HIGH-DENSITY LIPOPROTEINS AND ATHEROSCLEROSIS

HIGHLIGHTS
This publication is based on the lectures presented by international experts during the satellite symposium entitled “High-Density Lipoproteins and Atherosclerosis,” held as a satellite meeting from June 19-20, 2009, in Newport, RI, USA following the XV International Symposium on Atherosclerosis (June 14-18, 2009 in Boston, MA, USA).

DISTINGUISHED FACULTY

Bela F. Asztalos, PhD  
Human Nutrition Research Center on Aging  
Tufts University  
Boston, MA, USA  
A New Aspect of HDL Remodeling

H. Bryan Brewer, Jr., MD  
Department of Lipoprotein and Atherosclerosis Research  
Cardiovascular Research Institute  
Washington Hospital Center  
Washington, DC, USA  
ABCA1 Function and Deficiency  
Novel HDL Raising Therapies for the Treatment of High Risk Cardiovascular Patients

John D. Brunzell, MD  
Department of Medicine  
University of Washington  
Seattle, WA, USA  
Role of Hepatic Lipase in HDL Metabolism and Coronary Artery Disease

Laura Calabresi, PhD  
Center E. Grossi Paoletti  
Department of Pharmacological Sciences  
University of Milan  
Milan, Italy  
Human ApoA-I Variants  
Human LCAT Deficiency

M. John Chapman, PhD, DSc  
Dyslipoproteinemia and Atherosclerosis Research Unit  
National Institute for Health and Medical Research  
Paris, France  
Functions of HDL: New Insights

Monty Krieger, PhD  
Biology Department  
Massachusetts Institute of Technology  
Cambridge, MA, USA  
Analysis of the HDL Receptor SR-BI and Its Cytoplasmic Adaptor Protein PDZK1

Jan A. Kuivenhoven, PhD  
Department of Vascular Medicine  
Academic Medical Center  
Amsterdam, The Netherlands  
The Genetics of High-Density Lipoproteins

Stefania Lamon-Fava, MD, PhD  
Human Nutrition Research Center on Aging  
Tufts University  
Boston, MA, USA  
Effects of Alcohol, Estrogens, Niacin, and CETP Inhibition on HDL Metabolism

Hiroshi Mabuchi, MD  
Kanazawa University School of Medicine  
Kanazawa, Japan  
Human CETP Deficiency and CETP Inhibition

George H. Rothblat, PhD  
Children’s Hospital of Philadelphia  
University of Pennsylvania School of Medicine  
Philadelphia, PA, USA  
Cholesterol Flux between Cells and Lipoproteins

Kerry-Anne Rye, PhD  
The Heart Research Institute  
Sydney, Australia  
The Structure, Function, and Anti-inflammatory Properties of HDL
Ernst J. Schaefer, MD
Human Nutrition Research Center on Aging
Tufts University
Boston, MA, USA

Human ApoA-I Deficiency
Conclusion

Alan R. Tall, MD
Division of Molecular Medicine
Department of Medicine
Columbia University
New York, NY, USA

Role of ABCA1 and ABCG1 in Atherogenesis

Gerald F. Watts, DSc, PhD, DM
Metabolic Research Centre
School of Medicine and Pharmacology
University of Western Australia
Perth, Australia

Therapeutic Regulation of HDL Metabolism: Statins and Fibrates

Vassilis I. Zannis, PhD
Molecular Genetics
Boston University School of Medicine
Boston, MA, USA

Apolipoprotein A-I, HDL Biogenesis, and Sites of Regulation

EDITORS OF THE PUBLICATION

Margaret R. Diffenderfer, PhD
Human Nutrition Research Center on Aging
Tufts University
Boston, MA, USA

Ernst J. Schaefer, MD
Human Nutrition Research Center on Aging
Tufts University
Boston, MA, USA
While reduction of low density lipoprotein (LDL) cholesterol is the primary target of cardiovascular prevention, increasing attention has focused on high density lipoprotein (HDL) cholesterol as a secondary target in order to address residual risk. Low HDL cholesterol is widely prevalent in the United States and Europe and is one of the defining features of the metabolic syndrome.

Studies confirm that low HDL cholesterol is independently predictive of cardiovascular risk, even at low levels of LDL cholesterol. However, there is much that is unknown about HDL; and an increasing need to understand, validate, and quantify its role in the atherosclerotic process is emerging, in order to improve diagnosis, prevention, and treatment of cardiovascular diseases. The anti-atherogenic properties of HDL are thought to derive primarily from its role in reverse cholesterol transport, but there is evidence also of antioxidant and anti-inflammatory properties. It is known that several genetic mutations can affect the structure, function, and plasma concentration of HDL; but it is unclear how the mutations affect cardiovascular risk. Lifestyle interventions that reduce cardiovascular risk require long-term commitment; existing drug therapies that raise HDL cholesterol are limited; and it is not clear which pharmacological agents actually augment reverse cholesterol transport. Moreover, the development of treatments intended to raise HDL cholesterol needs to take into account not only the absolute levels of HDL cholesterol, but more importantly the functional quality of HDL.

A need for expert consensus on these and other issues surrounding HDL persists, in order to understand better the pathophysiology of atherosclerosis, direct the future course of research, and design interventions that effectively reduce residual risk in various patient populations.

This satellite symposium presented state-of-the-art knowledge on HDL and HDL particles. Its aim was to:

- Describe the role of HDL in the atherosclerotic process.
- Identify the steps involved in the metabolism of HDL and reverse cholesterol transport.
- Discuss mechanisms of cholesterol efflux with increased knowledge of the underlying biological processes.
- Define the genes affecting HDL structure, function, and metabolism.
- Assess recent experimental, observational, and clinical studies related to HDL function and augmentation in terms of their potential applicability in clinical practice.
- Present existing and potential strategies for increasing HDL cholesterol levels.
HDL has antioxidant, anti-inflammatory, and anti-thrombotic properties which also contribute to its atheroprotective effect.

