Reverse Cholesterol Transport: High-density Lipoprotein's Magnificent Mile

Peter P. Toth, MD, PhD

Address
Sterling Rock Falls Clinic, 101 East Miller Road, Sterling, IL 61081, USA.
E-mail: pptoth@essex1.com

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High-density lipoproteins (HDLs) are among the most structurally complex and functionally versatile forms of circulating serum lipoproteins. HDLs undergo extensive enzymatic remodeling during their maturation in serum, interact with highly specific receptors in peripheral tissues and the liver, and are able to exert a variety of antiatherogenic effects (*eg*, inhibit inflammation, oxidation, and apoptosis). Considerable epidemiologic, clinical, and basic scientific investigation supports the conclusion that HDLs as a molecular class are atheroprotective. One of the most important antiatherogenic functions of HDL is its capacity to drive reverse cholesterol transport, the process by which excess cholesterol in peripheral tissues is extracted and delivered to the liver for disposal. Despite the rapid expansion in our understanding of how HDL antagonizes many of mechanisms etiologic for atherosclerosis and the observation that a low serum level of HDL is the most frequent lipid abnormality in patients with premature coronary artery disease, many physicians still focus inadequate attention on recognizing and treating hypoalphalipoproteinemia. Our current understanding of reverse cholesterol transport reinforces the clinical importance of including HDL screening when evaluating any given patient's risk for cardiovascular disease.

Introduction

High-density lipoproteins (HDLs) have become an enormous focus of scientific and clinical investigation. A large number of prospective, epidemiologic studies performed throughout the world consistently demonstrate that patients with low levels of serum HDL have increased risk for the development of atherosclerotic disease and its sequelae, including myocardial infarction, stroke, intermittent claudication, and sudden death [1]. The Veteran's Administration HDL Intervention Trial (VA-HIT) demonstrated that raising serum HDL lowers risk for cardiovascular endpoints independent of LDL reduction [2]. Multivariate analyses of numerous other clinical intervention studies also suggest that at least part of the efficacy of statin, fibrate, and niacin therapies stems from the ability of these drugs to raise HDL [3••]. Given the scope of evidence, The National Cholesterol Education Program [4] defines an HDL level less than 40 mg/dL as an independent and categorical risk factor for the development of cardiovascular disease. A low level of serum HDL is widely prevalent (35% of men, 15% of women) [5] and frequently poses a formidable challenge to raise into the normal range. The frequency of low HDL will likely increase sharply as the incidence of diabetes mellitus and the metabolic syndrome continues to rise.

High-density lipoproteins mediate a variety of antiatherogenic effects. HDL stimulates endothelial cell nitric oxide production, inhibits adhesion molecule expression, mediates antioxidant effects via paraoxonase and methionine residues in apoprotein A-I, promotes prostacyclin production, and inhibits thrombosis and endothelial cell apoptosis among other processes [6•]. HDL is a highly heterogeneous and metabolically active class of lipoproteins. It is currently believed that one of HDL's most important functions is its ability to promote reverse cholesterol transport (RCT). RCT appears to be the primary mechanism by which excess peripheral cholesterol is delivered back to the liver for disposal as bile salts or biliary cholesterol and to steroidogenic organs for conversion into steroid hormones.

This review traces the individual steps of the RCT pathway and summarizes some of the most recent advances in our understanding of how HDL prevents excess accumulation of cholesterol within the vasculature. Whenever possible, known alterations in the individual steps of RCT and their net effect (*ie*, facilitative or inhibitory) on atherogenesis are also addressed. Given the number of currently identified polymorphisms in the various molecular components of RCT, examples of mutations are intended to be illustrative rather than comprehensive. The overall scheme for RCT is depicted in Figure 1.

Apoprotein A-I

Apoprotein A-I (apoA-I) is the predominant apoprotein constituent of HDL. ApoA-I is produced by both the intestine and liver and can be secreted into plasma in its free form, as a component of hepatically derived HDL, or as a surface coat constituent of chylomicra and very low-

