Abhimanyu Garg *Editor*

Dyslipidemias

Pathophysiology, Evaluation and Management

Humana Press

Contemporary Endocrinology

Series Editor:
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Abhimanyu Garg
Editor

Dyslipidemias

Pathophysiology, Evaluation and Management

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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

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

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
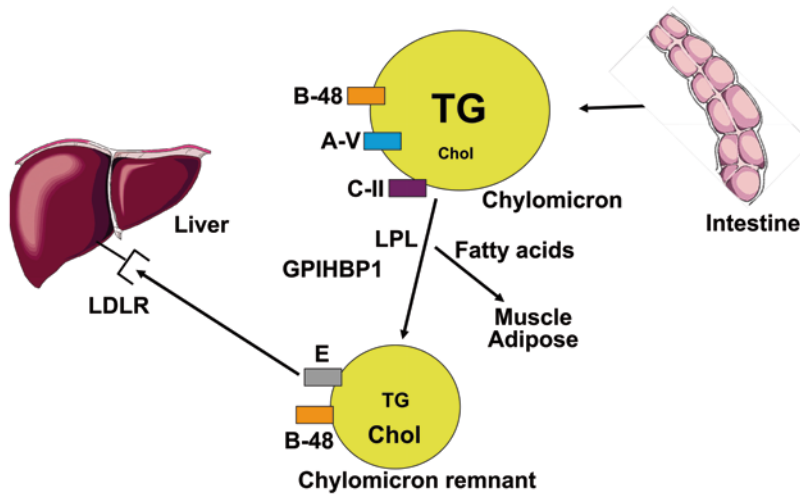
Fig. 1.1 Chylomicron metabolism. *A-V* apolipoprotein A-V, *B-48* apolipoprotein B-48, *C-II* apolipoprotein C-II, *Chol* cholesterol, *E* apolipoprotein E, *LDLR* LDL receptor, *LPL* lipoprotein lipase; *TG* triglyceride, *GPIHBP1* glycosylphosphatidylinositol-anchored high-density lipoprotein-binding 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
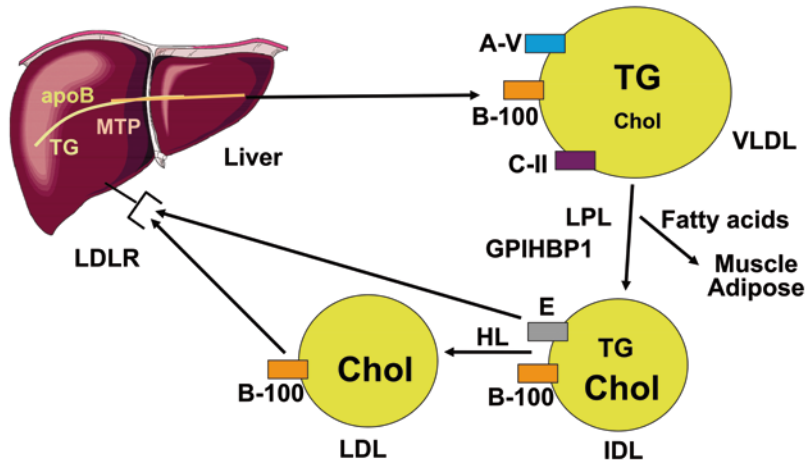
Fig. 1.2 Very low density lipoprotein (VLDL), interme-diate-density lipoprotein (IDL), and LDL metabolism. *apoB* apolipoprotein B, *B-100* apolipoprotein B-100, *HL* hepatic lipase, *MTP* microsomal triglyceride (TG) trans-fer protein. *LDL* low-density lipoprotein, *A-V* apolipo-protein A-V, *C-II* 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 dur-ing 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-lipo-lytic pathways are activated that lead to the hy-drolysis 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 uti-lized by cardiac and skeletal muscle). VLDLs are also synthesized by the liver during the fed state. In particular, excess dietary carbohydrate is con-verted 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 es-terified 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 diac-ylglycerol to form TGs [3]. In addition, synthesis of CE appears to be important for VLDL assem-bly 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 ma-ture VLDL particles. The key roles of apoB-100 and MTP in VLDL assembly and secretion are demonstrated by the existence of inherited con-ditions 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 im-portant determinant of hepatic TG synthesis and VLDL production. Insulin is an important posi-tive regulator of hepatic TG synthesis and VLDL production. The inherited condition familial com-bined hyperlipidemia (FCHL) is characterized by VLDL overproduction, although the molecular mechanisms remain poorly understood [21].