ApoE is another significant protein found in HDL particles. Studies have documented that apoE can form its own HDL particle, separate from those containing apoA-I. As the only medium-sized apolipoprotein present in the brain, apoE may be crucial for regulating lipid homeostasis in the central nervous system. The interaction of apoE with ABCA1 in the brain promotes cholesterol efflux and leads to the biogenesis of apoE-containing HDL. Once formed, apoE-containing HDL can interact with SR-BI; and this interaction leads to both the selective uptake of cholesteryl esters and the efflux of free cholesterol with virtually no degradation of the HDL particle. ApoE-containing HDL appears to have antioxidant and anti-inflammatory functions in the brain, similar to those attributed to apoA-I-containing HDL in the plasma. It has been shown that apoE-containing HDL prevents amyloid deposition and plaque formation, key processes that can cause dementia and Alzheimer’s disease. A better understanding of the role of apoE in the central nervous system may be critical to research in the area of dementia prevention.

The APOAI gene belongs to a gene cluster on chromosome 11 which encodes the genes for apoA-I, apoC-III, and apoA-IV and is important for HDL metabolism. The
regulation of the genes in this cluster appears to be linked. An enhancer 3′ to APOC3 is known to affect APOAI expression as well as that of APOC3, and both the APOC3 enhancer and the APOAI promoter are essential for the normal expression of APOAI. When the APOC3 enhancer was mutated in vivo in mice, the intestinal expression of APOAI was abolished; and hepatic expression was reduced to ~20% of the wild-type control. Mutations in the APOAI promoter alone reduced hepatic and intestinal expression to approximately 15% of the WT control.

Deletions in the APOAI/APOC3/APOA4 gene cluster are associated with severe HDL deficiency and marked coronary risk. Patients in which the entire gene complex is deleted have plasma HDL cholesterol levels below 10 mg/dL, low levels of triglycerides, moderate fat malabsorption, and premature heart disease. Those lacking APOAI and APOC3 but expressing APOA4, due to a rearrangement of DNA, also have greatly reduced HDL cholesterol levels but no abnormality in fat absorption. Patients who lack only APOAI have normal triglyceride levels and relatively normal amounts of apoE- and apoA-IV-containing HDL particles. The plasma levels of HDL cholesterol in these patients, however, are less than 5 mg/dL; and the patients are at increased risk of developing coronary heart disease (CHD). These data indicate that the expression of APOAI is essential for the normal formation of HDL subparticles but not necessarily for the efflux of cholesterol from cells.

Low plasma concentrations of HDL cholesterol can also be attributed to molecular variation in the APOAI gene; but unlike complete apoA-I deficiency which is definitely associated with premature coronary disease, the marked reductions of HDL cholesterol caused by subtle mutations in the APOAI gene sequence do not necessarily lead to enhanced coronary risk. To illustrate, the apoA-I (R173C) substitution in apoA-I Milano results in greatly reduced plasma concentrations of HDL cholesterol, apoA-I, and apoA-II. Carriers of the mutation, however, show no evidence of preclinical atherosclerosis; and their plasma LDL cholesterol levels are normal. One explanation for this apparent paradox may stem from the properties of the HDL particles isolated from the apoA-I Milano carriers. Lipid-free apoA-I Milano HDL particles are more efficient than HDL particles isolated from healthy controls in promoting cellular cholesterol efflux through both the ABCA1 and SR-BI pathways, respectively, and in preserving vascular function and endothelial cell homeostasis. In contrast, carriers of other missense apoA-I mutations leading to hypoalphalipoproteinemia, like the apoA-I (L178P), present with accelerated carotid arterial wall thickening and endothelial dysfunction. This dramatic difference in the clinical phenotype of two point mutations in the APOAI gene, both leading to similar HDL deficiency, demonstrates that HDL cholesterol levels do not necessarily reflect the efficiency of reverse cholesterol transport or the functionality and atheroprotective potential of HDL particles.
The atheroprotective properties of HDL are thought to relate primarily to the ability of HDL and its major apolipoprotein, apoA-I, to remove excess cholesterol from macrophage foam cells in atheromatous blood vessels as well as from other tissues. Four apparently distinct and unrelated pathways for the efflux of cholesterol have been defined: (1) ABCA1-mediated unidirectional efflux to lipid-poor apoproteins or preβ1-migrating HDL particles; (2) ABCG1-mediated unidirectional efflux to mature (α-migrating), phospholipid-containing HDL; (3) SR-B1-mediated bidirectional efflux and influx to and from mature HDL particles; and (4) passive aqueous diffusion, a relatively inefficient bidirectional efflux and influx to phospholipid-containing HDL particles. Each of these pathways and, specifically, the efficiency of the pathway in regulating the cholesterol content within the cell, is key to the composition, functionality, and metabolism of HDL.

The impact of cholesterol efflux on HDL composition is clearly illustrated by the genetic defect in Tangier Disease. Named after the Chesapeake Bay island home of the two original probands, Tangier Disease is a rare familial HDL deficiency state characterized by the accumulation of cholesteryl esters in various tissues of the body, most notably in macrophages. The accrual of cholesteryl ester leads to hyperplastic orange tonsils, lymphadenopathy, hepatosplenomegaly, and, often, peripheral neuropathy and premature CHD. Homozygotes typically have extremely low HDL cholesterol and apoA-I levels, modest hypertriglyceridemia, and LDL cholesterol levels that are about 50% of normal. The disease is caused by mutations in the gene encoding ABCA1 which prevent the transporter from functioning properly. Without normal cholesterol efflux, the plasma of Tangier Disease patients contain only preβ1-migrating HDL composed of apoA-I and phospholipid. These abnormal
particles are rapidly catabolized, resulting in the absence of normal circulating HDL in homozygotes. These data indicate a crucial role for ABCA1 in facilitating cellular cholesterol efflux.