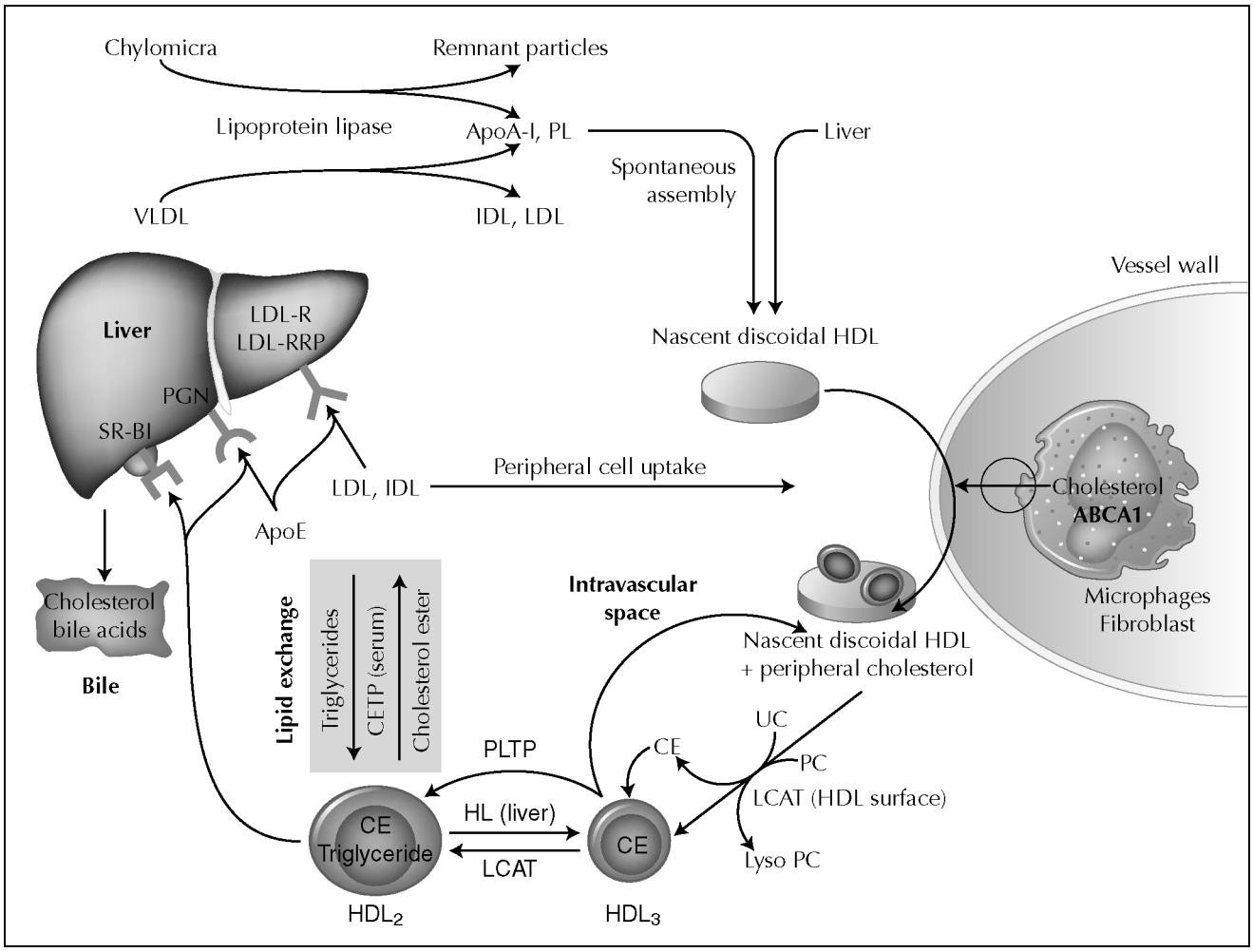

Figure 1. Molecular machinery for reverse cholesterol transport. (ABCA1—ATP-binding membrane cassette transporter A1; ApoA-I—apoprotein A-I; ApoE— apoprotein E; CE—cholesteryl ester; CETP—cholesterol ester transfer protein; HL—hepatic lipase; IDL— intermediate-density lipoprotein; LCAT— lecithin:cholesterol acyltransferase; LDL—low-density lipoprotein; LDL-R—low-density lipoprotein receptor; LDL-RRP—low-density lipoprotein receptor-related protein; Lyso PC—lysophosphatidylcholine; PC— phosphatidylcholine; PGN—proteoglycans; PL— phospholipid; PLTP—phospholipid transfer protein; SR-BI—scavenger receptor BI; UC— unesterified cholesterol; VLDL—very low-density lipoprotein.) (*Adapted from* Toth [61]; with permission. Copyright © 2001, Lawrence DellaCorte Publications.)

density lipoprotein (VLDL). ApoA-I can interact with macrophages and fibroblasts and stimulate the extracellular translocation of phospholipid and cholesterol. After binding phospholipid, apoA-I interacts with cholesterol in the extracellular space or in plasma membranes to form pre–β-HDL or nascent discoidal HDL (ndHDL). Both apoA-I and ndHDL are efficient acceptors of cholesterol translocated out of peripheral cells. The overexpression of apoA-I in a variety of animal models substantially reduces risk for atherosclerotic disease and can stimulate atheromatous plaque regression.

Low serum levels of apoA-I [7,8] and some mutant forms of this apoprotein are associated with increased risk for atherosclerosis in humans. In a recent survey of apoA-I mutations, 23 were associated with some degree of hypoalphalipoproteinemia [9]. An example of such a mutation is ApoA-IZavalla (Leu159→Pro), which results in a fourfold decrease in serum HDL and is associated with increased risk for coronary artery disease (CAD) [10]. Not all apoA-I mutations resulting in hypoalphalipoproteinemia potentiate risk for CAD. ApoA-IMilano (Arg173→Cys) is associated with hypoalphalipoproteinemia and reduced risk for CAD. This apparent paradox is at least partially explained by the observation that this apoA-I variant is antithrombotic by virtue of its ability to inhibit platelet aggregation [11].

ATP-binding Membrane Cassette Transport Protein A1

Cholesterol trafficking within cells is a complex, dynamic, and highly regulated process involving a variety of enzymes, cell signaling molecules, and organelles [12]. Excessive cellular accumulation of cholesterol leads to changes in cell membrane fluidity, disturbances in the functions of specialized membrane domains, increased

lipid droplet and cholesterol crystal formation, and ultimately apoptosis and necrosis [13]. In the presence of high concentrations of cholesterol, the scavenging function of activated macrophages in the subendothelial space promotes the formation of foam cells, fatty streaks, and atheromatous plaque. Maintaining intracellular cholesterol homeostasis and overall cellular integrity depends on the ability to balance cholesterol uptake with the capacity to mobilize and efflux excess cholesterol back into the extracellular space.

