A
n inverse relationship between the plasma concentration of high-density lipoprotein (HDL) cholesterol and the risk of having a cardiovascular event was first reported in the mid 1970s.1 During the ensuing 4 decades, this relationship has been investigated in a plethora of human, animal, and basic scientific studies. The outcomes of these studies have led to the development of new therapies that increase HDL levels and have the potential to reduce the morbidity and mortality associated with atherosclerotic cardiovascular disease. However, the situation has turned out to be much more complex than originally envisaged. This is partly because the HDL fraction consists of multiple subpopulations of particles that vary in terms of shape, size, composition, and surface charge, as well as in their potential cardioprotective properties. This heterogeneity is a consequence of the continual remodeling and interconversion of HDL subpopulations by multiple plasma factors. Evidence that the remodeling of HDLs may impact on their cardioprotective properties is beginning to emerge. This serves to highlight the importance of understanding not only how the remodeling and interconversion of HDL subpopulations is regulated but also how these processes are affected by agents that increase HDL levels. This review provides an overview of what is currently understood about HDL metabolism and how the subpopulation distribution of these lipoproteins is regulated. (Circ Res. 2014;114:143-156.)

Key Words: lipoproteins ■ high-density lipoprotein

Abstract: There is compelling evidence from human population studies that plasma levels of high-density lipoprotein (HDL) cholesterol correlate inversely with cardiovascular risk. Identification of this relationship has stimulated research designed to understand how HDL metabolism is regulated. The ultimate goal of these studies has been to develop HDL-raising therapies that have the potential to decrease the morbidity and mortality associated with atherosclerotic cardiovascular disease. However, the situation has turned out to be much more complex than originally envisaged. This is partly because the HDL fraction consists of multiple subpopulations of particles that vary in terms of shape, size, composition, and surface charge, as well as in their potential cardioprotective properties. This heterogeneity is a consequence of the continual remodeling and interconversion of HDL subpopulations by multiple plasma factors. Evidence that the remodeling of HDLs may impact on their cardioprotective properties is beginning to emerge. This serves to highlight the importance of understanding not only how the remodeling and interconversion of HDL subpopulations is regulated but also how these processes are affected by agents that increase HDL levels. This review provides an overview of what is currently understood about HDL metabolism and how the subpopulation distribution of these lipoproteins is regulated. (Circ Res. 2014;114:143-156.)

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between this circumstantial evidence and the absence of positive results in human clinical outcome trials highlights the importance of performing additional research to advance our understanding of the complex inverse relationship between HDLs and cardiovascular disease.

This review is concerned with the regulation of HDL metabolism. It summarizes current understanding of HDL biogenesis and maturation, the processes responsible for generating and regulating the distribution of HDL subpopulations, and how HDL clearance is regulated.

HDL Biogenesis

Biogenesis of Discoidal HDLs

The majority of the HDL particles circulating in human plasma are spherical, although they mostly originate as discoidal particles that are either generated in the liver before secretion into the extracellular space or assembled in the circulation from individual lipid and apolipoprotein constituents (Figure 1). Discoidal HDLs consist of a phospholipid bilayer surrounded by ≥2 apolipoprotein molecules. These particles acquire unesterified cholesterol from cell membranes and other plasma lipoproteins. The unesterified cholesterol in discoidal HDL particles partitions between the phospholipid acyl chains.

ABCA1 exports phospholipids from cell membranes to lipid-free/lipid-poor apoA-I in the extracellular space, forming a discoidal phospholipid/apoA-I complex that further accepts cholesterol from cell membranes in a process that is also dependent on ABCA1 (Figure 1B). These are the initiating events in the process of HDL biogenesis. Recent reports have indicated that the ABCA1-dependent export of lipids from adipocytes and the intestine to apoA-I also makes a significant contribution to HDL biogenesis.

ApoA-I is also secreted from the liver into the extracellular space in a lipid-free or lipid-poor form. After secretion from the liver, the C-terminal domain of lipid-free apoA-I interacts with an extracellular loop of ABCA1 in a process that initiates the biogenesis of discoidal HDLs (Figure 1B). Recent reports have indicated that the ABCA1-dependent export of lipids from adipocytes and the intestine to apoA-I also makes a significant contribution to HDL biogenesis. It is not known, however, whether these events involve processes comparable with those reported for hepatic HDL biogenesis.

Regulation of Discoidal HDL Biogenesis by Apolipoproteins

The 4 main apolipoproteins in human HDLs, in order of decreasing abundance, are apolipoprotein A-I (apoA-I), apolipoprotein A-II (apoA-II), apolipoprotein A-IV (apoA-IV), and apolipoprotein E (apoE). ApoA-I is synthesized in the liver and intestine. Hepatic apoA-I is initially generated as a preproprotein that is cleaved intracellularly by a signal peptidase. The resulting propeptide is secreted before cleavage by bone morphogenetic protein-1 in a process that is facilitated by procollagen C-proteinase enhancer-2. The initial lipidation of apoA-I occurs in the endoplasmic reticulum and is independent of the ATP-binding cassette transporter A1 (ABCA1).

