HDL: The Metabolism, Function, and Therapeutic Importance

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1. Introduction

Epidemiological studies point to an inverse relationship between high-density lipoprotein (HDL) levels and the risk of coronary artery disease (CAD).[1−5] Clinical trials such as the Helsinki Heart Study and the Veterans Administration HDL Intervention Trial (VA-HIT) demonstrated a significant reduction in the risk of CAD with increased HDL levels.[6,7] Further biochemical and pharmacological studies indicated that HDL has a variety of activities that may be responsible for its cardioprotective effects. To characterize these effects, it is important to understand the origin, composition, and metabolism of HDL. HDL is a lipoprotein species in the plasma that carries cholesterol, phospholipids, and apolipoproteins. Its composition and metabolism are complex. While sufficient knowledge on the functions of the key HDL components have been revealed, the exact importance of the particle size, ratio of the various constituents, and roles of the different subspecies in reducing cardiovascular risks remain to be further investigated. The inhibitory effect of HDL on the development and progression of atherosclerosis may contribute to the reduction in cardiovascular risks. There are several proposed mechanisms through which HDL attenuates the formation and progression of atherosclerotic lesions in the artery wall, one of which involves its role in mediating cholesterol efflux in the reverse cholesterol transport pathway. In addition, the anti-oxidation effect and anti-inflammatory properties also contribute to the anti-atherogenic property of HDL.

The increased understanding of the reverse cholesterol transport pathway has provided new opportunities to dissect the mechanisms that control the plasma HDL level, and new approaches to raise HDL have been discovered. Some of these new methods have been applied in the discovery of new pharmaceutical targets and agents for HDL modulation. This paper will review the beneficial effects of HDL revealed in clinical studies. In addition, since the reverse cholesterol transport (RCT) pathway is the main pathway for HDL metabolism, it is reviewed in detail. A number of key molecules involved in the RCT pathway are reviewed separately with special focus. The importance of HDL in reducing CAD risk and the mechanism of its preventive effect on atherosclerosis are also covered. Finally, current pharmaceutical approaches and agents will be discussed

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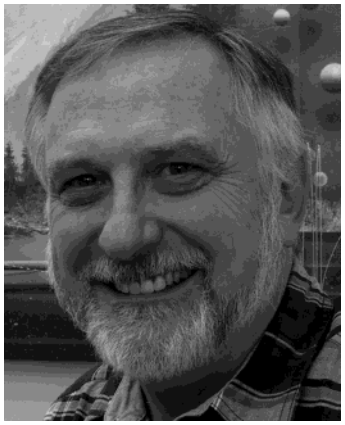

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for modulation of HDL metabolism. The paper covers the data in the literature before the end of 2002.

2. Benefits of HDL Levels: Epidemiological Studies and Clinical Trials

Plasma cholesterol molecules are associated with lipoproteins. There are four major classes of lipoproteins: chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). There are also less commonly measured classes such as lipoprotein (a) and subtypes of the main classes. Generally about 65% of the total plasma cholesterol is carried in LDL and about 25% in HDL particles in man. The level of LDL is directly associated with risks of CAD and has been a primary focus of treatment. The inverse relationship of HDL level with the risk of coronary artery disease has been revealed in epidemiological studies over the last several decades. In the Framingham Study, 2815 men and women between the ages 49 and 82 were monitored for 4 yr for their lipid levels and development of coronary artery disease.[1] The HDL level was found to be a potential risk factor, especially among the subjects of older ages. The association of low HDL with each of the major manifestations of coronary artery disease is significant even when other risk factors are considered. The participants were followed up for 8 more yr after the initial 4-yr study, and low HDL was confirmed as an independent risk factor of CAD.[2] In a separate study with 321 male patients with angiographically documented CAD, low HDL alone was found to be the most frequent dyslipidemia.[3] The inverse relationship between HDL level and the risk of CAD was further supported by both the Prospective Cardiovascular Munster (PROCAM)[4] and the Quebec Cardiovascular Studies,[5] which suggest that HDL cholesterol can be used as a predictor for CAD.

While reducing LDL with statins has successfully reduced coronary events and cardiovascular risks, HDL remains as an independent risk factor for CAD. This notion was evidenced in several statin trials, where increases in HDL levels with statin therapy were associated with mild reductions in coronary events.[8] In the West of Scotland Coronary Prevention Study (WOSCOPS), pravastatin-treated patients were monitored for 5 yr for the incidence of major event rates. Despite the fact that statin treatment lowered the risk of coronary events, patients with a lower baseline HDL still had higher risks than those with a higher baseline HDL, suggesting that statin treatment did not alter the risk of low HDL. The effect of HDL levels on cardiovascular risks was examined in the Helsinki Heart Study, in which 4081 asymptomatic middle-aged men with primary dyslipidemia were treated with gemfibrozil, and major event rates were monitored in a period of 5 yr.[6] It is evident from this study that the increased HDL by gemfibrozil is associated with a 34% decrease in CAD. However,

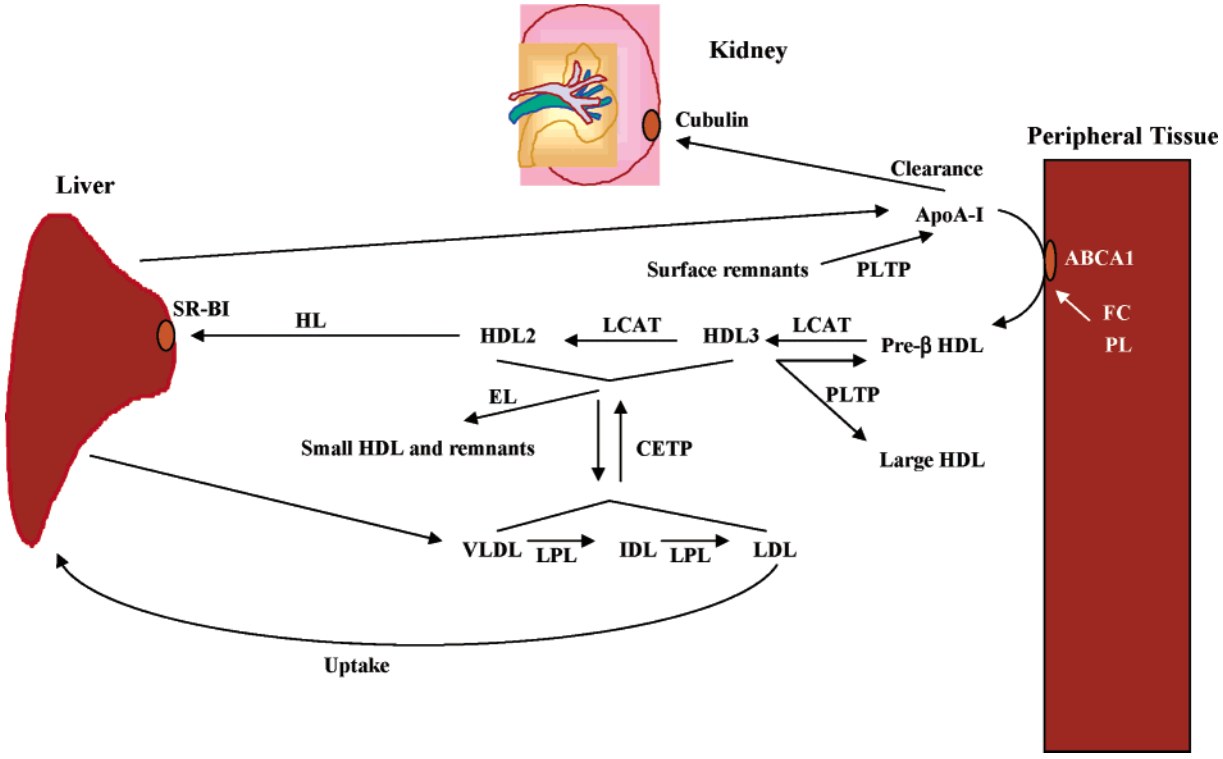

Figure 1. Reverse cholesterol transport pathway with the major proteins involved.

the HDL levels of the patients selected for the trial were not low (average 47 mg/dL), and the LDL levels of these patients were high (average 189 mg/dL).[6,9] Hence, the benefit of HDL elevation is combined with that of LDL lowering in the clinical end points and is not independently addressed. In the VA-HIT trial, patients with low HDL but normal LDL were selected to examine the independent effect of HDL raising on cardiovascular risks.[7] In this study, 2531 patients with an average HDL of 40 mg/dL and LDL of 140 mg/dL were treated with gemfibrozil, and the rates of nonfatal myocardial infarction or death from coronary artery disease were monitored for 5 yr. A 24% reduction in the combined end points (including death from coronary heart disease, nonfatal myocardial infarction, and stroke) was achieved in the treatment group. In light of these clinical trials, possible treatment thresholds have been proposed for different groups of patients characterized by age, symptoms, and other medical conditions.[10] These clinical trials independently demonstrated that HDL level is an independent risk factor of cardiovascular disease and that elevation of HDL has beneficial effects.