The ATP-binding membrane cassette transport protein A1 (ABCA1) regulates cholesterol translocation into the extracellular space. This transporter is defective in the macrophages and fibroblasts of patients with Tangier's disease and familial hypoalphalipoproteinemia [14]. Patients with Tangier's disease are characterized by excessive tissue cholesterol deposition and extremely low serum levels of apoA-I and HDL, resulting in hyperplastic orange tonsils, hepatosplenomegaly, and increased risk for atherosclerotic disease. The regulation of ABCA1 activity is complex. One important mechanism regulating ABCA1 expression involves increased intracellular oxysterol formation, resulting in the formation of liver X receptor α/retinoid X receptor heterodimers and the activation of ABCA1 gene transcription so as to augment cholesterol exporting [15].

ATP-binding membrane cassette transport protein A1 mediates the initial step in reverse cholesterol transport. ABCA1 transports phospholipid into the extracellular space in an energy-dependent manner [16]. ApoA-I in serum and the subendothelial space functions as a reservoir for externalized phospholipid. The phospholipidated apoA-I can then bind cholesterol, leading to the formation of ndHDL. Inadequately lipidated apoA-I and ndHDL are rapidly catabolized and cleared from serum, resulting in hypoalphalipoproteinemia. The specific mechanism(s) and membrane dynamics by which ABCA1 facilitates cholesterol transport are not yet fully elucidated, but this area of investigation is advancing rapidly. Cellular exposure to apoA-I increases the delivery of vesicles enriched with cholesterol from the Golgi apparatus to the plasma membrane, resulting in increased cholesterol efflux, possibly via exocytosis [17]. Phospholipidated apoA-I may also produce net cholesterol efflux by interacting with such plasma membrane microdomains as rafts and caveolae [18]. More recent work supports a direct interaction between apoA-I and ABCA1 [19]. In this study, amino acid residues 220–231 in apoA-I facilitate specific binding to ABCA1 and are required for cellular lipid efflux and ndHDL formation. At least a portion of the cholesterol bound to ApoA-I may arise from passive diffusion down a concentration gradient into the extracellular space.

ATP-binding membrane cassette transport protein A1 activity is unequivocally associated with serum levels of HDL, capacity for RCT, and risk for atherosclerotic disease. Transgenic mice overexpressing human ABCA1 demonstrate 1) increased serum levels of HDL and apoA-I and increased RCT as demonstrated by increased biliary cholesterol excretion [20]; and (2) increased capacity to efflux cholesterol and phospholipid from macrophages and decreased risk for atherosclerotic disease [21]. In the latter study, as apoA-I became progressively more enriched with phospholipid, its capacity to stimulate cholesterol efflux increased. Dutch men enrolled in the Regression Growth Evaluation Statin Study and screened for the R219K variant of ABCA1 demonstrated higher HDL, lower triglyceride, slower progression of CAD, and lower risk for acute coronary events. Patients heterozygous for ABCA1 mutations, which decrease capacity for cholesterol efflux, have decreased serum HDL and increased triglycerides and experience a threefold increased risk for CAD with earlier onset compared with unaffected family members [23••]. In this study, each 8% change in ABCA1-mediated cholesterol efflux was associated with a 0.1-mmol/L change in serum HDL. ABCA1Alabama (C254→T) is also associated with low HDL and increased risk for premature CAD [23••].

Lecithin:Cholesterol Acyltransferase

Lecithin:cholesterol acyltransferase (LCAT) catalyzes the esterification of cholesterol on the surface of HDL using the sn-2 fatty acid of phosphatidylcholine as an acyl chain donor. The esterified cholesterol partitions into the hydrophobic core of the HDL particle. This reaction creates a local concentration gradient for free cholesterol out of peripheral cells. As the amount of esterified cholesterol within the HDL particle increases, it becomes progressively rounder and larger, leading to the successive formation of HDL_3 and then HDL_2 (Fig. 1).

A variety of mutations are known to affect LCAT activity. Familial LCAT deficiency can arise from such mutations as Lys209→Pro and Met252→Ile, and in homozygous patients results in complete or nearly complete loss of enzyme activity. Fish eye disease is characterized by the development of corneal opacities and proteinuria, with variable reductions in enzyme activity. A novel LCAT mutation, Pro260→Stop, produces a truncated protein with significant reductions in serum HDL and apoA-I [25]. Patients with impaired LCAT activity have decreased capacity for cholesterol ester formation, moderate to severe reductions in serum HDL, and increased catabolic rates of serum apoA-I and ndHDL. Despite the resulting hypoalphalipoproteinemia and expected reductions in rates of RCT, there is, as yet, no clear relationship between the severity of LCAT deficiency and risk for CAD in humans. There are no known examples of human LCAT overexpression. However, in one rabbit model, LCAT overexpression decreased risk for atherosclerotic disease [26].

The activation of LCAT is apoA-I–dependent. The ability of apoA-I to activate LCAT depends on three strictly conserved arginine residues (positions 149, 153, and 160)

found within the α-helical repeat formed by amino acid residues 143–164 [27]. ApoA-IMallorca has reduced ability to activate LCAT and is associated with familial hypoalphalipoproteinemia and decreased capacity for cellular cholesterol efflux [28].