VLDL are secreted into the blood and trans-ported 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 chylo-micron TG. The particle generated after lipolysis of VLDL is known as a VLDL remnant or IDL, which also acquires apoE from HDL. In a simi-lar 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
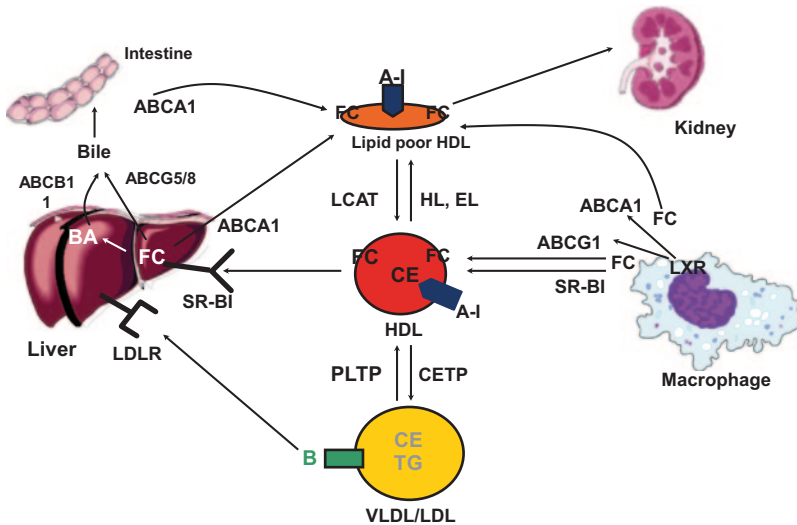
Fig. 1.3 HDL metabolism. *ABCA1* ATP-binding cassette transporter A1, *ABCB11* ATP-binding cassette transporter B11, *ABCG1* ATP-binding cassette transporter G1, *ABCG5/8* ATP-binding cassette transporter G5 and G8, *A-I* apolipoprotein A-I, *CE* cholesterol ester, *CETP* Cholesteryl ester transfer protein, *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 macro-

phage 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.

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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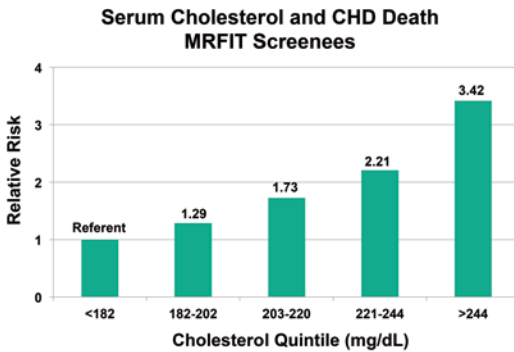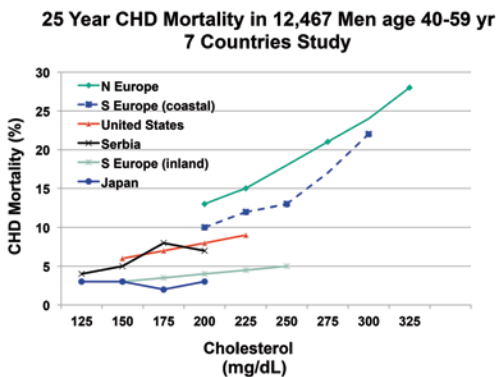e-mail: pwwilso@emory.edu

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])
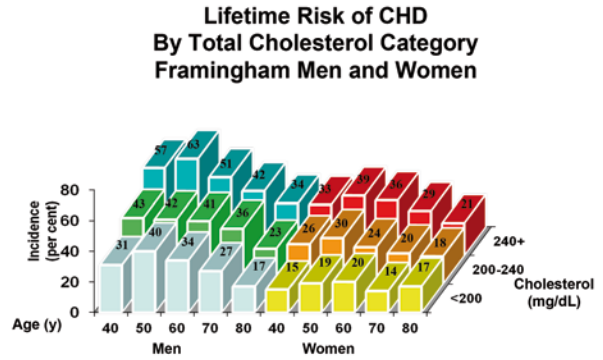

Fig. 2.4 Twelve-year risk of myocardial infarction shown for Framingham men according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol
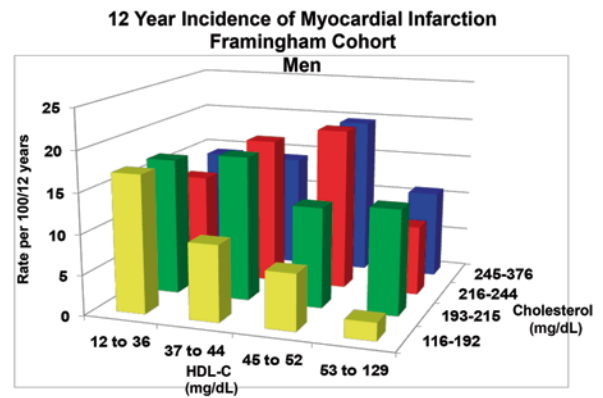
Fig. 2.5 Twelve-year risk of myocardial infarction shown for Framingham women according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol
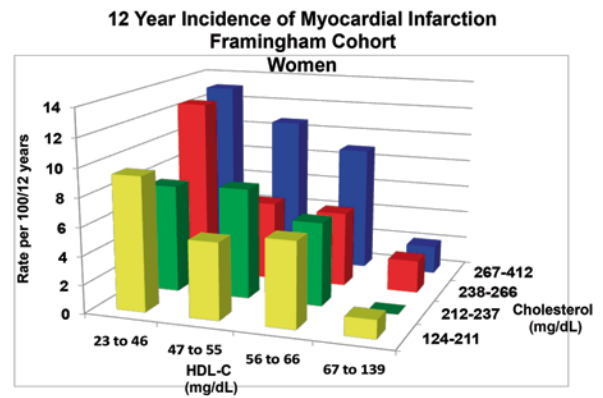
Table 2.1 Determinants of LDL-cholesterol

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