3. Reverse Cholesterol Transport (RCT) Pathway

The metabolism of HDL involves sophisticated dynamics in its own pathway as well as balances with other lipoproteins. The RCT pathway regulates the formation, conversion, transformation, and degradation of HDL. A variety of tissues and important molecules play key roles in this pathway. The liver is the primary site for the formation of nascent HDL whereas both the liver itself and the peripheral tissues are involved in further lipidation of the nascent HDL with phospholipids and cholesterol. The hepatic secreted apoA-I becomes associated with phospholipids and forms the discoidal pre-β HDL, the nascent form of HDL in plasma. This form of HDL, also termed lipid-poor apoA-I, is involved in the removal of cholesterol and phospholipids from the peripheral tissues.[11] This process of lipid transfer from the plasma membrane of cells to the small and large HDL particles (namely, cholesterol and phospholipids efflux) is mediated by the membrane transporter ATP-binding cassette transporter 1 (ABCA1 or ABC1), which transfers cholesterol and phospholipids to the lipid-poor apoA-I (Figure 1). The ABCA1 transporter is important in apoA-I lipidation as well as in the entire RCT process because ABCA1 deficiency results in little or no plasma HDL in human or experimental animals.[12] After the efflux, the free cholesterol on the surface of the lipidated apoA-I is esterified by lecithin cholesterol acyl transferase (LCAT), and the particle undergoes morphological change from discoidal disk to spherical shape, with cholesterol ester in the hydrophobic core region and phospholipid and the remaining free cholesterol on the surface. LCAT is a secreted esterase synthesized in the liver and located on the surface of HDL particles. The spherical HDL (namely, HDL_3) continues to become larger HDL_2 as it accepts more free cholesterol from cells that are subsequently esterified by LCAT. This stage of cholesterol transfer from cells to the HDL_3 is mediated by SR-BI or passive diffusion, both distinct from that mediated by ABCA1[13] (Figure 1).

The larger HDL_2 exchanges cholesterol and triglyceride (TG) with LDL/VLDL particles. This process is mediated by cholesterol ester transfer protein (CETP), a cholesterol ester, and TG transfer protein in the plasma. In a second mechanism, HDL_3 can

accept free cholesterol and phospholipids from VLDL hydrolysis mediated by lipoprotein lipase (LPL), a process facilitated by phospholipids transfer protein (PLTP). The link between HDL and LDL/VLDL is characterized by cholesterol ester, phospholipids, and TG exchange mediated by CETP and PLTP (Figure 1). The lipid exchange between these lipoproteins is important in that it moves excess cholesterol from the periphery to metabolic disposal or recycling processes and has a major impact on the composition, size, and level of HDL and thus regenerates functional HDL for additional rounds of RCT.

The degradation of the larger HDL_3 takes place in the liver following the CE-selective uptake mediated by SR-BI.[12] The apoA-I from the degradation is recycled for new HDL formation. The uptake and degradation of HDL involve hepatic lipase that hydrolyzes the TG on HDL particles.[11,12] Another member of the lipase family located on the surface of endothelium, endothelial lipase (EL),[14] is also postulated to regulate the level and function of HDL but mainly by hydrolyzing the phospholipids on HDL. A different pathway for HDL degradation is the renal clearance of apoA-I by cubulin, a receptor highly expressed in kidney and yolk sac.[11]

3.1. ATP-Binding Cassette Transporter 1 (ABCA1) and Cholesterol Efflux

Cholesterol efflux is the process of transfer of cholesterol from a variety of peripheral cells to lipid-poor apolipoproteins or HDL particles. This process can occur through several mechanisms. The transfer of free cholesterol to lipid-poor apoA-I is mediated by ABCA1 while the transfer of cholesterol to HDL particles involves several molecules such as SR-BI and LCAT via mechanisms such as passive diffusion and/or membrane microsolubilization.[13,15] ABCA1 is a transporter protein that mediates the transfer of cholesterol and phospholipid from peripheral cells to lipid-poor apolipoproteins, the initial step in reverse cholesterol transport. ABCA1 is a member of the ATP-binding cassette (ABC) transporter superfamily.[16,17] ABC transporters contain two ATP-binding domains or nucleotide binding folds (NBF) and 12 transmembrane (TM) domains. This family of large transmembrane proteins transport a large variety of molecules such as proteins, ions, and lipids across the plasma membrane.[18]

The substrates of ABCA1 are phospholipids and cholesterol. The ABCA1-mediated transfer of phospholipid and cholesterol from cells to apoA-I follows a two-step mechanism.[19] Fielding et al. discovered that ABCA1-mediated phospholipid efflux precedes free cholesterol efflux to apoA-I and that the complex of apoA-I and phospholipid is a much better acceptor of free cholesterol than apoA-I.[19] In addition, only the free cholesterol transfer (the second step) can be inhibited by okadaic acid and vanadate, suggesting that the transfers of phospholipid and free cholesterol involve different mechanisms.[19] The transfer of lipids from cell membrane to apoA-I requires physical interaction of apoA-I and cell surface.[20] The interaction appears to be dependent on ABCA1 function because ATPase-deficient ABCA1 failed to elicit a specific cell association of apoA-I.[20] The functional ABCA1 appears to induce modifications of the lipid distribution in the membrane which facilitate the docking of apoA-I at the cell surface.[20]

The ABCA1 gene is contained within a 149-kb chromosomal locus on human chromosome 9 and comprises 50 exons encoding a 250-kDa protein.[21−23] ABCA1 is ubiquitously expressed in tissues and is transciptionally regulated by nuclear receptors and cAMP,[24−27] which has become a primary target mechanism to increase ABCA1-mediated cholesterol efflux to apoA-I. Interestingly, the cAMP-dependent induction of ABCA1 expression was primarily found in mouse macrophage cell lines.[24−26] Mutations in ABCA1 gene have been identified in familial hypoalphalipoproteinemia characterized by low plasma levels of HDL cholesterol.[27−29] In more severe cases, ABCA1 defects have been identified in patients with Tangier Disease (TD), an autosomal recessive disorder characterized by extremely low plasma HDL levels.[28,30−32] There is a good correlation between the ABCA1-mediated cholesterol efflux fibroblasts to apoA-I and the HDL levels in patients with ABCA1 mutations.[28,29] Patients with heterozygous nonfunctional ABCA1 alleles have about 50% reduction in HDL cholesterol whereas TD patients, with two nonfunctional ABCA1 alleles, have extremely low HDL.[28,29] In addition to low levels of HDL, TD patients have high plasma levels of TG and may have increased risk of cardiovascular disease, but further evidence is needed to support this notion.[29] Mutations or deletions that severely affect the ability of ABCA1 to mediate phospholipid and cholesterol efflux have been found in regions across the ABCA1 molecule.[30−32] Slight variations of the ABCA1 sequence appear to have impact on HDL cholesterol as well. One polymorphism study has identified at least one ABCA1 sequence variation that is associated with higher plasma HDL cholesterol as compared with subjects with other genotypes.[33]

The importance of ABCA1 in HDL metabolism was further proved in ABCA1 knockout mice. If both alleles of the ABCA1 gene are deleted, the mice have extremely low HDL cholesterol.[31,32] Whereas, if only one allele of the ABCA1 gene is deleted, the mice have about 50% the HDL levels of normal mice.[34−36] These results are consistent with the findings from human TD patients[28,29] and suggest that the level, or potentially activity, of ABCA1 is critical for plasma HDL levels. While a reduction or loss of ABCA1 activity affects cholesterol efflux and the plasma levels of HDL, induction of ABCA1 expression can result in an increased cholesterol efflux and elevated levels of plasma HDL. Three research groups have independently made ABCA1 transgenic mice, in which the human ABCA1 gene was overexpressed. Among these researchers, two groups have shown that overexpression of human ABCA1 in mice resulted in increased cholesterol and phospholipid efflux and elevated HDL levels.[37,38] However, the third group did not observe any change in plasma HDL levels in ABCA1 transgenic mice they produced.[39] One of the key differences in these studies is that the first two groups used mice with partial or

full C57BL/6 background while the third group used FVB background in their study.[37−39] The different results could be due to the different genetic backgrounds that may mask the effect of increased cholesterol efflux on plasma HDL levels. Overall, the transgenic studies demonstrate the feasibility of elevating HDL levels by inducing ABCA1 expression and the ABCA1-mediated cholesterol and phospholipid efflux. The overexpression of ABCA1 in animals has been shown to be anti-atherogenic.[40] In addition, bone marrow transplantation between wildtype and ABCA1-deficient animals demonstrated that the level of ABCA1 in macrophage cells is critical for the development of atherosclerosis.[41] This is consistent with the finding that inactivation of ABCA1 in hyperlipidemic animals increased the progression of atherosclerotic lesions.[42]

Other ABC transporters may also play roles in HDL homeostasis. ABCG1 or ABC8 is the human homologue of the *Drosophila* White gene, a half-sized transporter with 6 transmembrane regions.[43] ABCG1 is induced in monocyte-derived macrophages during cholesterol influx mediated by acetylated low-density lipoprotein. ABCG1 appears to mediate HDL_3-dependent phospholipid and cholesterol efflux from macrophage cells.[44] The involvement of ABCG1 in HDL_3-mediated phospholipid and cholesterol efflux implicates its role in HDL homeostasis. Two other half ABC transporters, ABCG5 and ABCG8, have been implicated in efflux of dietary sterols from intestinal epithelial cells back into the gut lumen and from the liver to the bile duct.[45,46] The genes for ABCG5 and ABCG8 are controlled by a bidirectional promoter and share common regulatory elements,[45] and mutations in both transporters have been identified as the primary cause of sitosterolemia, an autosomal recessive disorder characterized by increased intestinal absorption and decreased biliary excretion of dietary sterols.[45] The stimulation of hepatic cholesterol excretion by LXR is mediated by both ABCG5 and ABCG8.[47]

3.2. Lecithin Cholesterol Acyltransferase (LCAT)

Following the efflux of free cholesterol from cells to discoidal pre-β HDL particles, the free cholesterol on the surface of the small HDL particles are progressively converted to cholesterol ester and stored in the core of HDL. This process converts smaller pre-β HDL to larger spherical HDL_3 and then to much larger HDL_2 particles. During this transition, the morphology of the discoidal pre-β HDL is changed to spherical shape with cholesterol ester in the core and phospholipid on the surface. This is one of the critical steps in the RCT pathway and is mediated by lecithin cholesterol acyltransferase (LCAT).