ABCG1, like ABCA1, has a major role in promoting cholesterol efflux. Being unidirectional, both ABCG1- and ABCA1-mediated efflux leads to the net removal of cell cholesterol. Recent studies from several different laboratories indicate that, despite the difference in the acceptor HDL particle, the efflux activities of these two transporters are mutually compensatory. When one transporter is deficient, the other is induced as the result of sterol accumulation and the activation of the liver X receptor (LXR).

Macrophages with combined deficiency of ABCA1 and ABCG1 were shown in vitro by Tall et al. to have major defects in the efflux of cholesterol to apoA-I, apoE, HDL, and whole sera. Net efflux was reduced as much as 60-100%. Likewise, mice with a combined deficiency of ABCA1 and ABCG1 showed a marked acceleration of atherosclerosis. Compared to wild-type mice or mice with a single knockout, the double-knockout animals had a much greater accumulation of cholesteryl esters in the peritoneal macrophages and developed a dramatic myeloproliferative disorder, with marked leukocytosis, splenomegaly, and foam cell and myeloid cell infiltration in various organs. Moreover, transplantation of Abca1<sup>-/-</sup> Abcg1<sup>-/-</sup> bone marrow into LDL receptor-deficient (Ldlr<sup>+/−</sup>) mice fed either a Western-type diet (15% cholesterol and saturated fat) or a chow diet led to increased atherosclerosis, compared to mice receiving bone marrow with the single-knockout of ABCA1 or ABCG1; and the severity of the atherosclerosis was relative to the level of the plasma cholesterol. It has been observed, however, that the overexpression of apoA-I reduces dietary atherosclerosis and protects against foam cell infiltration in both animal models and humans, independent of the absence of the two transporters. This suggests that multiple cellular and metabolic mechanisms, beyond that of cholesterol efflux, are relevant in promoting plaque regression and stabilization.

In addition to a key role in reverse cholesterol transport, ABCG1-mediated cholesterol efflux appears to have anti-inflammatory and antioxidant properties which are specific to this efflux pathway. ABCG1 and HDL can facilitate the efflux of 7-ketocholesterol in transfected macrophages. ABCA1 and apoA-I have no ability to stimulate the removal of this oxysterol. 7-ketocholesterol is a spontaneously formed cholesterol oxidation product that is present in processed foods and high-cholesterol diets and is the most abundant oxysterol in oxidized LDL and in human atherosclerotic plaques. Dietary 7-ketocholesterol is normally absorbed on chylomicrons, rapidly cleared from the circulation in remnants, and converted into bile salts in the liver. However, 7-ketocholesterol is apparently cytotoxic at some concentrations found in vivo, inducing the apoptosis and necrosis of endothelial cells and macrophages. The ability of ABCG1 to stimulate the removal of 7-ketocholesterol, coupled with the fact that ABCG1 is highly expressed in endothelial cells, suggests that large HDL<sub>2</sub> particles that promote sterol efflux via ABCG1 may have a particular role in protecting endothelial cells and macrophages from the deleterious effects of oxysterols consumed in the diet or formed on LDL. This could be important in maintaining normal endothelial functions and in stabilizing atherosclerotic plaque.

A number of questions have been raised by Rothblat and colleagues regarding the movement of free cholesterol between macrophages and HDL, among them (1) how the intracellular cholesterol content influences cholesterol efflux and (2) whether cholesterol efflux is associated with carotid intimal-medial thickness (CIMT), a well-recognized index of preclinical atherosclerosis. To address the first question, the change in cellular cholesterol mass was measured in vitro, using cholesterol-normal and cholesterol-enriched macrophages exposed for eight hours to HDL isolated from

In addition to a key role in reverse cholesterol transport, ABCG1-mediated cholesterol efflux appears to have anti-inflammatory and antioxidant properties which are specific to this efflux pathway.
normolipidemic sera. It was observed that the incubation of the cholesterol-normal cells with HDL resulted in a small increase in cell cholesterol mass, whereas the incubation of the cholesterol-enriched cells with the same HDL preparations effected a decrease in cell cholesterol content. Such data indicate that the net flux of cholesterol mass is a function of the initial cholesterol content of the macrophages.

To determine whether cholesterol efflux predicts CIMT, cholesterol efflux from macrophages was assessed using HDL isolated from normolipidemic individuals with no clinically-evident heart disease but who had been examined and measured for CIMT. Cholesterol efflux in these subjects was negatively correlated with CIMT. The inverse association became stronger when known risk factors - age, gender, and blood pressure, and apoA-I and HDL cholesterol levels assessed independently - were included in the statistical model. The results support the concept that plasma HDL cholesterol and apoA-I concentrations do not fully reflect the functionality of HDL particles.

Hence, a measure of HDL functionality, like cholesterol efflux, may have value in predicting atherosclerotic risk beyond determinations of HDL cholesterol or apoA-I levels. It has been demonstrated in vitro that HDL isolated from individuals with the same plasma concentration of HDL cholesterol or of apoA-I can efflux cholesterol from macrophages with different efficiency and, moreover, the HDL with high efficiency efflux has enhanced efflux through the ABCA1 pathway. ABCA1-mediated efflux is associated with small, preβ1-migrating HDL particles, and increased levels of these particles have been associated with the presence of atherosclerotic lesions.

**Session 3: Cholesterol Esterification and HDL Functions**

Kerry-Anne Rye, PhD  
*The Heart Research Institute, Sydney, Australia*

Laura Calabresi, PhD  
*University of Milan, Milan, Italy*

Evidence from *in vitro* and *in vivo* studies points to HDL as a potent anti-inflammatory agent.

After more than five decades of research, atherosclerosis is no longer regarded as simply the excessive accumulation of lipids in the artery wall. It is now understood in terms of a chronic inflammatory disorder that is characterized by the presence of macrophages and other inflammatory cells in the arterial intima. Evidence from *in vitro* and *in vivo* studies points to HDL as a potent anti-inflammatory agent.

