1999 George Lyman Duff Memorial Lecture
Lipid Transfer Proteins, HDL Metabolism, and Atherogenesis

Alan R. Tall, Xian-cheng Jiang, Yi Luo, David Silver

Abstract—Plasma high density lipoprotein (HDL) levels show an inverse relationship to atherogenesis, in part reflecting the role of HDL in mediating reverse cholesterol transport. The transfer of HDL cholesterol to the liver involves 3 catabolic pathways: the indirect, cholesteryl ester transfer protein (CETP)–mediated pathway, the selective uptake (scavenger receptor BI) pathway, and a particulate HDL uptake pathway. The functions of the lipid transfer proteins (CETP and phospholipid transfer protein) in HDL metabolism have been elucidated by genetic approaches in humans and mice. Human CETP deficiency is associated with increased HDL levels but appears to increase coronary artery disease risk. Phospholipid transfer protein deficiency, produced by gene knockout in mice, results in decreased HDL levels, reflecting decreased transfer of phospholipids from triglyceride-rich lipoproteins into HDL. Obese (ob/ob) mice have markedly increased HDL levels and represent an interesting model of defective HDL catabolism in the liver. In hepatocytes of wild-type mice, there is extensive uptake and resecretion of HDL and selective uptake of cholesteryl ester from HDL during recycling. In ob/ob mice, these processes are defective, suggesting that HDL recycling plays an important role in holo-HDL catabolism, selective uptake, and the determination of plasma HDL levels. (Arterioscler Thromb Vasc Biol. 2000;20:1185-1188.)

Key Words: HDL ♦ phospholipid transfer proteins ♦ liver x receptor ♦ scavenger receptor BI ♦ obese mouse

The inverse relationship of plasma HDL levels to coronary heart disease (CHD) was first established on a population basis in a cross-sectional study of men of Japanese ancestry living in Hawaii.1 The reverse cholesterol transport (RCT) hypothesis, initially enunciated by Glomset,2 provides a cogent explanation for the protective effect of HDL. According to this proposal, HDL removes cholesterol from peripheral cells, such as arterial wall macrophages. Particular subfractions of HDL, such as the pre-β-HDL fraction,3 may be especially efficient at mediating cholesterol removal from peripheral cells. HDL cholesterol is then esterified by lecithin:cholesterol acyltransferase, forming cholesteryl esters (CEs). HDL CE is subsequently returned to the liver by 3 distinct pathways. In the first pathway, HDL CE is transferred to triglyceride-rich lipoproteins (TRLs) by cholesteryl ester transfer protein (CETP), and then CE-enriched remnants of TRLs are removed from the circulation by receptors in the liver (Figure 1). The second pathway involves the selective uptake pathway, ie, the uptake of HDL CEs without concomitant degradation of HDL protein. This pathway was first elucidated by Pittman and coworkers (Glass et al4) and has recently been shown by Acton et al5 to be mediated by scavenger receptor BI. Finally, there is a pathway that leads to uptake of holo-HDL particles and degradation of HDL-associated proteins such as apoA-I. In part, this pathway may involve the formation of large HDL, enriched in apoE.

The role of CETP in lipoprotein metabolism has been elucidated by human genetic deficiency of CETP. Subjects with homozygous CETP gene–null mutations have massively elevated HDL levels, 3 to 5 times normal.6,7 Heterozygotes have moderate but significant HDL elevations of 10% to 30%. Remarkably, 2 CETP gene mutations (an intron 14 gene-splicing defect and an exon 15 [D442G] missense mutation) are found in ~5% to 7% of the general Japanese population.8 This high mutation frequency allowed us8 to ask the question: would elevated HDL resulting from a defect in the RCT pathway (ie, CETP deficiency) be associated with protection against CHD? To answer this question, we turned to the Honolulu Heart Program cohort of Japanese-American men.1 We found that ~6% of these men had CETP gene mutations, which resulted in slightly elevated HDL levels. However, there was an overall excess of CHD in these men. After adjusting for HDL levels and other risk factors, the odds ratio for CHD was 1.7 in men with CETP gene mutations. The relationship of the mutation to CHD was modified by HDL levels. The entire excess risk of CHD was seen in men with CETP mutations who also had HDL cholesterol levels of 40 to 60 mg/dL, whereas men with HDL cholesterol levels >60 mg/dL had a lower CHD prevalence, irrespective of the presence of the mutation. The overall implication of these findings is that both HDL levels and the flow of cholesterol through the HDL fraction (ie, RCT) are important in determining atherosclerosis risk. These findings along with a large...
body of other work\textsuperscript{3,10–12} support the RCT hypothesis. However, this is not the only explanation for the protective role of HDL, because other properties of HDL, such as its anti-inflammatory and antioxidant effects, are also likely important.\textsuperscript{13,14} In subjects with CETP deficiency and high HDL levels (\textgtr 60 mg/dL), there is a low prevalence of CHD,\textsuperscript{9} perhaps because other beneficial properties of HDL counteract the defect in RCT.