Additional lipidation of apoA-I takes place in the golgi and at the plasma membrane in processes that are dependent on a dimeric form of ABCA1. ApoA-I is also secreted from the liver into the extracellular space in a lipid-free or lipid-poor form. After secretion from the liver, the C-terminal domain of lipid-free apoA-I interacts with an extracellular loop of ABCA1 in a process that initiates the biogenesis of discoidal HDLs (Figure 1B). The 4 main apolipoproteins in human HDLs, in order of decreasing abundance, are apolipoprotein A-I (apoA-I), apolipoprotein A-II (apoA-II), apolipoprotein A-IV (apoA-IV), and apolipoprotein E (apoE). ApoA-I is synthesized in the liver and intestine. Hepatic apoA-I is initially generated as a preproprotein that is cleaved intracellularly by a signal peptidase. The resulting propeptide is secreted before cleavage by bone morphogenetic protein-1 in a process that is facilitated by procollagen C-proteinase enhancer-2. The initial lipidation of apoA-I occurs in the endoplasmic reticulum and is independent of the ATP-binding cassette transporter A1 (ABCA1). Recent reports have indicated that the ABCA1-dependent export of lipids from adipocytes and the intestine to apoA-I also makes a significant contribution to HDL biogenesis. It is not known, however, whether these events involve processes comparable with those reported for hepatic HDL biogenesis.

ABCA1 exports phospholipids from cell membranes to lipid-free/lipid-poor apoA-I in the extracellular space, forming a discoidal phospholipid/apoA-I complex that further accepts cholesterol from cell membranes in a process that is also dependent on ABCA1 (Figure 1B). These are the initiating events in the process of HDL biogenesis. Lipid-free/lipid-poor apoA-I can also form discoidal complexes with the phospholipids and cholesterol that dissociate from the surface of triglyceride-rich lipoproteins that are undergoing lipolysis by lipoprotein lipase.
lipase (Figure 1B).25 Because lipid-free/lipid-poor apoA-I is continually being generated during the remodeling of mature HDLs by plasma factors, it follows that the assembly of HDLs in the circulation has the potential to make a significant contribution to the total HDL pool. The importance of these events in HDL biogenesis comes from studies of individuals with loss-of-function mutations in the ABCA1 gene that inhibit the lipidation of apoA-I, causing apoA-I to be cleared rapidly from the circulation via the kidney. This results in low plasma HDL levels.26–28

The discoidal HDL particles that are either secreted from the liver or assembled in the plasma are rapidly converted into mature spherical HDLs by lecithin:cholesterol acyltransferase (LCAT), the enzyme that generates almost all of the cholesterol,31 establishing a concentration gradient whereby additional cholesterol is transferred from very-low-density lipoproteins (VLDLs), low-density lipoprotein (LDLs), and cell membranes to the HDL fraction subsequent to the hydrolysis of VLDL triglycerides by lipoprotein lipase. The lipidation of apoE is also dependent on ABCA1,34,44 with in vivo and in vitro studies indicating that the N-terminal domain of the apolipoprotein is important for this interaction.45,46

**Regulation of Discoidal HDL Biogenesis by ABCA1**

**Mutations in the ABCA1 Gene**

Loss-of-function mutations in the ABCA1 gene, which reduce the export of cellular cholesterol and phospholipids to apolipoproteins, inhibit HDL biogenesis. This is exemplified by the low levels or complete absence of HDLs in people with Tangier disease.26,27 Individuals with a loss-of-function ABCA1 mutation in a single allele have low HDL cholesterol levels, further supporting the role of this transporter as a key regulator of HDL biogenesis.47–50 The capacity of ABCA1 to regulate HDL biogenesis also extends to people with more common polymorphisms in the ABCA1 gene,51 although not all investigators have found this to be the case.52

**Regulation of ABCA1 Gene Transcription**

ABCA1 gene transcription and cholesterol efflux to apoA-I are both markedly enhanced when the nuclear liver X receptor complex is activated by oxysterols and peroxisome proliferator-activated receptor α/γ ligands.53–55 More recently, activation of the VLDL receptor and the apoE receptor 2 have also been shown to increase ABCA1 mRNA levels and protein expression, as well as cholesterol efflux to apoA-I.16 Conversely, macrophage ABCA1 gene expression and the efflux of cholesterol to apoA-I are suppressed by members...
of the oxysterol-binding protein–related protein family.\textsuperscript{57,58} Overexpression of activator protein 2α also suppresses ABCA1 gene transcription, whereas blockade of this protein with the α(1)-adrenoceptor blocker, doxazosin, increases HDL cholesterol levels in mice.\textsuperscript{69} ABCA1 gene transcription as well as cholesterol efflux to apoA-I and the generation of discoidal HDLs are also impaired in people with lysosomal acid lipase deficiency.\textsuperscript{70} Inhibition of extracellular signal-regulated protein kinases 1 and 2 and overexpression of the brefeldin A-inhibited guanine nucleotide-exchange protein-1, however, increase macrophage ABCA1 mRNA levels, ABCA1 protein stability, and cholesterol efflux to apoA-I.\textsuperscript{81,82}