HDL appears to inhibit the inflammation associated with atherosclerotic plaque development, including the initial step, where circulating leukocytes become tethered to the endothelial surface and migrate into the artery wall. Under normal circumstances the endothelium is resistant to leukocyte adhesion. Pro-inflammatory stimuli, like diets high in saturated fat, hyperglycemia, hypercholesterolemia, obesity, insulin resistance, and hypertension, can activate the endothelium by increasing the levels of circulating cytokines, like tumor necrosis factor-α (TNF-α). Activation of the cytokines, in turn, up-regulates endothelial expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and facilitates the tethering of the captured cells to the endothelial surface.
In a series of recent experiments looking at the role of HDL in acute vascular inflammation, Rye and colleagues administered intravenous infusions of saline, lipid-free apoA-I, or discoidal apoA-I reconstituted HDL (rHDL) consisting of a single apoA-I complexed with phospholipids to normocholesterolemic New Zealand White rabbits that had a non-occlusive Silastic periarterial collar inserted around the left common carotid artery. The infusion of the discoidal (apoA-I)rHDL essentially abolished the infiltration of neutrophils into the intima/media of the collared arteries as well as the collar-stimulated increase in endothelial expression of VCAM-1 and ICAM-1. Moreover, lipid-free apoA-I was found to inhibit acute arterial inflammation as effectively as (apoA-I)rHDL. These anti-inflammatory effects cannot be explained simply in terms of an increase in the concentration of plasma HDL, since inflammation was inhibited even when apoA-I was administered at a dose no greater than 10% of the plasma apoA-I pool. Rather, the results indicate that when lipid-free apoA-I is infused into animals, it assumes anti-inflammatory properties that are much greater than those of the bulk, endogenous HDL.

A similar series of experiments looked at the anti-inflammatory properties of apoA-II, the other main apolipoprotein found on HDL. In normal human plasma, most of the apoA-II is associated with apoA-II on a particle termed LpA-I:A-II. Infusions of saline and of lipid-free apoA-I were used as the negative and positive controls, respectively. Since the plasma of New Zealand white rabbits contains no endogenous apoA-II, the experimental conditions were clearly defined. The lipid-free apoA-II infusion decreased neutrophil infiltration into the intima/media of the collared arteries significantly, reduced the endothelial expression of VCAM-1 and ICAM-1 by 65% and 55%, respectively, and displaced lipid-free apoA-I from the endogenous rabbit HDL, thereby, producing lipid-free apoA-II and enhancing the anti-inflammatory response. The discoidal (A-II)rHDL infusion was less effective than lipid-free apoA-II in inhibiting acute vascular inflammation. Moreover, the discoidal (A-II)rHDL, not being a substrate for LCAT, fused rapidly, within a minute, with small spherical endogenous (A-I)rHDL to make spherical (A-I/A-II)rHDL and did not displace the apoA-I or produce its anti-inflammatory effects.

In vitro studies in TNF-α-activated human coronary artery endothelial cells have further defined the mechanisms responsible for the anti-inflammatory properties of apoA-II. In these cells the discoidal (A-II)rHDL reduced neutrophil adhesion to the cells and the expression of interleukin-8 (IL-8) on the cell surface. The discoidal (A-II)rHDL also decreased neutrophil expression of the IL-8 receptors, CXCR1 and CXCR2, and, thereby, inhibited neutrophil chemotaxis and trans-endothelial migration.
Both apoA-I and apoA-II therefore, inhibit acute vascular inflammation and do so, in part, by inhibiting the expression of chemokines and their receptors. These observations substantiate the existence of a minor, highly active subpopulation of HDL with profound anti-inflammatory effects. They also suggest that rHDL has potential as a therapeutic agent for treating the acute vascular inflammation that occurs in acute coronary syndromes and stroke.

A fundamentally key aspect of HDL metabolism, in terms the ability of the particle to protect against atherosclerosis, is the recycling of apoA-I and the other HDL apolipoproteins between the lipid-free and the lipid-associated forms, a process known as particle remodeling. This recycling delays the clearance of lipid-free apolipoproteins via the kidney and, thus, maintains HDL levels in the plasma.

LCAT facilitates HDL recycling by converting discoidal particles into larger, spherical ones. As LCAT esterifies the free cholesterol in the HDL discs, the resulting cholesteryl esters are sequestered into the center of the particle; and the discs become spheres. This remodeling depletes the particle surface of cholesterol and establishes a concentration gradient down which additional unesterified cholesterol moves from the cell membranes and other lipoproteins into the HDL fraction. In maintaining the gradient between the cholesterol content of the peripheral cell and HDL in plasma, LCAT has a critical role in the initial steps of reverse cholesterol transport.

LCAT is activated primarily by apoA-I, but it can also esterify cholesterol in discoidal HDL particles that contain other apolipoproteins, like apoA-IV and apoE. It does not, however, esterify cholesterol in discoidal apoA-II-containing particles.

Mutations in the LCAT gene lead to two rare autosomal diseases: familial LCAT deficiency (FLD) and fish-eye disease (FED). In FLD homozygotes, LCAT is absent or lacks catalytic activity. As a result, the plasma of affected individuals contain very little cholesteryl esters. FED is characterized by the inability of LCAT to esterify free cholesterol in the HDL particle, but the enzymatic activity in the apo-B-containing lipoproteins is normal. The plasma of FED carriers has subnormal levels of cholesteryl esters. Carriers of LCAT deficiency have severe corneal opacification and are predisposed to kidney failure.

Recently Calabresi and colleagues identified 16 Italian families with LCAT deficiency, carrying 22 different mutations gene. All eighteen carriers of two mutant LCAT alleles had remarkably low HDL cholesterol, apoA-I, and apoA-II levels, a selective depletion of LpA-I:A-II particles, a predominance of small preβ-migrating HDL, and a complete lack of large, α-migrating HDL_2 particles. Over half of the 47 carriers of one mutant LCAT allele had HDL cholesterols and apoA-I levels significantly lower than the non-carrier controls and an increased number of preβ1-migrating HDL, indicating that LCAT activity is essential in producing larger, spherical HDL particles.