Phospholipid Transfer Protein

Phospholipid transfer protein (PLTP) facilitates the transfer of phospholipids from apoB lipoproteins into HDL. PLTP also remodels HDL via fusion reactions into larger and smaller lipoprotein species in a process termed HDL conversion [29]. During HDL conversion, two molecules of HDL_3 can fuse, resulting in the formation of HDL_2 and pre–β-HDL (Fig. 1). Because pre–β-HDL is an important systemic cholesterol acceptor, it has been proposed that PLTP overactivity could be antiatherogenic by facilitating the net flux of cholesterol back to the liver [30]. To date, no human polymorphisms resulting in PLTP overexpression have been identified. Transgenic mice that overexpress (2.5- to 4.5-fold increase in activity) human PLTP experience a 30% to 40% reduction in plasma HDL and a two- to threefold rise in pre–β-HDL compared with wild-type mice [31]. When macrophage monolayers incubated with LDL are exposed to the plasma of these transgenic mice, there is significantly less accumulation of intracellular cholesteryl esters compared with cells exposed to the plasma derived from wild-type mice. Based on these studies, the authors concluded that PLTP overexpression is likely antiatherogenic. In a more recent study, these investigators found this was not the case when PLTP overexpression was evaluated in vivo [32]. In mice fed a high-fat and high-cholesterol diet, PLTP overexpression (two- to ninefold increase) resulted in progressive reductions of serum HDL and a dose-dependent increase in the severity of aortic atherosclerosis compared with wild-type mice. The substantial reductions in serum HDL levels (2.4- to 8.3-fold) appear to outweigh the increase in pre–β-HDL expected by PLTP overexpression.

Cholesteryl Ester Transfer Protein

Cholesteryl ester transfer protein (CETP) is a hepatically derived, hydrophobic glycoprotein that binds to HDL. CETP mediates the equimolar exchange of cholesteryl esters from HDL for triglycerides in apoB lipoproteins (chylomicra, VLDL, and low-density lipoprotein [LDL]). The cholesteryl ester transferred into apoB lipoproteins can subsequently undergo two divergent metabolic fates: 1) delivery to the liver via the LDL receptor and the LDL receptor-related protein, a process that facilitates RCT and would be expected to be antiatherogenic unless the cholesterol is partitioned into resecreted apoB-containing lipoproteins; and (2) delivery to such peripheral tissues as macrophages resident in blood vessel walls, a lipid transfer process that is atherogenic (Fig. 1).

A variety of polymorphisms result in CETP deficiency and hyperalphalipoproteinemia and are the subject of intensive investigation. CETP deficiency states are best characterized in the Japanese. People living in the Omagari region of Japan have a high incidence of a total CETP deficiency secondary to homozygosity for a G→A substitution mutation in the 5′ splice donor site of intron 14 in the CETP gene. These patients can experience a three- to fourfold elevation in serum HDL. Another important mutation involves a D442→G in exon 15, which decreases CETP activity by 30% and increases HDL one- to twofold. Although some studies have demonstrated reduced capacity for macrophage lipid extraction and RCT by the HDL of patients with CETP deficiency states [33], CETP deficiency can be associated with decreased risk for CAD. In the Honolulu Heart Study, Japanese-American men with the D442→G mutation and HDL levels greater than 60 mg/dL experienced a significant reduction in risk for coronary events relative to those participants whose HDL was less than 60 mg/dL [34]. In another study evaluating Japanese men with either D442→G or Int14 +1 G→A mutations living in Kochi Prefecture, CAD-related events were significantly lower in patients with HDL greater than 80 mg/dL [35]. Among American men participating in the VA-HIT study [36], the presence of the TaqI B2B2 genotype was associated with reduced CETP activity, significantly higher baseline HDL levels, and a 48% lower CHD risk compared with patients homozygous for the B1 allele. An example of a CETP polymorphism that reduces HDL and increases risk for CAD is EcoN1 G/G [37].

Given the implications of these studies, efforts have been made to raise serum HDL levels by manipulating CETP activity. In rabbits vaccinated with anti-CETP sera and fed an atherogenic diet, there is a significant decrease in CETP activity, increase in serum HDL levels, and reduced risk for aortic atherosclerosis [38]. After 1 month of treatment with the CETP inhibitor JTT-705, patients developed significant reduction in CETP activity and up to a 37% elevation in serum HDL [39•]. Clearly, CETP inhibition offers a potentially important and novel means by which to modulate serum HDL levels and possibly decrease risk for future CAD-related events. A prominent group of investigators in this area has called for the design and implementation of new, long-term outcome trials to more fully explore the clinical utility of these approaches [40].

Hepatic Lipase

Hepatic lipase (HL) is synthesized in hepatocytes and has both phospholipase A1 and triglyceride lipase activities and plays an important role in HDL metabolism. HL is bound to the heparan sulfate proteoglycans of hepatocytes in the space of Disse and the endothelium lining hepatic sinusoids. HL hydrolyzes triglycerides and phospholipids during the conversion of larger, more buoyant HDL_2 to the smaller and denser HDL_3. During

this conversion, HL is believed to participate in RCT by facilitating the release of apoA-I and the reformation of pre–β-HDL. However, as in the case of PLTP, this may yet prove incorrect when tested in vivo. Increased HL activity is also potentially proatherogenic by virtue of its ability to decrease circulating levels of HDL_2 (considered by some investigators to be more antiatherogenic than HDL_3) and catalyze the conversion of large, buoyant LDL to its more atherogenic small, dense form. Reductions in serum HDL_2 may also result in decreased hydrolysis of the triglycerides in VLDL by HL [41], thereby further potentiating the atherogenicity of a given patient's lipoprotein distribution. Consequently, the effects of HL on risk for CAD are difficult to quantify because polymorphisms in the gene for HL can generate simultaneous changes in multiple lipoprotein fractions and in the size distribution of a given class of lipoprotein.