Posttranscriptional Regulation of ABCA1 Protein Levels

Compelling evidence of posttranscriptional regulation of ABCA1 protein levels comes from studies showing that microRNA-33 (miR-33) represses ABCA1 protein levels, decreases cholesterol efflux to apoA-I, and reduces HDL cholesterol levels.\textsuperscript{83}–\textsuperscript{85} Inhibition of miR-33 in mice, by contrast, increases cholesterol efflux and HDL cholesterol levels.\textsuperscript{86}–\textsuperscript{88} Inhibition of miR-33 has also been shown to increase plasma HDL levels and to decrease plasma triglyceride levels in nonhuman primates.\textsuperscript{89} ABCA1 expression in macrophages and neuronal cells is also regulated by several other microRNAs, including miR-758,\textsuperscript{90} miR-106b,\textsuperscript{91} miR-144,\textsuperscript{92} and miR-26.\textsuperscript{93} Another aspect of the posttranscriptional regulation of ABCA1 comes from a recent report showing that the ATP-binding cassette transporter, ABCA12, increases ABCA1 stability and enhances macrophage cholesterol efflux in a liver X receptor-β–dependent manner.\textsuperscript{94}

Intracellular Trafficking of ABCA1

Factors that facilitate the trafficking of ABCA1 to the cell surface, or that regulate ABCA1 degradation, can further affect discoidal HDL biogenesis. The first evidence of intramolecular regulation of ABCA1 protein levels came from the discovery of a polypeptide sequence enriched in proline, glutamic acid, serine, and threonine sequence in the cytoplasmic domain of ABCA1 that mediates calpain protease–dependent degradation of the transporter. It is also noteworthy that the binding of apoA-I to ABCA1 inhibits this degradation.\textsuperscript{72,73}

The ability of ABCA1 to efflux cholesterol to apoA-I and generate discoidal HDLs is also impaired in people with lysosomal acid lipase deficiency.\textsuperscript{70} This effect has been attributed to reduced release of cholesterol from late endosomes/lysosomes.\textsuperscript{60}

Localization of ABCA1 in the plasma membrane and the efflux of cellular phospholipids and cholesterol to apoA-I in the extracellular space can also be enhanced by the palmitoylation of ABCA1 cysteine residues\textsuperscript{74} as well as by increased expression of the GTPase, Rab8.\textsuperscript{75} Inhibition of cathepsin D, by contrast, is associated with retention of ABCA1 in lysosomes and reduced efflux of cholesterol from both macrophages and hepatocytes.\textsuperscript{76,77}

Biogenesis of Spherical HDLs

As outlined, ABCA1 plays a critical role in the biogenesis of discoidal HDLs. However, this represents only the first step in the formation of mature, spherical HDLs. Most of the HDLs in human plasma are spherical particles that contain a core of neutral lipids (cholesteryl esters and a small amount of triglyceride) surrounded by a surface monolayer that consists of phospholipids, apolipoproteins, and limited amount of unesterified cholesterol.

The biogenesis of spherical HDLs is driven by the LCAT-mediated hydrolysis of phospholipid sn-2 acyl ester bonds in discoidal HDLs. This produces fatty acyl groups that are transferred by LCAT to the 3-hydroxyl group of cholesterol in a reaction that generates cholesteryl esters and lysophosphatidylcholine (Figure 2). Because cholesteryl esters are extremely hydrophobic, they partition into the center of the discoidal HDLs, which are thereby converted into spherical particles. The lysophosphatidylcholine that is generated by the LCAT reaction associates with albumin (Figure 2). The LCAT reaction depletes discoidal HDLs of unesterified cholesterol. This establishes a concentration gradient whereby additional cholesterol is transferred from other lipoproteins and cell membranes to the HDL surface, resulting in continuing cholesterol esterification and the ongoing generation of HDL cholesteryl esters (Figure 2).