The alternations in lipid profile and distribution of HDL subparticles contributed to marked differences in cellular cholesterol efflux. LCAT deficiency promoted ABCA1-mediated efflux in a gene-dependent manner, due to the increased number of preβ-migrating HDL. It reduced ABCG1-mediated efflux, due to the low plasma HDL cholesterol concentration, and decreased the number of α-migrating HDL. And it caused a reduction in SR-B1-mediated efflux. The result, however, was the net efflux of cholesterol. Despite the shift in HDL subpopulations, LCAT deficient patients were not significantly different from the non-carrier controls. Moreover, the LCAT deficient patients were found to have, in a gene-dose-dependent effect, smaller average- and maximum-CIMT values than age- and sex-matched healthy controls.
These data suggest that although a mutated LCAT genotype may cause a gene-dose-dependent alteration in the plasma lipid and lipoprotein profile, the changes are not necessarily atherogenic. Reverse cholesterol transport continues in an efficient manner, a lot of free cholesterol is returned to the liver, and carotid atherosclerosis is reduced. Patients with familial LCAT deficiency and hypoalphalipoproteinemia, unlike those with homozygous apoA-I deficiency or homozygous Tangier Disease, do not have the same markedly increased risk of heart disease.

HDL particles are extremely heterogeneous, consisting of a number of discrete subpopulations of entities that vary widely in density, size, shape, composition, and surface charge. Classified on the basis of density, there are two main subfractions: HDL2, having a density of 1.063\(<d\)<1.125 g/mL, and HDL3, having a density of 1.125\(<d\)<1.21 g/mL. In terms of size, HDL particles fall into five separate groups ranging from 10.6 to 7.6 nm in diameter. The most abundant apolipoproteins in human HDL, apoA-I and apoA-II, divide HDL into two main subpopulations: LpA-I, particles containing only apoA-I, and LpA-I:A-II, particles containing both apoA-I and apoA-II. LpA-I:A-II particles tend to be smaller and denser than LpA-I particles and prevail in the HDL3 subfraction. Other apolipoproteins associate with HDL particles, including apoE, apoA-IV, and the C-apolipoproteins, creating a number of subclasses which may or may include apoA-I. Indeed, HDL particles apparently serve as transporters of many proteins. A shotgun proteomic approach has recently identified a plethora of associated proteins, among which are oxidation inhibitors like paraoxonase, acute-phase proteins, protease inhibitors, complement factors, and complement regulatory proteins.

Session 4: HDL Metabolism and Remodeling

Bela F. Asztalos, PhD  
*Tufts University, Boston, MA, USA*

M. John Chapman, PhD, DSc  
*National Institute for Health and Medical Research, Paris, France*

Hiroshi Mabuchi, MD  
*Kanazawa University School of Medicine, Kanazawa, Japan*
When subjected to agarose gel electrophoresis, HDL particles can be resolved on the basis of surface charge into subpopulations with either α- or preβ-mobility. Spherical HDL migrate to an α position and include HDL₂ and HDL₃, as well as LpA-I and LpA-I:A-II particles. Lipid-free/lipid-poor apoA-I and discoidal HDL migrate to a preβ position. It is this classification of HDL subpopulations based on surface charge that perhaps best illustrates the HDL remodeling that occurs in the process of reverse cholesterol transport.

Remodeling defines any process that changes the size, shape, surface charge, or composition of HDL and, thereby, influences HDL metabolism. HDL particles are continuously being remodeled by plasma factors like the enzymes LCAT, LPL, HL, and endothelial lipase (EL) and the transfer proteins CETP and phospholipid transfer protein (PLTP) or by cell surface components like ABCA1, ABCG1, and SR-BI. Non-denaturing two-dimensional gel electrophoresis and image analysis of plasma from subjects with genetic mutations or deficiency in key HDL-associated metabolic factors have been used by Asztalos and colleagues to build a working model of HDL remodeling in vivo. Seven steps have been elucidated.

**Step 1. ApoA-I secretion and formation of preβ₁ (very small precursor HDL).**
Provided apoA-I is not deficient and the gene is expressed, the liver and, to a lesser extent, the intestine secrete apoA-I as a lipid-free or lipid-poor entity. Quite rapidly apoA-I picks up phospholipid in the plasma and is converted almost immediately to preβ₁ HDL, a discoidal particle containing 2 molecules of apoA-I. If apoA-I is not secreted, no apoA-I containing particles are present in plasma; there is marked HDL deficiency and severe premature CHD.

**Step 2. Preβ₁ to α₄ (very small α HDL) via ABCA1-mediated cholesterol efflux.**
If both apoA-I and ABCA1 are expressed and functional, the unidirectional efflux of cholesterol and phospholipid from the cell to the preβ₁ particles, promoted by ABCA1, turns the preβ₁ particle to a discoidal α₄ particle containing free cholesterol as well as phospholipid and apoA-I. If the ABCA1 transporter is deficient and cellular cholesterol efflux is impaired, as is the case in Tangier Disease, the plasma contains only preβ₁ particles.
Step 3. α4 to α3 (small spherical α HDL) via LCAT.  
LCAT-activated esterification of the free cholesterol in the α4-migrating particles results in the formation of larger, α3-migrating HDL. The α3-migrating particles contain apoA-II, which is probably secreted by the liver on different lipidated discoidal particles, as well as apoA-I. Hence, α3 particles could be classified as LpA-I:A-II particles. Without LCAT, the plasma consists only of preβ1-migrating HDL, a high concentration of α4-migrating HDL, and large non-lipidated particles formed by the aggregation of poorly lipidated discoidal apoA-I particles.