LCAT is a soluble enzyme that converts cholesterol and lecithins (phosphatidylcholines) to cholesterol esters (CE) on the surface of HDL particles.[48] In addition, it also catalyzes the esterification of other sterols.[49,50] The esterification of cholesterol by LCAT removes cholesterol from the surface of HDL particles and results in the accumulation of cholesterol esters in the core of HDL and change of HDL morphology. This process promotes cholesterol flux from cell surface to the surface of HDL. Therefore, LCAT plays a pivotal role in promoting cholesterol efflux from peripheral cells to HDL after the initial cholesterol efflux mediated by ABCA1 to apoA-I, through which the cholesterol molecules enter the RCT pathway. In addition to producing HDL cholesterol esters, LCAT also catalyzes the esterification of apolipoproteins B (apoB) lipoprotein cholesterol.[51] Most of LCAT protein is synthesized in the liver and circulates in blood. LCAT contains 416 amino acid residues, binds reversibly to lipid surface, and uses lipids as substrates.[52,53] On the basis of the amino acid composition, LCAT appears to be a hydrophobic protein but remains in the plasma and is bound to lipoproteins. Several residues of LCAT are glycosylated, but the role of the carbohydrate chains is not yet well-defined since removal of the glycosylation sites by mutagenesis has yielded equivocal effects on the enzyme activity.[54−56] LCAT shares the Ser/Asp-Glu/His catalytic triad with lipases, esterases, and proteases and therefore belongs to the α/β hydrolase fold family.[57] A "lid" domain, which also exists in lipases, might be involved in enzyme−substrate interaction.[10] Structural and functional studies have revealed additional residues important for the enzyme activity.[58−60] The enzymatic activity of LCAT is activated by apolipoproteins A-I, A-IV, E, and C-I.[61−64] The LCAT activation by HDL is mediated by its binding to HDL. Using recombinant HDL as substrates, Bolin and Jonas found that the lipid composition of HDL is critical for LCAT binding whereas the apolipoprotein composition has no effect on LCAT binding.[65] However, the apolipoproteins in HDL are important for LCAT activation.[66,67] Both binding and activation of LCAT on the surface of HDL are necessary for esterification of free cholesterol and accumulation of cholesterol esters in the central core of HDL. Mapping studies with apoA-I revealed that repeat 6 of apoA-I is important for LCAT activation. When repeat 6 is replaced with repeat 10, LCAT activity is reduced.[66] Genetic deficiencies of LCAT activity have been identified in humans.[68] There are two types of LCAT deficiencies: familial LCAT deficiency (FLD) and Fish-eye disease (FED). FLD patients have little or no plasma LCAT activity. Two classes of LCAT mutations have been identified in these patients with class 1 being null mutations and class 2 being missense mutations. The disorder is associated with HDL deficiency and abnormal VLDL and LDL fractions. These patients have corneal opacification, proteinuria, anemia, and renal disease.[68] FED patients have characteristic classes 3 and 4 LCAT missense mutations that partially impair LCAT activity.[68] They have no clinical manifestations except for the dense age-dependent coneal opacification. Although it is not clear if LCAT deficiency is directly linked to premature CAD, increased risks of atherosclerosis and CAD have been observed in some patients.[68]

The importance of LCAT in HDL metabolism has been evidenced by a study where LCAT was overexpressed in cholesterol-fed rabbits.[69] High levels of human LCAT expression in these rabbits increased HDL and apoA-I due to decreased catabolism of

larger HDL particles, suggesting that the size of HDL may modulate the selective HDL cholesterol ester uptake by the liver.[69] This was further shown in a LCAT transgenic mice study where the liver uptake of HDL was reduced 41% due to a substantial increase in large HDL particles that are atherogenic.[70] This is due to the fact that mice lack CETP and that continued increase of cholesterol ester on HDL by high levels of LCAT changes both the size and the lipid composition of HDL. When CETP was co-expressed in the LCAT transgenic mice, the size and the composition of HDL changed and the animals were protected from atheroslcerosis.[71] These data suggest that, under normal conditions where CETP is present as in humans, increased LCAT activity is likely to increase HDL coupled with reduced risk of atherosclerosis.

3.3. Scavenger Receptor Class B Type I (SR-BI)

Scavenger receptors are cell surface membrane proteins that bind chemically modified lipoproteins such as acetylated LDL (AcLDL) and oxidized LDL (oxLDL).[72] On the basis of sequence homology, scavenger receptor class B type I (SR-BI) was originally identified as a scavenger receptor closely related to CD36,[73] a member of the scavenger proteins.[74] When expressed in transfected cells, SR-BI mediates the binding of AcLDL, oxLDL, and unmodified LDL and VLDL.[75] However, SR-BI binds HDL with high affinity, resulting in the internalization of HDL-derived cholesterol esters without degradation of HDL apolipoproteins,[76,77] which allows the recycling of HDL apolipoproteins such as apoA-I. These data suggest that SR-BI is a HDL receptor that mediates the selective uptake of HDL cholesterol. Further, overexpression of SR-BI in cultured cells resulted in an increase in the HDL-dependent cholesterol efflux rate that correlated with SR-BI expression.[78] This finding suggests a new role of SR-BI in HDL metabolism.

SR-BI is a 509-amino acid and 82-kDa glycoprotein. SR-BI has a horseshoe-like topology with a large extracellular loop anchored to the plasma membrane via the amino and carboxyl terminal transmembrane domains with short intracellular extensions.[73] It is expressed mainly in the adrenal gland, but abundant levels are found in liver and testis as well as in monocytes.[77] In addition, SR-BI expression is regulated under several physiological conditions. The expression of SR-BI increases upon human monocyte differentiation.[79] SR-BI is expressed in macrophages of human atherosclerotic lesions,[79] which is consistent with a proposed role of SR-BI in mediating HDL-dependent cholesterol efflux in the arterial wall.[78] The expression of SR-BI is induced by PPARα and PPARγ activators in both cultured cells and in animals treated with PPAR activators.[79] An alternatively spliced form of SR-BI, termed SR-BII or SR-BI.2, has a completely different C-terminal cytoplasmic domain. SR-BII mediates both selective HDL-derived cholesterol uptake and HDL-dependent cholesterol efflux, but with lower efficiency,[80] suggesting the importance of the C-terminal cytoplasmic domain in SR-BI function.

Since SR-BI bind both LDL and HDL as well as modified lipoproteins, it is important to distinguish the structural requirements for the binding of these different ligands. Mutagenesis screens have generated evidence that the binding of HDL and LDL by SR-BI are independent of each other,[81] because some SR-BI mutants were capable of mediating LDL-derived cholesterol uptake but lost most of their HDL receptor activity.[81] It is widely believed that the selective uptake of HDL-derived cholesterol mediated by SR-BI is mechanistically distinct from the uptake of LDL cholesterol by LDL receptor, which involves the endocytocis of the intact LDL particle and its subsequent hydrolysis. Following the binding of HDL particles to SR-BI on the cell surface, the cholesterol esters from the hydrophobic core of HDL are transferred to intracellular compartments, but the apolipoproteins are not internalized.[82,83] However, recent evidence supports that SR-BI is an endocytic receptor that facilitates HDL cholesterol uptake and apolipoprotein recycling.[84] The presentation of HDL to SR-BI on the cell surface appears to be facilitated by apoE. In apoE knockout mice, the clearance of HDL cholesterol ester is significantly reduced.[85] The physiological role of SR-BI in the RCT pathway has been revealed in studies with SR-BI knockout or mice with attenuated expression of SR-BI.[86−88] The data demonstrate that SR-BI is a HDL receptor responsible for the selective uptake of HDL cholesterol in liver. When heterozygous and homozygous SR-BI null mutations were introduced into mice, plasma HDL cholesterol levels increased significantly,[86] suggesting a reduction in HDL catabolism. However, the plasma apoA-I level did not change. This is in agreement with the notion that SR-BI does not mediate the degradation of HDL apolipoproteins. In addition, the knockout mice data support the role of SR-BI in biliary cholesterol secretion and maintaining biliary cholesterol levels by mediating hepatic HDL uptake.[87,88] The potential effect of SR-BI on atherosclerotic lesions was revealed in a study where increased lesion size was observed in LDLR knockout mice crossed with SR-BI attenuated mice.[89] These data implicate the importance of SR-BI level in the accumulation of lipids in macrophage foam cells and lesion formation. In contrast, hepatic overexpression of SR-BI in LDLR-deficient mice reduced atherosclerotic lesions.[90,91] This could be due to the decreased levels of apoB-containing particles and accelerated rate of RCT in these animals.[92,93] However, overexpression of SR-BI does not automatically reduce atheroslcerotic lesions. It appears that the expression level of SR-BI is critical to its effect on the lesion size. Low-level overexpression of SR-BI inhibits atherosclerosis while high-level overexpression has little effect on lesion size.[94]

3.4. Endothelial Lipase (EL)

Endothelial lipase (EL) is a member of the TG lipase family, including lipoprotein lipase and hepatic lipase. It shares 45% identity with LPL and 40% identity with HL. This family of lipases also has differential phospholipid lipase activities. EL was isolated by differential display from THP-1 cells exposed to oxidized LDL[95] and also from cultured human umbilical vein endothelial cells (HUVECs)

undergoing tube formation by subtractive hybridization.[96] EL is a 500-amino acid secreted lipase after translation. The secretion involves the cleavage of an 18-amino acid signal peptide, and the remaining 482-amino acid fragment predicts a 55-kDa protein. However the mature EL is 68 kDa, suggesting the existence of post-translational modification. There are five potential glycosylation sites identified in EL. But the role of glycosylation in the enzymatic function is still unclear.[97] Endothelial lipase is anchored to the surface of endothelium via attachment to heparan sulfate proteoglycans on the surface. The full-length EL undergoes proteolysis to generate a 40-kDa amino terminal fragment containing the catalytic domain and a 28-kDa carboxyl terminal fragment.[97] However, the 40-kDa amino terminal catalytic domain does not appear to have independent phospholipid lipase activity,[97] and the function of the 28-kDa fragment is unknown. EL is highly expressed in placenta, liver, and lung as well as endothelial cells.