The pivotal role of LCAT in spherical HDL biogenesis and its impact on HDL cholesterol levels has been studied extensively in both animals and humans. Several investigators have reported a significant, positive correlation between LCAT gene expression and plasma HDL cholesterol levels in both mice and rabbits with transgenic overexpression of human LCAT.\textsuperscript{78–80} Deficiency of LCAT in humans, by contrast, is associated with low levels of HDL cholesterol.\textsuperscript{81}

Regulation of the Biogenesis of Spherical HDLs by Apolipoproteins

Studies of human plasma have established that the acyl ester hydrolase and cholesterol transesterification activities of LCAT are regulated by apolipoprotein cofactors. ApoA-I is the major activator of LCAT,\textsuperscript{82} with the N-terminal domain of the apolipoprotein being responsible for this effect in vitro and in vivo.\textsuperscript{83} In vitro studies of discoidal reconstituted HDLs (rHDLs) consisting of apoA-IV and apoE complexed with phosphatidylcholine have established that these apolipoproteins also activate LCAT, but with much lower catalytic efficiencies than apoA-I.\textsuperscript{84–86} However, because discoidal HDLs that contain apoA-IV and apoE have not been reported in normal human plasma, it follows that the LCAT activity in the plasma of these individuals is sufficient to convert all of the circulating apoE-containing and apoA-IV-containing discoidal HDLs into spherical particles, although these apolipoproteins activate LCAT less effectively than apoA-I.

The evidence regarding whether LCAT generates spherical apoE–containing HDLs that also contain apoA-I is conflicting. HDLs isolated from normal human plasma by immunoaffinity chromatography have been reported to contain both apolipoproteins.\textsuperscript{87} Studies of HDL subpopulation distribution by 2-dimensional gel electrophoresis and the isolation of plasma HDLs by gel filtration chromatography and isotachophoresis, by contrast, have found that most of the apoE-containing spherical HDLs in normal human plasma are deficient in apoA-I.\textsuperscript{88–90} A report showing that HDLs that contain apo-IV are also deficient in apoA-I is consistent with LCAT interacting specifically with apoA-IV–containing discoidal HDLs.\textsuperscript{90}

The HDLs in normal human plasma have been classified on the basis of their apolipoprotein content into those
that contain apoA-I, but not apoA-II, and those that contain apoA-I as well as apoA-II.91 People with low plasma HDL levels are likely to have more HDLs that contain apoA-I and apoA-II than HDLs that contain apoA-I without apoA-II.91 Naturally occurring variants of apoA-I can also influence the relative levels of apoA-I–containing and apoA-II–containing rHDLs that have established that spherical HDLs containing apoA-I as well as apoA-II are most likely produced by the LCAT-mediated fusion of apoA-II–containing discoidal HDLs with apoA-I containing small spherical HDLs.96

Further insights into the regulation of spherical HDL biogenesis by apolipoproteins have come from studies of people with CETP deficiency who have elevated plasma levels of large apoE-enriched HDL particles that also contain apoC-I, apoC-II, and apoC-III,88 and from studies of people with coronary heart disease who, relative to control subjects, have low HDL cholesterol levels, reduced levels of large HDL particles that contain apoA-I but not apoA-II, and high levels of HDLs that contain apoA-I as well as apoA-II.95 Pharmacological inhibition of CETP has also been reported to increase plasma levels of HDLs that contain apoA-I and apoA-II.96,97

Regulation of the Biogenesis of Spherical HDLs by Phospholipids

The phospholipid composition of discoidal HDLs regulates their ability to act as substrates for the LCAT reaction. For example, the presence of sphingomyelin in discoidal HDLs inhibits cholesterol esterification by LCAT.96–100 This is most likely a consequence of a strong interaction between unesterified cholesterol and sphingomyelin in the discoidal HDLs, which decreases the amount of cholesterol available for esterification by LCAT.

The length and unsaturation of the phospholipid acyl chains in discoidal HDLs also impact on the kinetics of the LCAT reaction, with mixed chain phospholipids that contain palmitic acid in the sn-1 position and a long unsaturated fatty acid in the sn-2 position being the most reactive substrates. These phospholipids presumably alter the conformation of apolipoproteins in ways that enhance access to the active site of LCAT.101 Phospholipids can also exert interfacial effects that influence the reactivity of discoidal HDLs with LCAT.101

Regulation of the Biogenesis of Spherical HDLs by Discoidal HDL Size

The size of discoidal HDLs is one of the main determinants of LCAT activation. This aspect of the LCAT reaction was elucidated in studies of discoidal rHDLs that contain apoA-I. The results of these studies indicated that intermediate-size discs are more effective LCAT substrates than either larger particles with a high phosphatidylcholine/apolipoprotein molar ratio or smaller discoidal rHDLs with a low phosphatidylcholine/apolipoprotein molar ratio.102,103

Regulation of Spherical HDL Biogenesis in Disease States

The conversion of discoidal HDLs to spherical HDLs by LCAT may be impaired in pathophysiological conditions such as diabetes mellitus, rheumatoid arthritis, and chronic kidney disease. Although these effects cannot be attributed to a common underlying mechanism, individuals with these disorders often have low plasma HDL cholesterol levels and are at increased risk for development of cardiovascular disease.