In human cross-sectional studies, plasma CETP levels are positively related to VLDL and LDL cholesterol levels.\textsuperscript{15} A similar relationship is observed in natural flanking region human CETP–transgenic mice.\textsuperscript{16} When the CETP transgene is bred into different backgrounds and the mice are studied while being maintained on atherogenic diets, plasma CETP levels vary over a 15-fold range, and CETP levels are highly correlated with plasma cholesterol levels. This correlation is due to increased transcription of the CETP transgene in the liver and peripheral tissues as a response to hypercholesterolemia. The promoter elements mediating this response have recently been analyzed by promoter-reporter gene analysis in adipocytes and by preparation of transgenic mouse strains with mutations in the CETP promoter.\textsuperscript{17} These studies have revealed that the positive sterol response of the human CETP gene is mediated by a nuclear hormone receptor direct repeat 4 element (i.e., with a spacing of 4 nucleotides between the 2 direct repeats). This element is activated by the transcription factors liver X receptor (LXR)-\textalpha and LXR-\textbeta, which act as heterodimers with retinoid X receptor-\textalpha. LXR-\textalpha has been shown to mediate upregulation of the cholesterol 7\alpha-hydroxylase (Cyp7a1) promoter by dietary cholesterol.\textsuperscript{18,19} Thus, LXRs may coordinate the CETP-mediated catabolism of HDL CEs in the liver, as well as hepatic excretion of cholesterol by its conversion to bile salts (Figure 2).

Biochemical studies as well as analysis of plasma from subjects with genetic CETP deficiency have revealed the existence of a second lipid transfer protein in human plasma.\textsuperscript{20} This protein transfers phospholipids but not neutral lipids between plasma lipoproteins. To evaluate the function of phospholipid transfer protein (PLTP) in lipoprotein metabolism, we recently developed PLTP knockout (PLTP\textsuperscript{0/0}) mice.\textsuperscript{21} In wild-type mice, there is rapid transfer of a major...
fraction of the phospholipids of TRLs into HDL as the TRLs undergo lipolysis. Remarkably, in PLTP0 mice there is an almost complete defect in this transfer process. Moreover, these mice have a major (70%) decrease in HDL phospholipids. There are also approximately proportionate reductions in other HDL components: cholesterol, CEs, and apoA-I. On a high–saturated fat (hydrogenated coconut oil), high-cholesterol diet, these mice accumulate phospholipid, free cholesterol, and apoA-IV–rich vesicular lipoproteins in the IDL-LDL size region (as determined by fast protein liquid chromatography). Detailed analysis indicates that the HDL in PLTP0 mice is protein-rich (primarily apoA-I) and specifically depleted in phosphatidylcholine.22 With the use of autologous HDL from PLTP0 mice, metabolic turnover studies show a 3- to 4-fold increase in the fractional catabolic rate of HDL protein and CEs. This profile is reminiscent of the defect in Tangier disease, wherein a mutation in ATP-binding cassette transporter 1 results in defective transfer of cellular phospholipids and cholesterol onto apoA-I and an associated hypercatabolism of apoA-I.23–25 Because the defect in Tangier disease is more severe than that of PLTP deficiency, PLTP may act downstream of ATP-binding cassette transporter 1 in the HDL maturation process (Figure 3).

As a further model to study the catabolism of HDL, we have been investigating HDL metabolism in ob/ob mice. These mice have markedly increased HDL levels due to a defect in hepatic catabolism.26 Recently, this defect in catabolism has been confirmed by studies on isolated hepatocytes from ob/ob mice.27 The defect is specific for the uptake of HDL, because the liver clears asialoglycoprotein normally and the hepatocytes take up normal or increased amounts LDL.27 In wild-type hepatocytes there is extensive binding, uptake, recycling, and resecretion of HDL. A relatively minor amount of HDL protein is targeted to lysosomes for degradation. HDL recycles through the perinuclear endosomal recycling compartment, where it colocalizes with transferrin. Normally, there is significant selective uptake of HDL CE and free cholesterol. HDL binding, uptake, and recycling are impaired in ob/ob mice, even though the levels of scavenger receptor BI mRNA and protein are normal. These studies suggest that HDL recycling plays an important role in HDL catabolism and selective uptake (Figure 4). The identity of receptors mediating HDL uptake and recycling in hepatocytes is presently unknown.

A challenge for the future will be to develop therapies that increase HDL levels, enhance RCT, and protect against atherosclerosis. Toward this goal, a deeper understanding of HDL metabolism and its regulation is likely to be helpful.

References


1999 George Lyman Duff Memorial Lecture: Lipid Transfer Proteins, HDL Metabolism, and Atherogenesis
Alan R. Tall, Xian-cheng Jiang, Yi Luo and David Silver

doi: 10.1161/01.ATV.20.5.1185
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/5/1185

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/