EL was originally thought to be a phospholipase without TG lipase activity as a result of the employment of a LPL assay to assess its activity in the presence of serum.[95,96] However, in a newly developed TG lipase assay without the presence of serum, EL exhibited low but significant TG lipase activity that is sensitive to a yet unidentified serum factor.[14] Unlike LPL, whose TG and phospholipid lipase activities are activated by apoC-II, EL activity is not changed in the presence of apoC-II.[14] The ratio of its phospholipid lipase activity to TG lipase activity is much higher than those of LPL and HL, suggesting that EL might be primarily involved in phospholipid hydrolysis.[14] Comparative studies with EL, LPL, and HL revealed that EL preferentially hydrolyzes HDL while LPL has highest lipase activity against apoB-containing lipoproteins.[14] This is further supported by a study in which EL is overexpressed in animals by an adenovirus delivery system. When expressed in wildtype or human apoA-I transgenic mice, EL significantly reduced HDL and apoA-I levels.[95] In the meantime, VLDL/LDL levels were also reduced but to a lesser extent.[95] These data suggest that EL functions as a HDL phospholipid lipase in vivo and might be important in the regulation of HDL homeostasis.

Although EL is closely related to LPL and HL, its catalytic domain differs from those of the other two enzymes. The lid domain (or the loop domain) in this lipase family covers the catalytic pocket and plays critical roles in substrate interaction and specificity.[98−100] Both LPL and HL have 22-amino acid loops while EL has a loop 3 residues shorter.[97] This distinct feature supports the idea of a unique substrate specificity of EL. In fact, EL has significantly higher phospholipid lipase activity relative to its TG lipase activity as compared with HL and LPL[14] and a distinct loop domain structure. In contrast, LPL is more active against TG than phospholipid, and HL has both intermediate activities. EL is up regulated in endothelial cells in response to the cytokines, IL-1β and TNF-α, as well as in response to shear and cyclic stress, all factors implicated in the pathogenesis of atherosclerosis and plaque instability. Up regulation of EL by these factors implicates a role of this novel lipase in atherogenesis.

3.5. Hepatic Lipase (HL)

Another member of the triglyceride lipase family that is involved in HDL metabolism is hepatic lipase (HL). Hepatic lipase is a 476-amino acid secreted protein produced in the liver.[101,102] HL has a 23-amino acid signal peptide, has four potential glycosylation sites, and shares 30−75% sequence homology with the other lipases.[101] The physical localization of HL was determined by both immunofluorescence and immunoelectron microscopy in either rat liver or transgenic rabbits with hepatic expression of human HL. In both cases, it was found that HL is bound to the surface of hepatocytes and hepatic endothelial cells.[103,104] The cell surface proteoglycans are likely involved in HL binding and internalization, a process involved in lipoprotein catabolism.[105] HL hydrolyzes monoglycerides, diglycerides, triglyceride, phospholipids, and cholesterol esters in vitro. However, the in vivo substrate specificity has not been fully elucidated due to its lack of requirement for an apolipoprotein cofactor. In vitro evidence suggests that HL enhances binding and degradation of VLDL via the LDLR-mediated endocytosis pathway by concentrating the lipoprotein particles on heparan sulfate proteoglycans (HSPG) on the cell surface.[106] In addition, HL facilitates the selective uptake of cholesterol esters from apoB-containing remnant lipoproteins.[107] The role of HL in HDL metabolism was evidenced in a study where both HL and SR-BI were co-expressed in embryonic kidney 293 cells and HDL cholesterol ester uptake was examined.[108] The investigators found that HL enhanced the uptake of HDL cholesterol esters by SR-BI and that the induction requires the interaction of HL with HSPG. The hydrolysis of triglyceride-rich HDL_2 by HL generates pre-β HDL; the resulting phospholipid-poor apoA-I is capable of accepting cholesterol, which then acts as a substrate for LCAT, thus repeating the RCT cycle. Concomitant with the pre-β HDL formation by HL, an α HDL particle of decreased size, termed "remnant HDL_2", has been shown to be generated and rapidly cleared.[109] As a result of the HDL hydrolysis by HL, the remnant HDL exhibits higher binding affinity for the surface of human hepatoblastoma ($HepG_2$) cells, which may trigger internalization and degradation,[110] suggesting an important role of HL in HDL remodeling. And the involvement of HL in this process appears to require cell surface anchorage by interaction with HSPG.

The activity of HL is regulated by apolipoproteins, but the results are contradictory due to the nature of the in vitro assays. The triglyceride lipase activity of HL was inhibited by both apoA-I and apoA-II when a substrate of triolein particles stabilized with gum arabic was used,[111] while in other studies its activity was activated by apoA-II and apoE.[112,113] The discrepancies are likely due to the fact that the effect of the apolipoproteins on HL activity in vitro appears to depend on the surface pressure of lipid monolayers.[114] Under a defined surface pressure of a lipid monolayer in vitro, apolipoproteins A-I, A-II, C-I,

C-II, and C-III all inhibited while apoE activated the triglyceride lipase activity of HL.[114] The data support the hypothesis that, under physiological conditions, the phospholilpids in apoE-rich HDL_1 and triglyceride in intermediate density lipoproteins are likely preferred substrates for HL.[114] This is consistent with both previous and recent findings that HL is involved in the metabolism of both HDL and apoB-containing lipoproteins[106−110] and that HL has intermediate triglyceride and phospholipid lipase activities as compared with LPL and EL.[14] The effect of apoA-II on HL activity was determined in vivo with apoA-II/HL knockout mice.[115] The activation of HL activity and the corresponding elevation of HDL in these animals suggest that AII inhibits HL in vivo and HL is involved in HDL catabolism. On the other hand, the affinity of HL for A-II rHDL is about 10 times higher than that for A-I rHDL, suggesting that HL might prefer interaction with A-I/A-II HDL in plasma to A-I HDL. This is consistent with the fact that A-I/A-II HDL in plasma are depleted of triglyceride while A-I HDL still contains triglyceride.[116] In addition, the composition of HDL appears to be important for HL selectivity. For example, diacylglycerol is the preferred substrate in HDL for HL.[117]

Inactivation of both alleles of the HL gene results in mild dyslipidemia.[118] Total cholesterol levels increased 30% coupled with elevation of plasma phospholipid and HDL but the triglyceride level did not change. Detailed analysis of HDL revealed that HDL_1 increased on a normal chow diet. When the animals were put on a high fat and high cholesterol diet, HDL was doubled. This study implicates the role of HL in HDL catabolism, and specifically, HL may be involved in the catabolism of large HDL particles. The unchanged triglyceride level is unexpected, and it could be due to the compensatory effect of other lipases in the absence of HL. To further support the role of HL in HDL metabolism, HL was overexpressed under the control of an inducible promoter in mice,[119] in which HDL was reduced by 34% coupled with a reduced HDL particle size, consistent with the findings from the knockout mice that HL may prefer the large HDL particles as substrates. This was further supported by data from transgenic rabbits where the level of large HDL, mainly HDL_1 and HDL_2, was reduced.[120] The HDL level was further reduced when apoE was coexpressed in the rabbits.[121] In addition, the intermediate-density lipoprotein (IDL) was also reduced, suggesting that HL is also involved in IDL metabolism. Genetic HL deficiency in human populations is associated with hypertriglyceridemia and elevated HDL.[122] Polymorphisms of HL associated with low HL activity and larger HDL have been identified, confirming the role of HL in the lipolysis of triglyceride-rich large HDL particles.

3.6. Lipoprotein Lipase (LPL)

Lipoprotein lipase is a N-linked glycoprotein synthesized in adipocytes, smooth muscle cells, and cardiac myocytes.[123] It is secreted and transported to and anchored on the luminal surface of the vascular endothelium by binding to heparan sulfate proteoglycans (HSPG).[123,124] Using in vitro assays for triglyceride and phospholipid lipase activities, McCoy et al. determined that LPL is primarily a triglyceride lipase while EL is mainly a phospholipid lipase.[14] The triglyceride lipase activity of LPL is activated by apoC-II. The free fatty acid generated by LPL-mediated lipolysis of triglyceride is an important source of triglyceride synthesis in adipocytes and an energy source in skeletal and myocardiac muscles. More importantly, hydrolysis of chylomicrons and large VLDL particles by LPL releases the surface components (mainly phospholipids, apolipoproteins, and free cholesterol), which are transferred to HDL.[124] The LPL activity is therefore related to HDL levels, and triglyceride level is inversely related to HDL level.