In the case of people with poorly controlled diabetes mellitus and chronically elevated plasma glucose levels, the formation of reactive α-oxoaldehydes that nonenzymatically glycate HDL apolipoproteins is particularly detrimental. The fact that nonenzymatic glycation of apoA-I by glucose and α-oxoaldehydes reduces LCAT activation was initially reported in the mid 1990s104 and was confirmed more recently by Nobecourt et al.105

The rate of LCAT-mediated cholesterol esterification in plasma is also decreased in people with rheumatoid arthritis.106 This has been attributed to elevated levels of haptoglobin in these individuals that bind to apoA-I and reduce its availability for the LCAT reaction.107 LCAT activity is also decreased in chronic renal disease,108 possibly as a consequence of reduced transcription of the LCAT gene in the liver.109

Regulation of HDL Subpopulation Distribution

The HDL fraction in human plasma consists of multiple subpopulations of particles that are continually being remodeled and interconverted by plasma factors. It has long been suspected that individual HDL subpopulations vary in terms of their cardioprotective properties, although progress in this area has been problematic because strategies for isolating and assessing the functions of individual subpopulations of HDL particles are limited.

The HDLs circulating in human plasma have been classified on the basis of hydrated density into 2 main subfractions: HDL2 and HDL3. HDLs that contain apoA-I, but not apoA-II, are mainly found in the HDL2 fraction, whereas HDLs that contain apoA-I as well as apoA-II are generally associated with smaller and denser particles in the HDL3 subfraction. An association of HDL2 and HDL3 levels with a decreased risk of myocardial infarction has been reported in the Physicians’ Study.110 Other investigators have, by contrast, reported that the inverse relationship between the HDL2 subfraction and the development of ischemic heart disease in participants in the Quebec Cardiovascular Study is greater than that of HDL3.111

Nondenaturing gradient gel electrophoresis is frequently used to separate HDLs on the basis of size into 5 distinct subpopulations of particles. In order of decreasing size, these are HDL2(a), HDL2(b), HDL3(a), HDL3(b), and HDL3(c).112 HDLs can also be resolved on the basis of surface charge into particles that migrate to a pre-β-position, α-position, or γ-position during agarose gel electrophoresis. Lipid-free apoA-I, lipid-poor apoA-I, and most discoidal HDLs migrate to a pre-β-position, whereas spherical HDLs exhibit α-migration.113 Spherical apoE-containing HDLs migrate to a γ-position.114
The subpopulation distribution of HDLs is extensively regulated by multiple plasma factors, including 2 members of the bactericidal permeability-increasing protein and lipopolysaccharide-binding protein family: CETP and phospholipid transfer protein (PLTP), 2 members of the triglyceride lipase gene family, hepatic lipase (HL) and endothelial lipase (EL), and the group IIA secretory phospholipase A2 (sPLA2).

**Cholesteryl Ester Transfer Protein**

Activity of CETP decreases plasma HDL cholesterol levels and reduces HDL particle size. Mice transgenic for human CETP have low plasma HDL cholesterol levels and small HDL particles relative to nontransgenic littermate controls. Polymorphisms in the CETP gene also regulate human plasma HDL levels, with the absence of a Taq1 B polymorphism being associated with increased HDL2 and HDL3 levels. Moreover, people with CETP deficiency have large HDLs and elevated plasma HDL cholesterol levels.

The underlying reasons for these changes relate to the ability of CETP to promote bidirectional transfers (and thus the equilibration) of both cholesteryl esters and triglycerides between the particles in all lipoprotein fractions. Because most of the cholesteryl esters in plasma originate in the HDL fraction as a consequence of the LCAT reaction, whereas most of the triglycerides enter the circulation as a component of VLDLs and chylomicrons, the overall effect of this equilibration is a net mass transfer of cholesteryl esters from HDLs to particles in other lipoprotein fractions and of triglycerides from chylomicrons and VLDLs into the LDL and HDL fractions.

The transfer of cholesteryl esters out of HDLs and the transfer of triglyceride from VLDLs into HDLs generate HDLs that are enriched in triglycerides. Because a triglyceride molecule is significantly larger than a cholesteryl ester molecule, the substitution of cholesteryl esters with triglycerides increases HDL size. Under circumstances in which plasma VLDL levels are elevated, the net mass transfer of cholesteryl esters from HDLs to other lipoprotein fractions and of triglycerides from chylomicrons and VLDLs into the LDL and HDL fractions.

**Effects of Apolipoproteins on the Remodeling of HDLs by CETP**

The rate of CETP-mediated transfers of core lipids between HDLs and triglyceride-rich lipoproteins is independent of HDL apolipoprotein composition, with comparable rates of transfer of core lipids between HDLs and VLDLs being reported in studies of spherical rHDLs containing either apoA-I or apoA-II as the sole apolipoprotein. Similar conclusions have been drawn from studies of mice transgenic for human apoA-II and CETP.

