, and inhibited the progression of atherosclerosis in rabbits.

Since then, development of new CETP inhibitors continued and includes 4 compounds studies in human trials: torcetrapib, anacetrapib, evacetrapib, and dalcetrapib. When administered to humans, HDL-C rises by as much as 130%.

The first of these agents, torcetrapib, increased rates of cardiovascular events and mortality due to both cardiovascular and noncardiovascular causes—primarily cancer and infection [62]. It failed to prevent the progression of atherosclerosis in imaging trials [63].

The major trial of dalcetrapib failed to reduce cardiovascular events and terminated early due to futility [64]. The study randomized 15,871 acute coronary syndrome (ACS) patients to receive either dalcetrapib or placebo. Dalcetrapib increased HDL-C by 30% (with minimal effect on LDL-C) but failed to affect the primary end point: a composite of death from CHD, a major nonfatal coronary event (myocardial infarction, hospitalization for unstable angina with objective evidence of acute myocardial ischemia, or cardiac arrest with resuscitation), or ischemic stroke.

Long-term cardiovascular outcome studies of evacetrapib and anacetrapib are ongoing [65]. In a 12-week trial, evacetrapib raised HDL-C by almost 130% and reduced LDL-C by 36% without any major adverse events [66]. In a 24-week trial, anacetrapib raised HDL-C by 138% and reduced LDL-C by almost 40% [67] without any major adverse events.

Several issues may explain the lack of benefit from CETP inhibition. First, the drugs may affect off-target organs. For example, torcetrapib may raise mineralocorticoid levels. Second, apolipoproteins in HDL play roles outside of lipid homeostasis, and alteration of HDL particles may influence nonlipid-related processes. For example, apolipoprotein L1 has been linked to the development of kidney disease and also protection

against African sleeping sickness [68]. Third, CETP inhibition may result in accumulation of only large cholesterol-rich HDL particles that are not involved in cholesterol efflux. Finally, by inhibiting reverse cholesterol transport, CETP inhibition may interfere with an important process that returns cholesterol to the liver.

Augmentation of Lipid-Poor APOA1

Apolipoprotein A1 (APOA1) constitutes the major apolipoprotein on HDL particles. Lipid-poor APOA1, also termed nascent HDL or preβ-HDL, initiates reverse cholesterol transport by accepting effluxed cholesterol.

A naturally occurring variant of APOA1, APOA1 Milano, was identified in individuals from rural Italy who demonstrated a decreased prevalence of CHD despite low levels of HDL-C [69]. In a pilot study, recombinant APOA1-Milano infusion led to reduction of atheroma volume in patients with ACS [70]. No major adverse events occurred, although one patient experienced a hypersensitivity reaction and withdrew from the study. Clinical development of this drug halted owing to manufacturing difficulties and was scheduled to resume in 2011 following completion of technology transfer to a different pharmaceutical company [65].

A larger ($n=145$) ACS study examined the effect of a reconstituted HDL product: CSL-111. CSL-111 consists of APOA1 from human plasma combined with soybean phosphatidylcholine [71]. The study randomized patients to receive placebo, 40 mg/kg or 80 mg/kg of the study drug. Atheroma volume failed to change as compared to placebo but improvement occurred in plaque characterization index and coronary score on quantitative coronary angiography (a secondary endpoint). With regard to safety, a mild self-limiting transaminase elevation occurred, prompting the termination of the higher-dosage CSL-111 treatment group. Also, hypotension occurred in more participants assigned to 40 mg/kg of CSL-111 compared with those receiving placebo (13.8 vs. 7.1%).

A different approach to increasing ApoA1 involves passing a patient's plasma through a delipidation device that selectively removes cholesterol. Lipid Sciences, Inc., developed a plasma delipidation system that can remove lipids from HDL particles resulting in lipid-poor ApoA1. Lipid-poor ApoA1 can then be reinfused. In a small pilot trial of ACS patients, seven weekly infusions of autologous delipidated HDL decreased total atheroma volume by 12% [72]. Adverse events included only hypotension related to the apheresis procedure.

Orally available HDL-mimetics are also under development. Such drugs mimic the structure of ApoA1 and bind lipids found in HDL [73]. However, little human data have been published thus far.