When mice were put on normal chow diet, increased LPL activity had no effect on plasma HDL level in LPL overexpressing mice.[125] But the plasma triglyceride and VLDL triglyceride were reduced. When the same mice were put on high cholesterol diet, higher HDL level was observed, suggesting that the effect of LPL activity on HDL level was indirect and likely depends on other factors. The absence of CETP in mouse may explain the difference. Increasing LPL activity is associated with elevated HDL in CETP transgenic mice.[126] These data are consistent with the notion that LPL is a key enzyme in mediating the lipolysis of VLDL triglyceride, the products of which are used as HDL components. The effect of tissue-specific overexpression of LPL has been studied in either all muscles or cardiac muscle only.[127,128] The cardiac muscle-specific expression of LPL in the LPL knockout background is sufficient to maintain whole animal plasma triglyceride and HDL levels.[128] LPL knockout animals developed severe hypertriglyceridemia and died within 48 h of birth. Heterozygotes have mild hypertriglyceridemia, and no change in HDL levels.[129,130] LPL deficiencies in humans are associated with hypertriglyceridemia and low HDL cholesterol levels.[131, 132]

The relationship between LPL expression and the risk of atherosclerosis is rather complicated and appears to be related to the localization of LPL. LPL is expressed in monocyte-derived macrophage cells, macrophage foam cells, and skeletal muscle cells in atherosclerotic lesions,[133,134] suggesting possible involvement of LPL in macrophage foam cell formation. This is due to the fact that LPL contributes to the degradation and internalization of triglyceride-rich lipoproteins by macrophages.[135] Furthermore, LPL also promotes chylomicron uptake by macrophages, which results in the accumulation of cholesterol esters in macrophages. The free fatty acid released from the hydrolysis is used in the esterification.[136] LPL increases monocyte adhesion to aortic endothelial cells and functions as a monocyte adhesion molecule.[137] These data are consistent with in vivo findings that the expression of LPL by macrophages promotes foam cell formation and atherosclerosis in the artery wall.[138] In addition, elevated LPL level in the arteries by perfusion also increased lipoprotein binding to the artery wall and increased the endothelial layer permeability.[139]

However, overexpression of LPL in either LDLR−/− or apoE−/− mice reduced atherosclerosis.[140,141] The beneficial effect of LPL in these studies is mainly from its effect on lipoprotein profiles. In LPL and LDLR double knockout (LDLR/LPL−/−) mice, triglyceride levels were markedly reduced coupled with a modest reduction in total cholesterol levels. Larger lipoprotein particles of IDL and LDL as well as remnant lipoproteins were significantly reduced.[140] In apoE and LPL double knockout (apoE/LPL−/−) mice on chow diet, triglyceride was markedly reduced without significant change in total cholesterol, but smaller non-HDL particles were found in these animals.[141] These favorable lipoprotein profiles are likely the reason for reduced size of atherosclerotic lesions in these animals. It is noteworthy that in these animals LPL is expressed in several tissues, including muscle, heart, adipose, and aorta. Conversely, expression of LPL in macrophage relative to reduced macrophage LPL level increased atherosclerosis in mice,[138] suggesting that the location of LPL may be involved in its effect on atherosclerosis. The LPL in macrophage increases cholesterol ester accumulation and induces foam cell formation,[138] while the LPL in other tissues helps reduce triglyceride and possible total plasma cholesterol, which has protective effect on atherosclerosis.[125] In the overexpression studies in LDLR and apoE knockout mice, it is apparent that the positive lipid effect overrides the negative effect of induction of foam cell formation.[140,141] The overall role of LPL in the reverse cholesterol transport pathway is to maintain the VLDL triglyceride hydrolysis and provide surface component for HDL production.[140,141] Elevation of LPL activity overall is beneficial, but the LPL activity in macrophage and the artery wall is atherogenic.[138]

3.7. Cholesterol Ester Transfer Protein (CETP)

Cholesterol ester transfer protein (CETP) is a protein mediating the net transfer of cholesterol ester and triglyceride between lipoproteins. Specifically, CETP mediates the net transfer of triglyceride from VLDL to LDL and HDL as well as the transfer of cholesterol esters from LDL and HDL to VLDL. Its substrate specificity is regulated by lipid transfer inhibitor protein (LTIP).[142] CETP is associated with HDL in the plasma. It is predominantly expressed in liver, spleen, and adipose tissue with lower levels in small intestine, adrenal gland, skeletal muscle, kidney, and heart.[142] The expression of CETP is stimulated by diet-induced hypercholesterolemia, possibly mediated by oxysterol-activated LXR.[143] The CETP expression is species-specific. For example, it is not expressed in rodents. The CETP mRNA level is negatively correlated with the adipocyte size.[144] Low levels of CETP is found in human preadipocytes, but the expression increases as the preadipocytes differentiate into small adipocytes,[145] suggesting that CETP may play a role in lipid metabolism in fat tissues. This is consistent with other findings that CETP activity increased in obesity but not weight matched NIDDM patients, and the levels correlate with total and LDL levels.[146]

As observed in CETP-deficient patients, reduced CETP activity is associated with higher HDL levels. CETP deficiency was found in Japanese siblings homozygous for a point mutation in the 5′-splice donor site of intron 14 of the gene.[147] These patients have significantly increased large HDL.[147] A dominant negative CETP mutation with the aspartic acid in residue 442 replaced by glycine inhibited the secretion of the wild-type molecule.[148] These data help explain that patients heterozygous for this mutation have 3-fold increased HDL.[148] In a study with 236 Japanese subjects, these two mutations account for 10% of the total variance of HDL cholesterol in the population.[149] CETP deficiency is associated with hyperalphalipoproteinemia, changes in size and composition of HDL and LDL.[149] For example, apoE-rich HDL increased in CETP-deficient patients.[150] CETP deficiency is also associated with smaller LDL with lower affinity for LDL receptor.[151] CETP concentrations were increased by the treatment of the hypolipidemic drug Probucol, which is accompanied by an increase in apoE and a decrease in HDL and apoA-I.[152] Injection of CETP neutralizing antibodies into normal and hypercholesterolemic animals resulted in a decrease in LDL and VLDL and an increase in apoE-rich HDL, consistent with the findings in CETP-deficient patients.[153−155] This raised the possibility that inhibition of CETP activity could be one of the approaches to elevate HDL. This notion was proved by the design and development of small molecules that specifically inhibit CETP.[156−158] Inhibition of CETP activity by injection of antisense oligonucleotides generated results consistent with that by inhibitor administration.[159]

The effect of increased plasma CETP levels on HDL was investigated with CETP transgenic animals, where human CETP was expressed in mouse under the control of mouse metallothionein-I promoter. Induction of the CETP transgene resulted in the significant reduction of HDL cholesterol, but there was no change in total cholesterol content in LDL and VLDL. In addition, the ratio of free cholesterol and cholesterol ester decreased, suggesting that CETP may induce cholesterol esterification.[160] The effect of CETP concentration on the levels of apoB-containing lipoproteins (LDL and VLDL) was examined in transgenic mice expressing different levels of CETP. With increasing CETP concentrations, cholesterol ester content in apoB-containing lipoproteins increased. This was associated with down-regulation of LDLR expression, increased cholesterol and cholesterol ester content in the liver, and reduced HMG Co-A reductase and 7-α hydroxylase mRNAs.[161] The same effect was also found in apoC-III transgenic mice where CETP transgene lowered HDL cholesterol, apoA-I, and HDL particle size.[162] These results are consistent with the finding that transgenic mice expressing simian CETP developed severe atherosclerosis, suggesting that the increase in lesions may be due to the change of lipoprotein profiles by CETP overexpression.[163]

3.8. Phospholipid Transfer Protein (PLTP)

Phospholipid transfer protein (PLTP) is an 81-kDa plasma glycoprotein partially associated with HDL

and mediates the transfer of phospholipds from VLDL to HDL as well as the exchange of phospholipids of VLDL and HDL.[164−166] The transfer and exchange of phospholipids by PLTP is stimulated by lipolysis by LPL.[164] The lipid transfer enhanced by PLTP was only associated with a slight increase in HDL particle size and the release of apolipoproteins (mainly apoA-I),[165] suggesting the formation of new HDL particles following the lipid transfer from VLDL. The generation of both larger and smaller HDL particles than the original HDL_3 population by PLTP was confirmed in both in vitro and in vivo studies.[167,168] In mice overexpressing PLTP and human apoA-I, HDL phospholipids increased by 26% with a major increase of apoA-I in pre-β HDL and a small increase in α-HDL.[167] This observation was confirmed in an assay where HDL_3 was incubated with PLTP and particles larger and smaller than HDL_3 after the reaction were found.[168] In vitro assays with reassembled HDL suggest that PLTP may also mediate the recycling of phospholipids from mature HDL to nascent HDL.[169] This is consistent with the finding that the ability of phospholipid transfer between HDL particles decreases with increasing free cholesterol content in the donor HDL and with decreasing donor HDL size.[169]