Although apolipoproteins do not influence the rate at which CETP transfers core lipids between donor and acceptor particles in the short-term, they have a marked influence on the CETP-mediated remodeling of HDLs. For example, the incorporation of apoA-II-containing into apoA-I-containing particles markedly inhibits the ability of CETP to remodel HDLs into large and small particles in vitro and in vivo. ApoA-II also inhibits the dissociation of lipid-free/lipid-poor apoA-I from rHDLs by forming salt bridges with apoA-I on the HDL surface.

**Effects of Phospholipids on the Remodeling of HDLs by CETP**

The CETP-mediated remodeling of HDLs into large and small particles is further influenced by phospholipid composition. HDLs that contain phospholipids with long, polyunsaturated sn-2 phospholipid acyl chains are remodeled more extensively by CETP than those with shorter, more saturated sn-2 acyl chains. This is most likely because of the long, polyunsaturated phospholipid acyl chains destabilizing the particle structure and excluding apoA-I from the surface.

**Phospholipid Transfer Protein**

In vitro studies have established that PLTP transfers phospholipids between HDLs and VLDLs, as well as between particles within the HDL fraction. PLTP also remodels HDLs into large and small particles by processes that are accompanied by the dissociation of lipid-free or lipid-poor apoA-I. Increased generation of lipid-free/lipid-poor apoA-I has also been observed in mice transgenic for human PLTP and apoA-I. Transgenic expression of PLTP in these animals modestly increased HDL levels but did not change in HDL size. The minimal effect on HDL levels and size was possibly because of the low level of PLTP expression in the animals because HDL size was increased to a greater extent in a later study of advenolar-mediated overexpression of PLTP in mice that were also transgenic for human apoA-I. The PLTP-mediated dissociation of apoA-I from HDLs has been reported to promote HDL biogenesis by enhancing cholesterol and phospholipid efflux from cells that express ABCA1. There is also evidence that PLTP is unable to remodel HDLs when its phospholipid transfer activity is inhibited, indicating that there is a high interdependence between the lipid transfer and HDL remodeling functions of PLTP.

Studies with spherical apoA-I-containing rHDLs have elucidated the mechanism by which PLTP remodels HDLs into large and small particles. The process involves an initial particle fusion, with subsequent rearrangement of the unstable fusion product via 2 distinct pathways (Figure 3). The first pathway (pathway 1) generates small HDL particles without
The dissociation of lipid-free or lipid-poor apoA-I. The second pathway (pathway 2) involves the dissociation of lipid-free/lipid-poor apoA-I from the fusion product and the formation of large spherical HDLs. A nested case–control study from the European Prospective Investigation of Cancer (EPIC)-Norfolk cohort has indicated that the overall effect of these events in vivo is an increase in the number of large HDL particles and a reduction in the number of small HDL particles.

PLTP also remodels spherical apoE-containing rHDLs into large and small particles by a mechanism that involves sequential particle fusions and rearrangements of the fusion products in processes that do not involve the dissociation of apoE (Figure 4).

Effects of Apolipoprotein and Lipid Composition on the Remodeling of HDLs by PLTP

The remodeling of HDLs by PLTP is regulated by both the apolipoprotein and the core lipid composition of the particles. The incorporation of apoA-II into apoA-I-containing HDL particles isolated from human plasma inhibits both the PLTP-mediated remodeling of HDLs into large and small particles and the dissociation of apoA-I. The PLTP-mediated remodeling of HDLs is, by contrast, enhanced in HDLs that are enriched with triglycerides. This effect is most likely a consequence of the triglycerides decreasing HDL particle stability. The ability of phospholipids to regulate the PLTP-mediated remodeling of HDLs is less clear, with the PLTP-mediated transfer of fluorescently labeled phospholipids from HDLs to phospholipid vesicles being independent of HDL phospholipid composition. It is not known whether this is also the case in more physiologically relevant systems.

Hepatic Lipase

HL preferentially hydrolyses HDL triglycerides, although it also has significant phospholipase activity. HDL particles that become triglyceride-enriched after interaction with CETP...
and VLDLs are excellent substrates for HL. The hydrolysis of HDL triglycerides by HL generates small core lipid-depleted particles and promotes the dissociation of lipid-free/lipid-poor apoA-I (Figure 5).139,140 This is in agreement with the results of human genetic studies showing that polymorphisms in the HL gene that decrease its activity are associated with increased HDL$_2$ levels,141,142 and results of animal studies in which transgenic overexpression of human HL in rabbits and mice decreases HDL levels.143,144