A variety of studies have attempted to evaluate the effects of HL polymorphisms on serum HDL levels. It is estimated that allelic variation in the gene for HL is responsible for 25% of the variation in HDL levels among humans [42]. The overexpression of HL is largely responsible for a very high incidence of low HDL among Turkish men (53%) and women (26%) [43], which likely contributes significantly to the high rate of premature CAD in this population. In one recent study of premenopausal, normolipidemic women, the presence of the C514→T allele was associated with significantly higher levels of HDL_2 and large, buoyant LDL compared with the CC allele control subjects [44]. Even though promoter polymorphisms in the HL gene such as G250→A and C514→T can give rise to elevations in HDL by producing reductions in enzyme activity, it is still not clear if the net effect of these mutations on lipoprotein metabolism yields reductions in risk for CAD. A number of studies have suggested that when low HL activity is combined with CETP deficiency, risk for CAD actually increases [45•]. Consequently, in order to positively impact CAD risk, the modulation of HL activity may require specific genetic and metabolic backgrounds. Clearly, this is an area that will require considerable additional investigation.

Increased HL activity does appear to be detrimental in patients with hypertriglyceridemia and insulin resistance. As demonstrated in the Familial Atherosclerosis Treatment Study, the inhibition of HL with antilipidemic medications in patients with CAD and hypertriglyceridemia can stimulate coronary artery plaque regression [46]. In patients with insulin resistance, HL activity is increased and they frequently have hypertriglyceridemia secondary to increased free fatty acid flux into the liver and increased VLDL secretion. In these patients, CETP enriches HDL and LDL with triglyceride. The increased triglyceride content renders these molecules better substrates for HL, resulting in augmented catabolism of HDL and increased production of small, dense LDL [47].

Endothelial Lipase

Endothelial lipase (EL) is a more recently identified lipase that preferentially hydrolyzes acyl chains at the sn-1 position of phospholipids. It is produced and secreted by endothelial cells. Two groups have shown that when EL activity is decreased in mice either by polyclonal antibody inhibition [48] or inactivation by gene targeting [49], serum HDL levels increase significantly (25% to 60%). Additional investigation will be required to more fully elucidate the precise role(s) of EL in human HDL metabolism and RCT. The regulation of this enzyme's activity and the effects of EL gene polymorphisms on risk for CAD will also have to be explored. Clearly, however, EL does pose an exciting and potentially important means of modulating serum HDL levels in the future.

Hepatic High-density Lipoprotein Receptors

The delivery of cholesteryl esters back to the liver for elimination in bile or conversion into bile salts is the final step in RCT. Scavenger receptor class B type I (SR-BI) is located on the hepatocytes' surface and is concentrated in caveolae [50]. SR-BI is a high-affinity receptor for HDL and mediates selective cholesteryl ester uptake into hepatocytes [51]. In murine models, SR-BI deficiency reduces biliary cholesterol, increases cholesteryl ester-enriched HDL, and leads to increased rates of atherogenesis [52]. In contrast, the overexpression of SR-BI in mice increases biliary cholesterol, markedly reduces serum levels of HDL, and is antiatherogenic [53]. These experiments elegantly illustrate the widely acknowledged concept that it is not the absolute level of HDL that determines risk for atherosclerotic disease, but rather the RCT pathway's overall capacity to affect adequate delivery of excess cholesterol from the periphery back to the liver for disposal.

The interaction of SR-BI with HDL is complex. Selective cholesteryl ester uptake by SR-BI is dependent on interactions with multiple α-helical segments in apoA-I [54], and targeted disruption of these sites leads to reduced capacity for lipid transfer [55]. Initial models for SR-BI suggested that this receptor tethered HDL to the external face of the hepatocyte plasma membrane and induced cholesteryl ester transfer with subsequent release of the delipidated HDL particle. A more recent model suggests that in polarized cells, SR-BI mediates selective transcytosis, with HDL trafficking through the endocytic compartment [56]. HDL transcytosis results in the secretion of HDL cholesterol across the apical membrane, whereas the delipidated HDL particle is secreted across the basal cell surface and back into the circulation. Cholesteryl ester internalized by SR-BI is channeled to a membrane compartment where it is hydrolyzed to free cholesterol by a neutral cholesteryl ester hydrolase [57].

Additional hepatic HDL receptors are beginning to be identified and characterized. The β-chain of the mitochondrial F1-ATP synthetase is expressed on the surface

of hepatocytes and functions as a high-affinity HDL receptor [58]. When this receptor binds apoA-I, HDL undergoes endocytosis in a reaction that hydrolyzes ATP. Another cell surface receptor, termed glycosylphosphatidylinositol-anchored HDL-binding protein 1, binds HDL with high affinity and mediates selective lipid uptake in mice [59]. It will be of interest to determine how many other hepatic HDL receptors participate in RCT and how, if at all, these receptors interact among themselves and with other cell regulatory systems to coordinate systemic cholesterol clearance.