PLTP activity can be evaluated as the rate of phospholipids transferred from liposome or isolated VLDL to plasma HDL.[170,171] The activity can also be determined as the plasma phospholipids mass transfer rate (PLTR),[171] which correlates positively with plasma HDL phospholipids and inversely with HDL free cholesterol.[171] In addition, the correlation of plasma PLTP activity with HDL cholesterol and particle size was determined in in-bred mouse strains.[172] It is evident that PLTP activity is associated with higher HDL cholesterol level and larger particle size,[172] suggesting the role of PLTP in HDL remodeling, which is enhanced in triglyceride-enriched HDL.[173] This is in agreement with another finding that HDL is enlarged by fenofibrate treatment in mice, which increased PLTP expression.[174] In the meantime, the level of the smaller pre-β HDL is also up-regulated by PLTP, as shown in PLTP transgenic mice.[175] On the other hand, PLTP deficiency in animals results in reduced HDL levels.[176,177]

3.9. Cubulin

Cubulin (also termed gp280) is a 460-kDa endocytic receptor involved in the catabolism of HDL and clearance of apoA-I.[178,179] It is co-expressed with megalin, a multi-ligand receptor of the low-density lipoprotein receptor gene family,[180,181] in the renal proximal convoluted tubule (PCT), visceral yolk sac, ileum, and placenta.[178,179] Cubulin contains a short N-terminal region with a putative amphipatic helix structure and eight epidermal growth factor repeats followed by a large cluster of complement C1r/C1s, Uegf, bone morphogenic protein 1 (CUB) domains at the carboxyl terminus.[179] Cubulin lacks a transmembrane domain and requires megalin for internalization. Initially identified as endocytic receptor for intrinsic factor-vitamin B12,[179,182] cubulin mediates HDL lipoprotein holoparticle endocytosis.[183] In yolk sac endoderm-like differentiated F9 cells that express high levels of cubulin, radiolabeled HDL was internalized and degraded in a cubulin-dependent manner. Smaller HDL_3 is more effective than the larger HDL_2 in the cubulin-mediated uptake. HDL apolipoproteins, apoA-I, apoA-II, and apoE can compete for the uptake while apoC-I and apoC-III cannot, suggesting that cubulin-mediated uptake is HDL selective.

Among the HDL apolipoproteins, apoA-I is the main ligand for cubulin[184] as shown in an affinity chromatography assay with immobilized cubulin. The high affinity binding of both apoA-I and HDL to cubulin was demonstrated by surface plasmon resonance.[184] The affinity of apoA-I for cubulin correlates with the renal uptake of apoA-I. Furthermore, urinary apoA-I loss is associated with some known cases of functional cubulin deficiency.[184−186] Several studies have shown that the circulating apoA-I and HDL levels are controlled by the rate of their renal catabolism.[187] The size and charge of HDL particles restrict their passage through the glomerulus. apoA-I has a much smaller size and therefore can be absorbed in the PCT.[188] These data suggest that cubulin plays a role in the renal clearance of apoA-I and HDL uptake.

4. Effect of HDL on Atherosclerosis and Coronary Artery Disease (CAD)

HDL has several effects on the formation of atherosclerosis: mediation of cholesterol efflux from macrophage cells and inhibition of foam cell formation, inhibition of endothelial cell dysfunction, inhibition of oxidation of LDL, and anti-inflammatory effects.

4.1. Cholesterol Efflux in Macrophages and Foam Cell Formation

Macrophages are part of the body's defense system and are involved in antimicrobial programs. Macrophages also collaborate with T cells through cell−cell interaction and cytokine-mediated interactions in inflammatory response. These functions also contribute to the mechanism of the development of atherosclerosis involving macrophage. Circulating monocytes are attracted to the lesion prone sites by cell adhesion molecules on activated endothelial cells on the vessel wall. Adherent monocytes migrate into the subendothelial space where they differentiate into macrophages in response to locally produced factors, which leads to the increased expression of SR-A, CD36, and other scavenger receptors that are capable of taking up oxidized LDL.[189] The oxidized LDL taken up by macrophages scavenger receptors is delivered to lysosomes where cholesterol ester is hydrolyzed to free cholesterol and fatty acids. The free cholesterol undergoes re-esterification and is stored intracellularly. The accumulation of cholesterol esters in macrophages leads to the formation of foam cells that, along with lymphocytes, smooth muscle cells, and endothelial cells, influence the development of atherosclerosis.[190]

The foam cell formation, characterized with accumulation of lipid droplets (mainly from oxidized

LDL and modified LDL) in macrophage cells, is one of the early events of atherogenesis. The interaction of LDL with arterial proteoglycans stimulates its uptake by macrophages,[191,192] suggesting that LDL modifications induced by proteoglycans may contribute to the formation of foam cells. This atherogenesis process is associated with inflammation and damage of endothelial cells. After the penetration of the vascular endothelial cell layer by monocytes, the expression of inflammatory mediators such as macrophage-chemoattractant protein 1 (MCP-1) and its receptor CXCR2 are upregulated.[193] The combined outcome of these changes is the formation of atherosclerotic plaques. The increased uptake of modified and oxidized LDL by macrophage cells marks the initial step of the accumulation of cholesterol esters in macrophages and the formation of foams cells. The uptake of the modified LDL and oxLDL is mediated by several scavenger receptors on macrophage cells.[73,74,194] Although the foam cell formation is an important part of the development of atherosclerosis, there is no direct evidence that foam cell alone is responsible for lesion formation. However, it is generally believed that delaying or prevention of foam cell formation can help prevent atherogenesis. This can be achieved, at least in part, by reducing cholesterol ester accumulation in macrophages cells via cholesterol efflux.

The cellular cholesterol efflux process to remove cholesterol from macrophage cells is mediated by HDL molecules. Although the efflux is mediated by either ABCA1 or SR-BI or alternative mechanisms, HDL particles play an important role as acceptors for the free cholesterol molecules from macrophage cells. Several mechanisms are involved in the efflux of cellular free cholesterol to HDL particles.[13] These involve the lipidation of pre-β HDL via cholesterol efflux to form larger HDL particles. Larger HDL particles continue to be active in removing cholesterol from macrophage cells. Cholesterol efflux from macrophage cells to larger HDL_3 and HDL_2 is mediated by SR-BI.[14] The movement of cholesterol from cellular plasma membrane to HDL is enhanced by overexpressing SR-BI.[78,195] The presence of SR-BI facilitates the movement of free cholesterol by increasing the rate of cholesterol exchange. In addition, HDL particles remove free cholesterol from cells through mechanisms of unmediated aqueous diffusion and membrane microsolubilization.[13] Overall, the direct inhibitory effect of HDL on the formation of atherosclerotic lesions is mediated by its ability to prevent cholesterol ester accumulation by stimulating cholesterol efflux from macrophage cells in the vessel wall. This notion is supported by several lines of experimental evidence. Hyperlipidemic animals (apoE−/− or LDLR−/−) with ABCA1 deficiency in only macrophage cells resulted in increased atherosclerotic lesions relative to controls.[42] On the other hand, increased ABCA1 expression protects animals against atherosclerosis.[40] These data demonstrated that lack of removal of the cholesterol esters from macrophages contributed to the formation of foam cells and atherosclerotic lesions. The deficiency of other members in the RCT pathway has led to similar conclusions. Macrophage-derived apoE has been implicated in contributing to macrophage cholesterol efflux and the overall HDL level. Expression of apoE in macrophages in hyperlipidemic animals reversed atherosclerotic lesion development.[196, 197]

4.2. Endothelial Dysfunction

In addition to its role in RCT to remove cholesterol accumulation in macrophage cells and preventing foam cell formation, the anti-atherogenic function of HDL includes inhibition of endothelial dysfunction during the development of atherosclerosis. Endothelial cells synthesize and release several relaxing factors, including nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI2), and several contracting factors (endothelium-derived contracting factors, EDCFs).[198,199] One of the risk factors in atherosclerosis is the impairment of endothelium-dependent vasodilations, the effect of which influences both NO- and EDHF-mediated responses.[200]

Several lines of evidence support the role of HDL in restoring and protecting endothelial dysfunction. Direct association of HDL level with endothelial function in hyperlipidemia has been observed.[201] In hypercholesterolemic patients, intravenous rHDL infusion rapidly normalized endothelium-dependent vasodilation by increasing NO bioavailability.[202] Similar study with apoA-I/phosphatidylcholine stimulated NO bioactivity.[203] In addition, the protective effect of HDL on endothelium-dependent vasodilation was also reported.[204] These effects are at least in part due to the stimulation of endothelial nitric oxide synthase expression by HDL.[205]

4.3. LDL Oxidation

The formation of modified and oxidized LDL is required for their uptake by macrophage cells and the formation of foam cells in the subendothelial space. The oxidation of LDL produces a variety of peroxidized lipids that induce the production of monocyte chemotractic protein-1 (MCP-1) and monocyte adhesion to the endothelium,[206] which contributes to the development of atherosclerosis. Since oxLDL promotes atherosclerotic events, the formation of oxLDL represents a key process for atherogenesis.[206] Two antioxidant enzymes are associated with HDL, paraoxonase, and platelet-activating factor acetylhydrolase.[207,208] These enzymes degrade deleterious oxidized phospholipids associated with LDL and inhibit the oxidation of LDL, which is thought to be one of the primary causes of lesion formation. HDL, as carriers of these enzymes, has anti-oxidant activity toward oxLDL and indirectly prevents the development of atherosclerotic plaques. In addition, HDL prevents the cell death of endothelial cells induced by oxidized LDL.[209] These data indicate that HDL had protective effect on the damage caused by oxLDL.