Effects of Apolipoproteins and Phospholipids on the Remodeling of HDLs by HL

The apolipoprotein and phospholipid composition of HDLs both impact on reactivity with HL. For example, the presence of apoA-II in HDLs inhibits the HL-mediated hydrolysis of both phospholipids and triglycerides in vitro145 and in vivo.121 On the contrary, apoA-I, irrespective of whether it is present in HDLs that also contain apoA-II, enhances the HL-mediated hydrolysis of HDL phospholipids and triglycerides.146 Moreover, the rate at which HL hydrolyses phospholipids and triglycerides in rHDLs is significantly greater in particles that contain apoE compared with particles that contain apoA-I.147 This is consistent with what has been reported for the HL-mediated hydrolysis of phospholipids and triglycerides in apoE-containing HDLs in human plasma.148 HDL phospholipid composition can also impact on both the phospholipase and triglyceride lipase activities of HL, with the enzyme having a clear preference for short saturated acyl chains.149

Endothelial Lipase

Although they belong to the same gene family, EL has a strikingly different substrate specificity from that of HL. Although HL preferentially hydrolyses HDL triglycerides, EL has a preference for HDL phospholipids and low triglyceride lipase activity.150,151 EL also differs from HL in its ability to remodel HDLs. The phospholipase activity of EL modestly decreases rHDL size in vitro in a process that is not accompanied by the dissociation of lipid-free or lipid-poor apoA-I.152 This reflects the inability of EL to hydrolyse triglycerides and reduce the core lipid content of the particles.

Overexpression of EL decreases the plasma concentrations of HDLs and apoA-I in mice,153 most likely as a consequence of increased HDL catabolism.154 HDL cholesterol levels are, by contrast, elevated in mice with complete deficiency of EL, in humans with decreased EL levels,154–156 and in individuals with polymorphisms in the EL gene that are associated with decreased activity.157 Loss-of-function mutations in the human EL gene have also been reported to increase plasma HDL cholesterol levels.158,159

Effects of Apolipoproteins and Phospholipids on the Remodeling of HDLs by EL

In vitro studies have established that the phospholipase activity of EL is regulated by both the apolipoprotein and phospholipid composition of HDLs, with spherical apoA-II–containing rHDLs being poor substrates for EL in vitro.160 This observation was subsequently confirmed in mice transgenic for human apoA-I and human A-II.161 The specificity of EL for HDL phospholipids is also distinct from that of HL, with EL preferentially hydrolyzing phospholipids with long polyunsaturated sn-2 acyl chains as opposed to HL, which has a preference for phospholipids with short saturated acyl chains.162

sPLA$_2$

The group IIA sPLA$_2$ is an acute phase protein that associates with HDLs under inflammatory conditions and hydrolyses HDL phospholipid sn-2 acyl ester bonds. Transgenic overexpression of sPLA$_2$ in mice increases the fractional catabolic rate of HDLs.162 It also reduces HDL cholesterol levels163 and particle size.162 sPLA$_2$ deficiency in mice, by contrast, does not affect plasma HDL levels.164

Regulation of HDL Catabolism

There is compelling evidence to suggest that most HDL components are catabolized individually, and that clearance of intact HDL particles from the circulation is minimal. For example, cholesteryl esters are selectively removed from HDLs when they bind to scavenger receptor B1 (SR-B1). Cholesteryl esters are also selectively removed from HDLs by CETP, which transfers them to other lipoprotein particles, as outlined. The lipid-free and lipid-poor apoA-I that dissociates from HDL particles as a consequence of CETP-mediated core lipid transfers to other lipoproteins may be cleared from the circulation by the endocytic receptors, megalin and cubulin, which are expressed in the kidney.

SR-B1 promotes the selective uptake of cholesteryl esters from HDLs into the liver and steroidogenic tissues.163,164 In vitro studies have indicated that HL facilitates this process by a mechanism that involves the binding of HDLs to the cell surface.165 The importance of SR-B1 in regulating plasma HDL cholesterol levels is evident from reports of mice in which hepatic overexpression of SR-B1 is associated with decreased

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Figure 5. Remodeling of high-density lipoproteins (HDLs) by cholesteryl ester transfer protein (CETP) and hepatic lipase. HDL particles that have become triglyceride-enriched by interacting with CETP and very low-density lipoproteins are substrates for hepatic lipase. Hepatic lipase hydrolyses the triglycerides in triglyceride-enriched HDLs, generating small core lipid–depleted HDL particles from which lipid-free/lipid-poor apolipoprotein A-I dissociates.
levels of HDL cholesterol\textsuperscript{168} and of SR-B1–null mice that have increased HDL cholesterol levels.\textsuperscript{169}

Studies of apoA-I–deficient mice have also established that the selective uptake of HDL cholesteryl esters by SR-B1 remodels HDLs into small particles and increases the rate of particle clearance.\textsuperscript{170} The selective uptake of core lipids from HDLs by SR-B1 and the accompanying reduction in HDL size might be predicted to mediate the dissociation of apoA-I from the particles, although this seems not to be the case.\textsuperscript{171} However, it is possible that the interaction of HDLs with SR-B1 does generate lipid-free or lipid-poor apoA-I that is rapidly reincorporated into preexisting spherical HDL particles in a process mediated by LCAT.\textsuperscript{172}

Studies using adenoviral vectors to induce hepatic expression of CETP in mice have suggested that CETP may also play a role in the selective hepatic uptake of HDL cholesteryl esters.\textsuperscript{173} This process seems to be independent of SR-B1 and has been attributed to the CETP-mediated transfer of HDL cholesteryl esters from HDLs to the hepatocyte surface or to a direct interaction of CETP/HDL complexes with the plasma membrane.\textsuperscript{174} It is not known whether these events also occur in humans.