Conclusions

High-density lipoproteins are highly complex lipoproteins with diverse antiatherogenic functions. Serum HDL levels are regulated by a large number of transcription factors, intracellular signaling pathways, cell surface receptors, and enzymes. The rate of RCT depends upon cellular capacity for cholesterol mobilization, HDL speciation and enzymatic remodeling in serum, and the efficiency of cholesterol transfer to the liver for disposal. Clearly, any number of polymorphisms during each step of the RCT pathway can positively or negatively impact this process. The level of HDL in serum also does not necessarily reflect the capacity for RCT. Advances in genomics [60] and proteomics will likely help elucidate key additional new molecular level insights into how RCT might be better modulated for preventing the development and progression of atherosclerotic disease. The growth of molecular cardiology will also likely assist the practicing clinician in more precisely identifying the molecular lesion(s) responsible for low circulating levels of HDL, thereby offering opportunities for more specific and efficacious therapeutic interventions aimed at increasing the rate of RCT.

A large number of studies support the institution of lifestyle modification and pharmacologic intervention for increasing serum HDL levels. When evaluated across populations, high levels of HDL are atheroprotective, whereas low levels appear to predispose patients toward the development of CAD. Many investigators feel that despite the results of VA-HIT and mechanistic information on HDL in both human and animal studies, the data from lipid intervention trials still do not adequately justify treating low serum levels of HDL. Because HDL metabolism is so intimately coupled to the metabolism of other lipoproteins and there is as yet no drug that specifically raises HDL independent of changes in other lipoprotein fractions, definitive proof for the HDL hypothesis will likely come from more detailed studies of RCT in vivo. Given the results of studies performed with ABCA1 and SR-BI, the induced overexpression of these cell surface constituents poses a potentially attractive therapeutic modality. There is good reason to support the call for large-scale clinical trials evaluating the effect of CETP inhibition on HDL levels and risk for CAD. However, the modulation of other enzyme components in the RCT pathway (PLTP, HL, EL, and LCAT), both alone and in combination, will require the availability of specific inhibitors and greater investigation in animal models. There is a great deal of information that could still be extracted from frozen plasma samples obtained from a number of the statin and fibrate trials. Perhaps these samples can be used to more precisely map out the effects of these drugs, if any, on HDL production and the component steps of RCT. There is also much more work that will have to be done to quantify the relative antiatherogenicity and effects on cardiovascular outcomes of various HDL subfractions (ndHDL, HDL_3, and HDL_2).

Even under the controlled circumstances of a clinical trial, only 20% to 35% of patients derive risk reduction for cardiovascular events with therapies targeted at LDL lowering. A huge gap remains, but perhaps a substantial portion of this gap will be closed by therapies more selectively aimed at raising HDL and augmenting its potential for RCT.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Maron DJ: **The epidemiology of low levels of high-density lipoprotein cholesterol in patients with and without coronary artery disease.** *Am J Cardiol* 2000, **86(suppl):**11L–14L.
2. Rubins HB, Robins SJ, Collins D, *et al.*: **Gemfibrozil for the secondary prevention of coronary heart disease in men with low HDL-cholesterol.** *N Engl J Med* 1999, **341:**410–418.
3. •• Sacks FM, for the Expert Group on HDL Cholesterol: **The role of high-density lipoprotein (HDL) cholesterol in the prevention and treatment of coronary heart disease: expert group recommendations.** *Am J Cardiol* 2002, **90:**139–143.

Expert group summary of epidemiologic and clinical intervention data on HDL. The paper clearly delineates the importance of using serum HDL levels for evaluating risk for atherosclerotic disease and supports pharmacologic intervention and lifestyle modification for raising HDL.

4. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: **Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).** *JAMA* 2001, **285:**2486–2497.
5. Johnson CL, Rifkind BM, Sempos CT, *et al.*: **Declining serum total cholesterol levels among US adults: the National Health and Nutrition Examination Surveys.** *JAMA* 1993, **269:**3002–3008.
6. • J Nofer, Kehrel B, Fobker M, *et al.*: **HDL and arteriosclerosis: beyond reverse cholesterol transport.** *Atherosclerosis* 2002, **161:**1–16.

A comprehensive review of the antiatherogenic effects of HDL. This paper addresses many of the detailed biochemical mechanisms involved in HDL's ability to inhibit oxidation, inflammation, thrombosis, endothelial cell dysfunction, adhesion molecule expression, and apoptosis. It also summarizes current insights into how HDL participates in intracellular signaling mechanisms.

7. Downs JR, Clearfield M, Weis S, *et al.*: **Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels. Results of AFCAPS/TexCAPS.** *JAMA* 1998, **279:**1615–1622.