Step 4. α3 to α2 and α1 (medium and large spherical α HDL) via LPL.  
Larger LpA-I:A-II HDL particles having α2 mobility in agarose gels are formed from the hydrolytic activity of both LCAT and LPL. LPL hydrolyzes the lipids in the apoB-containing lipoproteins and, thereby, converts chylomicrons and very low density lipoproteins (VLDL) into smaller remnant lipoproteins. It also induces the release of apolipoproteins, phospholipid, and free cholesterol for assembly in HDL, and, in turn, increases the size of the particle, forming α1-migrating HDL, a large, spherical LpA-I particle. In LPL-deficient patients, the plasma has some preβ1, preα, and α1 particles containing apoA-I; and apoA-II is generally found on its own particle.

Step 5. α1 converted back to α2 via HL.  
The enzymatic action of LCAT and LPL, increasing the size of the HDL particle, is offset by that of HL. Activation of HL converts the large α1-migrating particles back to α2- and even discoidal α4-migrating HDL. Without HL, this conversion does not happen. Together, the three enzymes catalyze the appearance of four distinct α-migrating particles: α4, a small discoidal LpA-I particle; α3, a relatively small spherical LpA-I:A-II particle; α2, a large spherical LpA-I:A-II particle; and α1, a very large spherical LpA-I particle.

Step 6. Exchange of lipids between HDL and apoB-containing lipoproteins via CETP.  
CETP facilitates the bidirectional net exchange of cholesteryl esters from HDL to apoB-containing lipoproteins and of triglycerides from apoB-containing lipoproteins to HDL. Through this transfer, very large α-mobility apoA-I-containing HDL particles, with the size of LDL, are converted back to α1-migrating particles enriched in triglycerides and a ready substrate for HL to make into α2-migrating particles. In the plasma of homozygous CETP deficient patients, very large abnormal HDL particles containing apoA-I, apoA-II, and apoE are present.

Step 7. Catabolism of apoA-I or recycling.  
Reverse cholesterol transport is a process of continuous recycling. Large HDL particles exchange cholesteryl esters for triglycerides through the action of CETP, and the cholesteryl esters returned to the liver on apo-B-containing lipoproteins are removed by the LDL receptor. Alternatively, HDL cholesterol is returned directly to the liver through the interaction of HDL and the HDL receptor SR-BI. As cholesterol is delivered to the liver for catabolism, cholesterol-enriched HDL particles are depleted of lipid. The particles become smaller in size and are available once again to receive cholesterol effluxed from the peripheral cell via ABCA1 and ABCG1. EL and secretory phospholipase A2 (sPLA2) act on α1-migrating particles and form HDL of preβ1-mobility, SR-BI-mediated uptake produces smaller α-migrating particles and the activity of CETP and of HL results in the formation of discoidal α4-migrating HDL. These smaller particles, especially preβ1 HDL, can be directly catabolized by the kidney.
HDL cannot, and should not be regarded as a single entity whose plasma levels are reflected by the measurement of HDL cholesterol.

Recent proteomic studies have both underscored and expanded the complexity of HDL metabolism. Davidson et al. have demonstrated the relationships that potentially exist between the proteome and lipidome of the HDL subfractions, and their anti-atherogenic activities. In their studies, normolipidemic human HDL was isolated by sequential density ultracentrifugation and then subfractionated by isopycnic density gradient ultracentrifugation into five physicochemically-defined...
particles: light HDL2b (d1.063-1.087 g/mL) and 2a (d1.088-1.110 g/mL) and dense HDL3a (d1.110-1.128 g/mL), 3b (d1.129-1.154 g/mL), and 3c (d1.154-1.170 g/mL). Some 28 distinct proteins were found to associate with these five subpopulations. Based on the observation that multiple proteins coisolate with human HDL – over 100 have been identified by liquid chromatography/electrospray mass spectrometry to-date – and that the plasma abundance of most of these proteins is insufficient to permit one copy for each HDL particle, it has been suggested that specific proteins might be bound to distinct particle species which are differentially distributed across the HDL mobility spectrum. Moreover, it is possible that the distinct subsets of HDL defined by the specific cluster(s) of bound proteins mediate the many biological functions of HDL.

To illustrate. Significant correlations have been found between the HDL3-enriched protein clusters and the capacity of HDL to attenuate LDL oxidation. Oxidized LDL (oxLDL) displays multiple pro-inflammatory activities, and oxidative modification of LDL in the arterial wall has been implicated in atherogenesis. In an in vitro oxidation assay, small, dense HDL3 displayed more potent antioxidative activity as compared to large, light HDL2. HDL3 also exhibited a greater potent capacity to protect human microvascular endothelial cells from the apoptosis induced by oxLDL, compared to large light HDL2; and the level of protection was determined to be independent of protein concentration, total particle mass, or the number of particles. Analysis of the mechanistic aspects of the antioxidative and anti-apoptotic potencies of HDL3 particles revealed further that both activities are significantly related to the apoA-I content of the particle. Such data support the hypothesis that small, dense HDL3 display particle-specific clusters of proteins which are intimately associated with their lipidome and confer this subset of particles with potent biological properties, notably anti-apoptotic and antioxidative.

The potential exists for both the proteome and lipidome of HDL particles to be used to differentiate between healthy populations and those at elevated cardiovascular risk. Patients with metabolic syndrome or type 2 diabetes have small dense HDL3 particles that are depleted of cholesteryl esters and enriched in triglycerides. This abnormal HDL3c lipid profile has been shown not only to decrease HDL particle stability and plasma residence time; it also has been found to correlate inversely with the ability of small HDL3 to decrease the rate of LDL oxidation.