The results of in vitro studies have indicated that the apolipoprotein composition of HDLs regulate the selective uptake of cholesteryl esters by SR-B1, although the results are conflicting. Although rHDL preparations that contain apoA-I, apoA-II, or the minor HDL apolipoprotein, apoC-III, have all been reported to bind equally well to SR-B1 in vitro,\textsuperscript{175} other investigators have reported that apoA-II increases the affinity of HDLs for SR-B1 but decreases the selective uptake of cholesteryl esters.\textsuperscript{176} It is not known whether this is also the case in vivo. HDLs that contain apoE also seem to have a reduced ability to participate in the selective uptake of cholesteryl esters by SR-B1, which may explain why HDL cholesterol levels are increased in apoE-deficient mice.\textsuperscript{177}

Cubulin, which is expressed in renal proximal tubules, clears lipid-free/lipid-poor apoA-I, and possibly intact HDL particles, from the circulation\textsuperscript{178} by processes that are dependent on megalin.\textsuperscript{179} These events have not been investigated systematically because of the high perinatal mortality of mice with global deficiency of megalin.\textsuperscript{180} A more insightful elucidation of these issues will require the development of tissue-specific knockout animals.

The turnover of apoA-I in mice is increased by overexpression of EL, most likely as a consequence of the formation of small, structurally compromised surface lipid–depleted HDL particles that are rapidly cleared by the kidney and liver.\textsuperscript{181} This observation is consistent with a reported increase in the rate of catabolism of apoA-I and apoA-II in individuals with the metabolic syndrome with HDL particles that tend to be small.\textsuperscript{182} The large HDL particles that accumulate in CETP deficiency are, by contrast, associated with decreased catabolism of both apoA-I and apoA-II.\textsuperscript{183} On the contrary, the fractional catabolic rates of the cholesterol and apolipoprotein components of HDLs are markedly increased in ABCA1-deficient mice\textsuperscript{184} and in humans with loss-of-function mutations in ABCA1. LCAT deficiency is also associated with an increased fractional catabolic rate of apoA-I and apoA-II in humans.\textsuperscript{185,186}

### Future Directions and Conclusions

Understanding of the regulation of HDL metabolism has increased significantly in recent years, although these advances have not, to date, translated into the development of HDL-raising therapies that decrease cardiovascular morbidity and mortality. Given the high economic cost and increasing burden of cardiovascular diseases worldwide, there is a compelling need to identify strategies and develop therapeutic agents that can begin to resolve these issues. This topic is addressed in another of the contributions to these series.

Although it is possible that the inverse relationship between plasma HDL cholesterol levels and cardiovascular disease is an epiphenomenon, this suggestion is not supported by numerous in vitro studies that show that HDLs possess a range of properties with the potential to inhibit the development of atherosclerosis, nor by the many preclinical studies that demonstrate that HDL-raising interventions in animals inhibit the development and, in some cases promote, the regression of atherosclerosis. The findings from clinical outcome trials of HDL-raising therapies that have, to date, failed to demonstrate a decrease in cardiovascular events suggest that we do not understand which HDL functions and which HDL subpopulations should be considered as therapeutic targets. Similarly, little is known about which HDL functions and which HDL subpopulations are altered by HDL-raising therapies that are currently undergoing investigation. There is also a growing awareness that simply measuring HDL cholesterol levels may be less informative than a direct assessment of specific HDL functions. To complicate matters even further, it is also possible that at least some of the known functions of HDL may be specific to particular subsets of HDL particles. It is important that future basic scientific and preclinical studies with animal models use targeted approaches, such as proteomics, lipidomics, and metabolomics, to specifically address these questions. Translation of the findings from such studies to the human situation has the potential to provide novel opportunities to develop next-generation HDL-raising agents that effectively decrease cardiovascular events.

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### References

1. Miller NE, Miller GJ. High-density lipoprotein and atherosclerosis. Lancet. 1975;1:1033


Atherosclerosis locus is not a major determinant of HDL-C levels in a population at high coronary artery disease. Circulation. 2001;103:1198–1205.


Chin et al., 1997;38:1859–1868.


containing HDL subspecies and apolipoprotein A-II metabolism.


Eckardstein A. Heterozygosity for apolipoprotein A-I(R160L)Oslo is associated with low levels of high density lipoprotein cholesterol and HDL subclass Lp(a)-A-II but normal levels of HDL subclass Lp(a)-A-I. J Lipid Res 1997;38:121–131.


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