8. Assman G: **Pro and con: high-density lipoprotein, triglycerides, and other lipid subfractions are the future of lipid management.** *Am J Cardiol* 2000, **87(suppl):**2B–7B.
9. Sorci-Thomas MG, Thomas MJ: **The effects of altered apolipoprotein A-I structure on plasma HDL concentration.** *Trends Cardiovasc Med* 2002, **12:**121–128.
10. Miller M, Aiello D, Pritchard H, *et al.*: **Apolipoprotein A-I Zavalla (Leu159—>Pro): HDL cholesterol deficiency in a kindred associated with premature coronary artery disease.** *Arterioscler Thromb Vasc Biol* 1998, **18:**1242–1247.
11. Li D, Weng S, Yang B, *et al.*: **Inhibition of arterial thrombus formation by apoA-I Milano.** *Arterioscler Thromb Vasc Biol* 1999, **19:**378–383.
12. Maxfield FR, Wustner D: **Intracellular cholesterol transport.** *J Clin Invest* 2002, **110:**891–898.
13. Tabas I: **Consequences of cellular cholesterol accumulation: basic concepts and physiological implications.** *J Clin Invest* 2002, **110:**905–911.
14. Brooks-Wilson A, Marcil M, Clee SM, *et al.*: **Mutations in ABCA1 in Tangier disease and familial high-density lipoprotein deficiency.** *Nat Genet* 1999, **22:**336–345.
15. Venkateswaran A, Laffitte BA, Joseph SB, *et al.*: **Control of cellular cholesterol efflux by the nuclear receptor LXRα.** *Proc Natl Acad Sci* 2000, **97:**12097–12102.
16. Attie AD, Kastelein JP, Hayden MR: **Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis.** *J Lipid Res* 2001, **42:**1717–1726.
17. Zha X, Gauthier A, Genest J, *et al.*: **Secretory vesicular transport from the Golgi is altered during ATP-binding cassette protein A1 (ABCA1) mediated cholesterol efflux.** *J Biol Chem* 2003, in press.
18. Oram JF: **ATP-binding cassette transporter A1 and cholesterol trafficking.** *Curr Opin Lipidol* 2002, **13:**373–381.
19. Chroni A, Liu T, Gorshkova I, *et al.*: **The central helices of ApoA-I can promote ATP-binding cassette transporter A1 (ABCA1)-mediated lipid efflux.** *J Biol Chem* 2003, **278:**6719–6730.
20. Vaisman BL, Lambert G, Amar M, *et al.*: **ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice.** *J Clin Invest* 2001, **108:**303–309.
21. Singaraja RR, Fiever C, Castro G, *et al.*: **Increased ABCA1 activity protects against atherosclerosis.** *J Clin Invest* 2002, **110:**35–42.
22. Clee SM, Zwinderman AH, Engert JC, *et al.*: **Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease.** *Circulation* 2001, **103:**1198–1205.
23.•• Clee SM, Kastelein JP, van Dam M, *et al.*: **Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes.** *J Clin Invest* 2000, **106:**1263–1270.

This paper evaluates the effect of ABCA1 mutations in 77 heterozygous patients and unaffected family members. It clearly demonstrates that decreased ABCA1 activity is associated with reduced serum HDL cholesterol and increased risk for CAD.

24. Hong SH, Rhyne J, Zeller K, *et al.*: **ABCA1Alabama: a novel variant associated with HDL deficiency and premature coronary artery disease.** *Atherosclerosis* 2002, **164:**245–250.
25. Kasid A, Rhyne J, Zeller K, *et al.*: **A novel TC deletion resulting in Pro260—>Stop in the human LCAT gene is associated with a dominant effect on HDL-cholesterol.** *Atherosclerosis* 2001, **156:**127–132.
26. Hoeg J, Santamarina-Fojo S, Berard A, *et al.*: **Overexpression of lecithin:cholesterol acyltransferase in transgenic rabbits prevents diet-induced atherosclerosis.** *Proc Natl Acad Sci* 1996, **93:**11448–11453.
27. Roosbeck S, Vanloo B, Duverger N, *et al.*: **Three arginine residues in apolipoprotein A-I are critical for activation of lecithin:cholesterol acyltransferase.** *J Lipid Res* 2001, **42:**31–40.
28. Martin-Campos JM, Julve J, Escola JJ, *et al.*: **ApoA-IMallorca impairs LCAT activation and induces familial hypoalphalipoproteinemia.** *J Lipid Res* 2002, **43:**115–123.
29. Husskonen J, Olkkonen VM, Jauhiainen M, *et al.*: **The impact of phospholipid transfer protein (PLTP) on HDL metabolism.** *Atherosclerosis* 2001, **155:**269–281.
30. Cheung M, Wolfbauer G, Brown B, *et al.*: **Relationship between plasma phospholipid transfer protein activity and HDL subclasses among patients with low HDL and cardiovascular disease.** *Atherosclerosis* 1999, **142:**201–205.
31. van Haperen R, van Tol A, Vermeulen P, *et al.*: **Human phospholipid transfer protein increases the antiatherogenic potential of high density lipoproteins in transgenic mice.** *Arterioscler Thromb Vasc Biol* 2000, **20:**1082–1088.
32. van Haperen R, van Tol A, van Gent T, *et al.*: **Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein.** *J Biol Chem* 2002, **277:**48938–48943.
33. Yamashita S, Maruyama T, Hirano K, *et al.*: **Molecular mechanisms, lipoprotein abnormalities and atherogenicity of hyperalphalipoproteinemia.** *Atherosclerosis* 2000, **152:**271–285.
34. Tall A, Jiang X, Silver D: **George Lyman Duff memorial lecture: lipid transfer proteins, HDL metabolism, and atherogenesis.** *Arterioscler Thromb Vasc Biol* 2000, **20:**1185–1188.
35. Moriyama Y, Okamura T, Inazu A, *et al.*: **A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency.** *Prev Med* 1998, **27:**659–667.
36. Brousseau ME, O'Connor JJ, Ordovas JM, *et al.*: **Cholesteryl ester transfer protein TaqI B2B2 genotype is associated with higher HDL cholesterol levels and lower risk of coronary heart disease endpoints in men with HDL deficiency. Veterans Affairs HDL Cholesterol Intervention Trial.** *Arterioscler Thromb Vasc Biol* 2002, **22:**1148–1154.
37. Wu JH, Lee YT, Hsu HC, *et al.*: **Influence of CETP gene variation on plasma lipid levels and coronary heart disease: a survey in Taiwan.** *Atherosclerosis* 2001, **159:**451–458.
38. Rittershaus CW, Miller DP, Thomas LJ, *et al.*: **Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2000, **20:**2106–2112.
39.• de Grooth GJ, Kuivenhoven JA, Stalenhoef AF, *et al.*: **Efficacy and safety of novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomised phase II dose-response study.** *Circulation* 2002, **105:**2159–2165.