It is also possible that the deficient anti-atherogenic activities of HDL in metabolic disease can be corrected by therapeutic approaches designed to raise HDL cholesterol and normalize intravascular metabolism. One HDL-raising strategy actively being explored is the inhibition of CETP. Optimism for CETP inhibition as a pharmacological target is generated, in part, by the favorable cardiovascular profile found in CETP deficient patients. CETP deficiency due to molecular defects in the CETP gene occurs frequently (9%) in the general Japanese population. As first reported by Mabuchi and his colleagues, both homozygous and heterozygous carriers of the mutant alleles have markedly elevated plasma levels of HDL cholesterol and apoA-I, decreased levels of LDL cholesterol, and an extremely low incidence of coronary heart disease. Such beneficial clinical outcomes prevail, irrespective of known coronary disease risk factors like age and gender, and are highly informative for the development of a CETP inhibitor.
Studies done in identical twins indicate that 50% of the variation in human HDL cholesterol levels is genetically determined, but the impact most of the genes have on HDL metabolism is unclear. To date, eleven genes are known to be key determinants for the synthesis, maturation, conversion, and catabolism of HDL. Five of the genes have been identified in humans, through classical linkage analysis in families with hereditary HDL disorders. The genes encoding apoA-I, LCAT, and ABCA1 are essential for *de-novo* synthesis of HDL. Those encoding CETP and HL (LIPC) promote the continual recycling of HDL, transferring cholesterol to apoB-containing lipoproteins and converting large spherical particles into smaller dense, discoidal particles. The other six “established HDL genes” have been identified through studies in mice: SCARB1 (the gene for SR-BI), ABCG1, ATP5B, PLTP, LIPG (the gene for EL), and APOM. Dramatic changes in HDL cholesterol levels occur when these genes are knocked out or overexpressed in murine models.

Genetic association studies have been employed to define further the roles genes have in human HDL pathways. Gene association aims to test whether a genetic variant is linked to a specific disease or trait: if association is present, the occurrence together of the particular allele, genotype, or polymorphism(s) and the disease will be seen more often than can be readily explained by chance. Analysis of single nucleotide polymorphisms (SNPs) in APOA1, APOE, ABCA1, CETP, LIPC, LCAT, and SCARB1 in 546 men with atherosclerosis, for example, showed that the variants together explained 12.4% of the variation in HDL cholesterol levels. Genetic variation in five LDL- and four HDL/triglyceride-associated genes of 5287 individuals were tested to determine whether a panel of nine SNPs could predict a first cardiovascular event during a 10-year follow-up. The results indicated a strong correlation with future cardiovascular events that was mainly driven by SNP s in LDL-associated genes LDLR, PCSK9, and APOE and in HDL-associated genes LIPC and LPL.

More recently, a large set of novel genes has been proposed to affect HDL, such as C/EBP, BPM1, PEMT, C/ABP, and VNN1; and genome wide association studies in humans have shown that variation at the ALNT-2, TRIB-1, MMAB/MVK, SIRT1, and TTC39B loci are linked with HDL cholesterol levels.

The integrated genomics approach has clinical application. It has been used by Kuivenhoven and colleagues to understand the HDL phenotypes observed in their patients. Approximately 50% of the low HDL phenotypes can be explained by specific genetic variation; but only 2% of the high HDL phenotypes. The technology may also lead to HDL-associated genes and their proteins being targeted pharmacologically as potential HDL-raising therapy.

SR-BI is one of several cell-surface receptors that play a major role in regulating lipoprotein metabolism and plasma cholesterol levels. It is predominantly expressed
in the liver, gastrointestinal tract, and steroidogenic organs; but it has also been
detected in macrophages and endothelial cells. SR-BI binding to HDL mediates the
selective bulk uptake of cholesteryl esters into the cell. It also stimulates the
bidirectional flux of unesterified cholesterol between cells and extracellular
lipoproteins. Targeted disruption of the SR-BI gene in mice leads to an approximately
2.2-fold increase in the amount of cholesterol in abnormally large, free-cholesterol-
enriched HDL particles and a decrease in the secretion of biliary cholesterol. In
mediating the delivery of cholesterol to steroidogenic tissues and the liver, SR-BI is
pivotal in the process of reverse cholesterol transport.

Hepatic expression of SR-BI is regulated by the cytoplasmic adaptor/scaffold-protein
PDZK1. PDZK1 is a four PDZ domain-containing protein that binds to the C termini
of numerous membrane-associated transporter proteins, including cell surface
receptors and ion channels, especially in the liver and kidney. The four domains
enable the protein to promote the clustering or scaffolding of groups of proteins; and,
therefore, PDZK1 could potentially affect the expression, function, and stability of
SR-BI at many different levels. PDZK1 knockout mice exhibit a >95% decrease in
hepatic SR-BI protein expression; and the subsequent effect on HDL metabolism,
particularly the massive accumulation of abnormally large, cholesterol-rich HDL
particles is similar to, but not identical to or as severe as, that observed in SR-BI
deficiency. Strikingly, too, the effect of PDZK1 global deficiency on SR-BI protein
expression is tissue specific. SR-BI protein levels are decreased by 95% in the liver
but only by 50% in the small intestine, and they are normal in steroidogenic adrenal
glands and ovaries. Hepatic overexpression of SR-BI in SR-BI/PDZK1 double
knockout mice restores the normal expression of SR-BI on the surface of hepatocytes.
Such data suggest that PDZK1 is required for maintaining the cell-surface levels of
SR-BI at an adequate steady state.