Overall, augmentation of ApoA1 is an attractive mechanism, but further large-scale studies are needed to establish the safety and efficacy of such agents.

Conclusions

With regard to treating dyslipidemias, a few challenging populations remain: statin-intolerant individuals; high-risk patients—nephrotic syndrome and heterozygous FH—who present treatment challenges and are unable to get to goal on existing drugs; and patients with rare diseases—homozygous FH and familial chylomicronemia syndrome—for whom existing drugs lack efficacy. A few promising therapeutic targets include APOB antisense inhibition, MTP inhibition, DGAT1-inhibition, liver-specific TR agonists, and PCSK9 inhibition. Although CETP inhibitors failed to demonstrate a cardiovascular benefit, augmentation of lipid-poor APOA1 may offer more promise. Overall, the future of lipid-lowering therapy offers several options that potentially can help fulfill unmet needs.

Addendum

Microsomal Triglyceride Transfer Protein Inhibitors Sacks et al [76] described a patient treated for 13 years with lomitapide (on a compassionate basis) for recurrent pancreatitis due to familial chylomicronemia syndrome. Her serum triglycerides decreased from 3000 mg/dL to 903 mg/dL, and pancreatitis episodes ceased. Of

concern, transaminases increased after 6 years of treatment and by the 13th year, liver biopsy showed cirrhosis. This case suggests that long-term therapy with lomitapide may potentially induce cirrhosis.

PCSK9 Inhibitors Several of the PCSK9 monoclonal antibodies have been assigned names: alirocumab (SAR236553/REGN727), evolocumab (AMG145), and bococizumab (RN316). Recent phase 2 and 3 studies of PCSK9 inhibitors have shown efficacy in patients with heterozygous FH [77, 78], statin intolerance [79], and hypercholesterolemia on statin therapy [80–82]. In all of these studies, LDL-C decreased by 40-70% without any reports of new major adverse events. In approximately 50 homozygous FH patients, the Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor Abnormalities (TESLA) [83] and the TESLA Part B [84] studies reported 31% reduction in LDL-C with evolocumab 420 mg every 4 weeks. However, it failed to reduce LDL-C in the few LDL receptor negative patients included in the studies.

In an exploratory extension study, evolocumab therapy, given to 2976 patients, reduced the rate of cardiovascular events when compared with standard therapy given to 1489 patients (hazard ratio [HR] 0.47; 95% CI, 0.28 to 0.78; p = 0.003) over a median follow up for 11.1 months [85]. Overall frequency of serious adverse events was similar in the two groups, and no neutralizing antibodies against evolocumab were detected. However, neurocognitive events were more frequently observed in evolocumab-treated patients (0.9%) compared to those on standard therapy (0.3%). Other side effects of evolocumab included arthralgia, limb pain, headache and fatigue.

In a post-hoc analysis of alirocumab-treated (n = 1553) versus placebo-treated (n = 788) patients at high risk of CHD, alirocumab reduced the risk of major adverse cardiovascular events (HR 0.52; 95% CI, 0.31 to 0.90; p = 0.02) over a median period of 78 weeks [86]. More patients on alirocumab experienced myalgia compared to placebo treated patients (5.4% vs 2.9%, p = 0.006). Interestingly, 37% of alirocumab treated patients achieved an LDL-C less than 25 mg/dL; their rate of adverse events resembled the overall alirocumab group. Although not statistically significant, more alirocumab-treated patients had neurocognitive disorders (1.2% vs 0.5%, p = 0.17) and ophthalmologic events (2.9% vs 1.9%, p = 0.65).

Drugs that affect LPL Activity A recent publication describes an APOC3 antisense inhibitor, ISIS 304801, administered (open-label) to 3 patients with familial chylomicronemia syndrome due to homozygous or compound heterozygous *LPL* mutations (baseline serum triglycerides 1406, 2083, and 2043 mg/dL) [87]. Patients were administered the study drug once weekly for 13 weeks and triglyceride levels dropped by 56-86% (617, 288, and 735 mg/dL, respectively). One patient developed pancreatitis during the study period; the study investigators felt it was not related to the study drug. Otherwise, one patient reported abdominal discomfort, diarrhea, flatulence, frequent bowel movements, headache, and hypoesthesia – all of which were mild to moderate in severity.

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