5. Pharmaceutical Agents and Targets for HDL Elevation

The new guidelines accepted and published by the international clinical community through the aus-

pices of the National Cholesterol Education Program known as the NCEP ATP III guidelines provide the most compelling impetus to aggressively manage not only high levels of LDL but also low levels of HDL and hypertriglyceridemia.[210] The guidelines may be found at http://www.nhlbi.nih.gov/guidelines/cholesterol/. Largely as a result of the failure of low LDL to identify the majority of patients at risk for CAD and reduce mortality below about 40%, low HDL is being evaluated as one of the more significant risk factors, independent of and in association with hypertriglyceridemia. While statin therapy for high LDL is being pushed to new limits, physicians for the first time are being encouraged to aggressively manage low levels of HDL. The issues are 2-fold. The first is the lack of a clear definition of the components that make up HDL and its multivariate role in the process of RCT, which has been the subject of the previous sections of this review. The second and most critical is the lack of efficacious and safe pharmacological agents to elevate "good" HDL. This section of the review will assess the current state-of-the-art available therapies, with known mechanism of action, and then consider the early clinical and preclinical experiences published to date on novel mechanisms to raise HDL and promote reverse cholesterol transport for therapeutic benefit.

5.1. Statins and Low HDL

While much of the data collected from the statin trials that were designed and powered to evaluate the effects on LDL have been through meta-analysis and post-hoc analysis, some significant data have been generated to demonstrate the independence of HDL as both a risk factor and a therapeutic target.[211] Statins are the weakest inducers of HDL as compared to the other therapies (e.g., the fibrates and niacin or its derivatives) with typical increases of HDL in the range of 5−15%. In addition to the dose-limiting side effects of statin therapy that have prevented greater effects on HDL elevation, their effects on HDL have been highly variable and not easily extrapolated from their effects on LDL. A key example is that high-dose simvastatin yields a greater effect on HDL elevation than does atorvastatin at equally efficacious doses for LDL.[212] In fact, a positive dose-dependent relationship is seen with simvastatin whereas with atorvastatin an inverse dependency is observed,[212] highlighting the fact that we are still at an early stage of understanding the complex relationships among lipoprotein subclasses and the drugs we use to treat them.

Most statins have at least some effect on HDL, while some have exhibited significant elevations approaching the fibrates. It has been difficult to clinically dissociate the benefits of the increased HDL from LDL lowering since the studies were designed to address LDL as the principal mechanism of action of the statins. Newer statins, so-called superstatins, are being evaluated not only for their role in lowering LDL but also for many other effects including their effects and mechanisms by which they might raise HDL. One such report shows that statins increased apoA-I in healthy subjects and a patient with CAD.[213] This is likely due to the statin-induced inhibition of the Rho-signaling pathway, which activates PPARα and induces apoA-I expression.[214] With an increased understanding of putative mechanisms for statin-mediated HDL effects coming from the recent literature, some of the clinical data indicating a contribution of HDL in the benefits of statin therapy are likely to be supported.

5.2. Fibrates and Low HDL

As with the statins, fibrates are indirect modulators of HDL that have produced an interesting but not clear-cut indication for HDL elevation and therapeutic benefit. They tend in general to be more efficacious at raising HDL than the statins, but their primary mechanism of action is thought to be lowering triglycerides. Bezafibrate has been the least attractive of the fibrate class of drugs, failing to demonstrate any but the most modest effects on HDL and no significant reduction of risk attributable to HDL in both the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and Bezafibrate Infarction Prevention study (BIP) trials, respectively showing a 9% and 15% increase in HDL.[215,216]

Risk prevention was assigned primarily to the triglyceride lowering. Gemfibrozil on the other hand has fared better, particularly in the recent VA-HIT trial in which 2531 men with low HDL and low LDL were treated and followed for approximately 5 yr.[217] Gemfibrozil treatment raised HDL on average by 7.5%, reduced triglycerides by 24%, and had no significant effect on LDL. Plasma HDL levels were inversely correlated to both nonfatal myocardial infarction and death attributable to CHD, and perhaps surprisingly, HDL was the only significant predictor of the beneficial outcome. Risk reduction with gemfibrozil achieved on the order of 2% reduction with every 1% increase in HDL, whereas on average in most statin trials only a 1% risk reduction is seen with a 1% LDL lowering.

Gemfibrozil has not always enjoyed such dramatic success. In the Lopid Coronary Angiography Trial (LOCAT) for prevention of the angiographic progression of coronary and vein-graft atherosclerosis after coronary bypass surgery in men with low levels of HDL cholesterol, it failed to affect progression of atherosclerosis in arteries that did not receive bypass or distal segments of bypassed arteries. Only after significant statistical effort was a beneficial outcome observed, and this correlated with triglyceride reduction but not HDL elevation.[218] Clearly the future dissection of HDL biology and its complex relationship to triglyceride levels will provide further insights into effective therapies.[219] An earlier clinical study with gemfibrozil, the Helsinki Heart Study, also showed a significant 11% increase in HDL that was independently identified as a predictor of reduced clinical events.[220]

5.3. Niacin

One of the oldest, least expensive, and still most efficacious treatments for low HDL cholesterol is niacin. This drug, actually a B vitamin, is the most

efficacious of any HDL-elevating therapy. This compound requires high doses, frequently greater than 1 g/day to provide beneficial effects on increasing HDL. In addition to increasing HDL, it also significantly lowers triglycerides, although not as much as fibrates, and LDL cholesterol. One additional risk factor addressed in a positive way by niacin is Lp (a), which is decreased significantly, and modest effects on fibrinogen, another putative risk factor for atherosclerosis and CAD.

Liabilities or side effects of niacin therapy include flushing and other skin disturbances. There is conflicting data on the exacerbation of hyperglycemia in diabetic patients versus benefits of co-administration of niacin with a statin in diabetes. Uric acid elevation is another side effect of niacin therapy as is some gastrointestinal effects, particularly in patients with ulcers or preexisting conditions. Niacin has been available for a number of years as a crystalline immediate-release form dosed up to 4 g/day. This form is efficacious but has some significant limitations due to the incidence and severity of flushing and itching that can occur. Extended-release (also called sustained-release) forms are available as an over-the-counter nutritional supplement and lessen the severity and occurrence of the flushing side effects but appear in some studies to have an increased risk for liver toxicity.[221] An intermediate-release formulation in prescription form is efficacious and appears to have less of either of the side effects seen with the immediate or slow-release forms of niacin.[222]

5.4. Peroxisome Proliferator Activated Receptor (PPAR) Family

The founding member of this family of nuclear receptor drug targets was identified by its activity in rodents to induce hepatic peroxisome proliferation in response to fibrates and other classes of fatty acid derivatives and, hence, came to be known as peroxisome proliferator-activated receptor α (PPARα). While this activity is a hallmark of activation of PPARα in the liver, it appears to be rodent-specific as this phenomenon does not occur in higher mammals including primates. One prevailing hypothesis is that this is a result of significantly higher expression of PPARα in rodent liver than human. Knockout animals that do not express PPARα confirm that this receptor is required for the action of PPARα agonists to induce hepatic peroxisome proliferation,[223] suggesting that it is a mechanism-based phenomenon.

As additional structural homologues were discovered, it assumed the α designation, and the others were named β and γ. β is also frequently identified by its rodent nomenclature δ. While these receptors are structurally related as close relatives in the nuclear hormone receptor family, they have very different tissue distribution and different apparent activities, although the exact nature and depth of their roles in normal and pathophysiology remain to be elucidated. PPARα compounds have therapeutic benefit in that they lower plasma triglycerides and increase fatty acid oxidation in the liver and skeletal muscle.

5.4.1. Fibrates and Other PPARα Agonists

The predominant effects of the fibrates or PPARα agonist class of drugs on HDL metabolism are thought to largely occur through increased apoA-I and apoA-II and decreased apoC-III expression.[224−226] As the major apoproteins constituent of HDL, apoA-I constitutes the nascent pre-β HDL with minimal levels of associated phospholipid. These small discoidal particles initiate cholesterol efflux, which forms the basis for HDL synthesis. In addition, apoA-I activates LCAT and stimulates the conversion of free cholesterol on the surface of HDL to cholesterol ester, which is subsequently transferred to and stored in the central core. Since these two steps are critical to both the level and the particle size of HDL, increased apoA-I level directly leads to HDL elevation as shown in transgenic animals.[227,228] The induction of apoA-II expression by fibrates through activation of PPARα also contributes to HDL elevation.[224] Peroxisome proliferator response elements (PPREs) have been identified in the promoter regions of human apoA-I, and apoA-II and transcriptional activation of apoA-I by PPARα agonists, such as fibrates, have been demonstrated.[224] It is important to note that although PPREs also exist in the apoA-I promoters in rodents, fibrates and other PPARα agonists do not stimulate but instead inhibit rodent apoA-I expression.[229] This is due to the existence of a negative regulatory element specific in rodent apoA-I promoter, and in response to PPARα activation, the suppression of apoA-I expression mediated by the negative element overrides the stimulation by PPARE.[229,230] The biological effects of fibrates lowering apoC-III are postulated to occur through its role as an inhibitor of lipoprotein lipase-mediated hydrolysis of VLDL triglyceride. Reduction of apoC-III expression would therefore enhance triglyceride lipolysis, which provides phospholipid and free cholesterol to HDL synthesis. Furthermore, increased triglyceride uptake from the circulation indirectly impacts HDL level.[226] The physiological role of apoC-III was revealed in apoC-III transgenic animals with reduced VLDL catabolic rate.[231] The utility of fibrates in raising HDL has been tested in both primate models and clinical trials.[232−235] Gemfibrozil has been used for clinical studies to demonstrate that elevated HDL levels resulted in a significant reduction of CAD risk.[6,7]

The effects on apoA-I are thought to be more direct with many experiments in animals showing significant positive correlation of HDL with apoA-I. Recent data are pointing to effects of the statins to increase PPARα levels in the liver as a possible explanation of triglyceride-lowering and HDL-raising effects of this class of drugs.[213,214] Newer agents, the so-called super-fibrates, have been discussed for a number of years, but limited preclinical success has all but abolished this line of research.