Demonstrates that specific CETP inhibition in humans increases serum levels of high-density lipoprotein.

40. Barter PJ, Brewer HB, Chapman MJ, *et al.*: **Cholesteryl ester transfer protein. A novel target for raising HDL and inhibiting atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2003, **23:**160–167.
41. Ramsany TA, Boucher J, Brown RJ, *et al.*: **HDL regulates the displacement of hepatic lipase from cell surface proteoglycans and the hydrolysis of VLDL triacylglycerol.** *J Lipid Res* 2003, in press.
42. Cohen J, Wand Z, Grundy S, *et al.*: **Variation at the hepatic lipase and apolipoprotein AI/CIII/AIV loci is a major cause of genetically determined variation in plasma HDL cholesterol levels.** *J Clin Invest* 1994, **94:**2377–2384.
43. Bersot TP, Vega GL, Grundy SM, *et al.*: **Elevated hepatic lipase activity and low levels of high-density lipoprotein in a normotriglyceridemic, nonobese Turkish population.** *J Lipid Res* 1999, **40:**432–438.
44. Carr MC, Ayobi AF, Murdoch SJ, *et al.*: **Contribution of hepatic lipase, lipoprotein lipase, and cholesteryl ester transfer protein to LDL and HDL heterogeneity in healthy women.** *Arterioscler Thromb Vasc Biol* 2002, **22:**667–673.
45.• Jansen H, Verhoeven AJ, Sijbrands EJ: **Hepatic lipase: a pro- or anti-atherogenic protein?** *J Lipid Res* 2002, **43:**1352–1362.

Exhaustive and penetrating review on how hepatic lipase activity may impact risk for atherosclerotic disease.

46. Zambon A, Hokanson JE, Brown BG, *et al.*: **Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density.** *Circulation* 1999, **99:**1959–1964.
47. Ginsberg H: **Insulin resistance and cardiovascular disease.** *J Clin Invest* 2000, **106:**453–458.
48. Jin W, Millar JS, Broedl U, *et al.*: **Inhibition of endothelial lipase causes increased HDL cholesterol levels in vivo.** *J Clin Invest* 2003, **111:**357–362.
49. Ishida T, Choi S, Kundu RK, *et al.*: **Endothelial lipase is a major determinant of HDL level.** *J Clin Invest* 2003, **111:**347–355.
50. Trigatti BL, Rigotti A, Braun A: **Cellular and physiological roles of SR-BI, a lipoprotein receptor which mediates selective lipid uptake.** *Biochim Biophys Acta* 2000, **1529:**276–286.
51. Krieger M: **Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems.** *J Clin Invest* 2001, **108:**793–797.
52. Van Eck M, Twisk J, Hoekstra M, *et al.*: **Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver.** *J Biol Chem* 2003, in press.
53. Kozarsky KF, Donahee MH, Glick JM, *et al.*: **Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse.** *Arterioscler Thromb Vasc Biol* 2000, **20:**721–727.
54. Williams DL, Llera-Moya M, Thuahnai ST, *et al.*: **Binding and cross-linking studies show that scavenger receptor BI interacts with multiple sites in apolipoprotein A-I and identifies the class A amphipathic α-helix as a recognition motif.** *J Biol Chem* 2000, **275:**18897–18904.
55. Liu T, Krieger M, Kan HY, *et al.*: **The effects of mutations in helices 4 and 6 of apoA-I on scavenger receptor class B type I (SR-BI)-mediated cholesterol efflux suggest that formation of a productive complex between reconstituted high density lipoprotein and SR-BI is required for efficient lipid transport.** *J Biol Chem* 2002, **277:**21576–21584.
56. Silver DL, Wang N, Xiao X, *et al.*: **High density lipoprotein (HDL) particle uptake mediated by scavenger receptor class B type I results in selective sorting of HDL cholesterol from protein and polarized cholesterol secretion.** *J Biol Chem* 2001, **276:**25287–25923.
57. Connelly MA, Kellner-Weibel G, Rothblat GH, *et al.*: **Scavenger receptor, Class B, type I (SR-BI)-directed HDL-cholesterol ester hydrolysis.** *J Lipid Res* 2003, in press.
58. Martinez LO, Jacquet S, Esteve JP, *et al.*: **Ectopic β-chain of ATP synthase is an apolipoprotein A-I receptor in hepatic HDL endocytosis.** *Nature* 2003, **421:**75–79.
59. Ioka RX, Kang MJ, Kamiyama S, *et al.*: **Expression cloning and characterization of a novel glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein, GPI-HBP-I.** *J Biol Chem* 2003, **278:**7344–7349.
60. Wang X, Paigen B: **Quantitative trait loci and candidate genes regulating HDL cholesterol. A murine chromosome map.** *Arterioscler Thromb Vasc Biol* 2002, **22:**1390–1401.
61. Toth PP: **High-density lipoprotein metabolism and reverse cholesterol transport.** *Mediguide to Heart Diseases* 2001, **4:**1–8.