5.4.2. PPARβ/δ

One of the more striking pieces of data to emerge recently was the finding that PPARδ agonists have marked effects on HDL. Because of its widespread expression and little knowledge of specific biology

associated with it as compared to the other PPAR isoforms, it was thought to be less important in lipid and lipoprotein metabolism than α or γ. In preclinical studies on obese Rhesus monkeys, it was found that, in addition to significant elevations in HDL, there was a significant lowering of the levels of small-dense LDL, fasting triglycerides, and fasting insulin.[236] In addition, it promoted expression of the ABCA1 and apoA-I-dependent cholesterol efflux from macrophages in relevant cells.[236] If safety concerns based on its widespread distribution and less-well-known biology can be effectively addressed as this and other compounds progress through preclinical and clinical development, this may offer a significant therapeutic opportunity for HDL and RCT.

Interestingly, in PPARα knockout mice, it was found that PPARδ could at least partially compensate for the lack of PPARα in skeletal but not cardiac muscle.[237] This may be partially accounted for by the fact that expression level of PPARδ is very high in skeletal muscle as compared to heart but still speaks to functional redundancy of the two PPARs. In addition to the PPARs, other RXR partners may offer attractive targets for HDL modulation, although they are much earlier in the preclinical pipeline.

5.5. CETP Inhibitors

There has been a great controversy over the utility and efficacy of CETP inhibitors in the elevation of HDL for therapeutic benefit for some time. It was known that blocking the transfer of cholesterol esters from HDL to the LDL clearance pathway would increase the level of HDL in the plasma, and this was assumed to be beneficial. However, in studies of Japanese Americans with mutations in the CETP gene leading to reduced levels of the protein, HDL was increased but their cardiovascular risk was paradoxically higher.[238] While controversy remains as to exact mechanisms for the discrepancy, recent advances with preclinical compounds have shown beneficial results in rabbit atherosclerosis models.[239] CETP inhibitors are making their way into clinical settings where their safety and efficacy is being assessed in the human disease setting.[240] It will be that the ultimate proof of concept may be realized as revealed in clinical trials.

5.6. Direct Infusion of HDL and/or apoA-I

One of the more ambitious proposals for managing HDL and promoting RCT is to inject patients directly with the beneficial species. This concept is supported by the finding that a mutant form of apoA-I, also A-$I_{Milano,}$ was found in a population with very low plasma levels of HDL and moderate hypertriglyceridemia but yet low incidence of CAD.[241−243] This raised the possibility that A-I_{Milano} might be more effective in removing lipids from atherosclerotic plaques. The A-I_{Milano}/phospholipid complex was injected into atherosclerotic animals.[244,245] Significant reduction of plaque lipid and macrophage content was observed.[244,245] In addition, A-I_{Milano} removed lipid from fatty streaks in cholesterol-fed rabbits and aortic atherosclerosis was reduced.[246]

5.7. Combination Therapies for Low HDL

5.7.1. Old Standard: Fibrates and Statins

One of the longest running combinations used by clinicians treating mixed dyslipidemia has been adding a fibrate to statin therapy to address the hypertriglyceridemia often present in this patient population.[220] This has historically been a very successful combination, and the recent data demonstrating a mechanism for induction of PPARα by statins may provide additional impetus to revisit this strategy since the induction of a nuclear receptor and addition of a synthetic ligand to activate it seem to make good sense. The principal limitation of this combination therapy has been the requirement for close physician−patient monitoring of the potentially life-threatening complication of rhabdomyolysis, which is the primary reason for the recent withdrawal of cerevistatin from the market.[247] Approximately 25% of the deaths attributable to cerevistatin were due to co-administration with gemfibrozil.

It appears that the liabilities of the combination of various statins with the fibrates commercially available are dependent on both the properties of the statin and the fibrate used. No good guidelines as to safety have been offered, although statins with lower safety margins, such as the potent cerevistatin, need to be closely monitored when used in any combination or multi-drug therapy.

5.7.2. Niacin and Statin Combinations

The main limitation of the statins is that they, in general, have little efficacy against low HDL and hypertriglyceridemia, which represent a significant contribution to the at-risk population. In addition, even with aggressive statin therapy, the risk of cardiovascular-related death is still significant. As early statins come off patent protection, we are seeing a move toward improving therapeutic efficacy in these high-risk patient populations through combinations of statin and niacin drugs. In a pioneering study, the HDL-Atherosclerosis Treatment Study (HATS) compared niacin plus simvastatin against placebo and found that the combination provided marked clinical improvements that were measurable angiographically.[248] While not compared directly to individual treatment alone, the data provided compelling evidence for an improved therapeutic regimen with LDL decreased by 42% and HDL increased by 26%. Interestingly in this study, concomitant antioxidant therapy seemed to diminish the effects of niacin plus statin.

The use of lovastatin as it becomes generic in combination with niacin is expected to provide significant new therapeutic benefit. Reviews of University of Pennsylvania's clinical databases to retrospectively define a subset of the patient population who had concurrently received both a statin and niacin showed for the first time a significant efficacy in further reducing LDL and triglycerides and raising HDL, supporting the benefit of combination therapy.[222] More direct evidence comes from an open-label combination trial with lovastatin and extended-release niacin at doses up to 40 and 2000 mg,

respectively.[249] At the highest dose, which was reasonably well tolerated, dramatic lipid profile changes were observed. LDL dropped by more than 45%, HDL increased by more than 40%, and triglycerides also dropped by more than 40%.[249] Dose-related reductions in lipoprotein (a) and high-sensitivity C-reactive protein were also observed. Clearly there is a need for a prospective and randomized outcomes trial with this therapy. Recently, the first combination form of lovastatin and Niaspan was introduced as Advicor, marketed by Kos Pharmaceuticals.[250]

5.7.3. PPAR Combinations for Lipid Disorders and Hyperglycemia in Diabetes

One of the most frequently observed phenotypes in patients with type 2 diabetes is the pre-insulin-resistant state known as the metabolic syndrome or Syndrome X.[251] Two predominant pathophysiological disorders of Syndrome X are low HDL and hypertriglyceridemia. In addition to controlling hyperglycemia, physicians treating type 2 diabetics are aggressively seeking to manage hypertriglyceridemia and low HDL in their patients. One of the primary actions of PPARα agonists, a class of drugs represented by the fibrates, is to lower triglycerides and raise HDL, at least in part through the actions of PPARα to lower apoC-III and thus triglycerides as previously discussed and to raise the levels of apoA-I through transcriptional mechanisms. As pharmaceutical companies have continuously sought to improve therapies, one emerging concept has been to combine the insulin sensitization and glucose lowering of the PPARγ agonist class of drugs, known currently as the thiazolidinediones (or TZDs) with triglyceride and HDL -raising of the PPARα agonists. Since both are members of a close related family of nuclear receptors, it is a reasonable hypothesis to search for a co-agonist of both receptors. Another intellectually stimulating possibility is that through the actions of PPARα on β-fatty acid oxidation, newer generation PPARα compounds, alone, in combination with PPARγ agonists or as co-agonists, will enhance fat metabolism. This may provide a solution to the increased weight gain, a significant liability of the current TZD insulin-sensitizing drugs for diabetes.

6. Conclusions

The past decade has seen great advances in understanding the underlying mechanisms for HDL metabolism, which has been achieved by the application of molecular biology, the use of transgenic and knockout animals, and the new findings in human genetics. The identification of ABCA1 as one of the first steps in HDL biosynthesis represents one of the major breakthroughs in mapping out the entire HDL pathway, especially when linking clinical phenotypes to genetic mutations or polymorphisms. Transgenic animals, though with their own limitations, have provided important information in dissecting the pathways and identifying individual proteins that regulate HDL synthesis and catabolism. Currently, very few small compound molecules exist that elevate HDL to substantial levels in animals and man as tools to prove that therapeutic elevation of HDL is achievable. Recently, new data on HDL structure have emerged, revealing the complex nature of the HDL biology and presenting new challenges ahead. Despite the progress made in understanding the effect of HDL on the development of atherosclerosis, many aspects of the functions and characteristics of HDL remain to be elucidated. First, there are many HDL species that exist at low levels that have not been well-defined. Second, among the HDL species that have been identified to date, the exact relationship between their constituents, size, and anti-atherosclerotic function has not been established. In addition, these characteristics may differ significantly on an individual base. It is therefore difficult to profile HDL populations for cardiovascular benefits. It is not clear yet as what subspecies are "good" HDL that should be raised to achieve therapeutic target.

The future research lies in the further understanding of the biological functions of HDL subspecies; the effect of constituents, size, and ratio of the major constituents; and the overall effect of these characteristics on the development of atherosclerosis. Critical questions regarding the formation of foam cells and atherosclerotic lesions and the exact role of different HDL populations in preventing this process will be the key to understand the beneficial cardiovascular effects of HDL.

7. References

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