The mechanism(s) by which PDZK1 controls the hepatic and intestinal levels of
SR-BI and the specific role of each domain, if any, are not yet fully understood.
PDZK1 and SR-BI interaction occurs at the most C-terminal amino acid in SR-BI.
Hepatic overexpression of full-length PDZK1 corrects SR-BI-mediated cholesterol
efflux in PDZK1 knockout mice, whereas the expression of the PDZ1 domain alone
only partially restores SR-BI levels and has no effect on SR-BI cell surface expression
or function. Krieger et al. have reported the results of a study in PDZK1 knockout
mice in which were expressed one of four transgenes containing combinations of the
PDZ domains with nested truncations of the remaining C-terminal sequence, namely,
pTEM, which lacks the putative PDZ-binding motif, and PDZ1.2, PDZ1.2.3, or
PDZ1.2.3.4. Hepatic overexpression of pTEM restored normal hepatic SR-BI
abundance, localization, and function. Hepatic overexpression of PDZ1.2 and of
PDZ1.2.3 restored SR-BI abundance to 12% and 30%, respectively, of levels in
wild-type animals but either did not (PDZ1.2.) or only slightly (PDZ1.2.3) restored
hepatic SR-BI cell surface expression and function. Hepatic overexpression of
PDZ1.2.3.4 completely restored SR-BI protein abundance and cell surface expression
and normalized the levels of plasma cholesterol. These results indicate that all four
PDZ domains of PDZK1, but not the C-terminal region, are necessary for maintaining
normal SR-BI abundance, localization, and function.

It seems likely that other adaptor proteins will be found to control the activities of
other cell-surface receptors and transporters and, furthermore, have implications for
lipoprotein metabolism. Indeed, the influence of PDZK1 on SR-BI is comparable to
that reported for ARH on the activity of the LDL receptor, though the mechanisms
by which each adaptor influences the activity of the associated receptor are distinct.
It is also probable that such adaptors may prove to be attractive targets for
pharmacological intervention in coronary disease.
Hepatic lipase (HL), like SR-BI, is important for regulating lipoprotein metabolism and plasma cholesterol levels. HL is a glycoprotein that catalyzes the hydrolysis of triglycerides and phospholipids in HDL and apoB-containing lipoproteins. The enzyme is synthesized and secreted primarily by the liver. Bound to heparin sulfate proteoglycans on the surface of cells in the space of Disse, it participates, along with SR-BI, the LDL receptor-like protein, and surface proteoglycans, as a ligand in promoting the hepatic uptake of HDL, LDL, and TRL remnants. Its catalytic activity contributes to the remodeling of HDL and LDL, resulting in small, dense particles. The enzyme, therefore, plays a key role in the process of atherosclerosis. The exact nature of the relation between HL and atherosclerosis, though, is unclear. HL may exert an anti-atherogenic effect by accelerating reverse cholesterol transport; it may also induce a pro-atherogenic effect by converting large, buoyant triglyceride-enriched LDL into small, dense particles.

Postheparin plasma HL activity varies widely in normal individuals and can be correlated with common CHD risk factors like gender, menopausal status and age, obesity, and lifestyle. Men have elevated HL activity, decreased HDL₂ levels, and an increased incidence of premature coronary disease. Women have much lower levels of HL activity, higher HDL₂ concentrations, and reduced CHD risk; but HL activity is 24% higher in postmenopausal women than in premenopausal women. Visceral adiposity and insulin resistance increase HL activity, while exercise lowers it. Hypertriglyceridemia elevates HL activity and plasma levels of atherogenic small dense LDL and small HDL particles. These observations would suggest that low levels of HL activity are associated with a lower risk of coronary disease.

Assessment of the atherogenic potential associated with genetic variations in the HL gene \textit{LIPC} fosters a different conclusion. It is estimated that 30-45% of the variability in HL activity is genetically determined. Four common polymorphisms in the promoter region of \textit{LIPC}, all in complete linkage disequilibrium, have been reported: G-250A, C-514T (i.e., C-480T), T-710C, and A-763G. Carriers of the -514T allele present a 30-40% decrease in post-heparin HL activity and a favorable lipoprotein phenotype characterized by large, buoyant LDL and HDL₂. The inverse relationship between HL activity and particle size points to a lower atherogenic potential in carriers of the variant allele. Yet, paradoxically, this assumption is not supported by evidence from clinical trials in which no benefit or even a slightly increased risk of CHD has been reported.

A possible explanation for this discrepancy is that the relationship between HL and atherosclerosis is modulated not only by mutations in the HL gene affecting the
concentration and activity of the enzyme, but also by related metabolic factors, like the concentration of LDL cholesterol. High HL activity in individuals who have high levels of LDL particles would lead to an elevated level of small, dense LDL upon the action of HL. The presence of high concentrations of small, dense LDL override the potential beneficial effect that high HL activity would have on reverse cholesterol transport. In individuals with low levels of LDL, high levels of HL could be atheroprotective, as reverse cholesterol transport and TRL remnant catabolism would be enhanced. Conversely, low HL activity could have atheroprotective effects in individuals who have high LDL by limiting the formation of large numbers of small, dense LDL particles. Low HL activity could be atherogenic among subjects with low LDL cholesterol levels, as reverse cholesterol transport is attenuated and TRL remnant catabolism is impaired. Therefore, high or low HL activity could be either atheroprotective or atherogenic, depending on the plasma concentration of small, dense LDL particles.

Two common syndromes in which elevated HL activity is associated with increased numbers of small, dense LDL particles are type 2 diabetes and familial combined hyperlipidemia. Patients manifesting these syndromes also have been shown to have an increased risk for atherosclerosis.

The association, in humans, of HL with the remodeling of HDL and LDL particles and, hence, with a subsequent risk for premature coronary disease appears to depend on concomitant lipid abnormalities, like increased LDL cholesterol levels, and on the underlying genetic dyslipidemia. Characterization of patients by their LIPC genotype may contribute to a better definition of an individual’s cardiovascular risk, especially in patients with qualitative (small, atherogenic LDL and low HDL2 cholesterol) rather than quantitative lipid abnormalities, for whom the routine lipid profile may underestimate risk.

Session 6: HDL Raising Strategies

Stefania Lamon-Fava, MD, PhD  
*Tufts University, Boston, MA, USA*

Gerald F. Watts, DSc, PhD, DM  
*University of Western Australia, Perth, Australia*

H. Bryan Brewer, Jr., MD  
*Cardiovascular Research Institute, Washington, DC, USA*

Epidemiological studies have established that low plasma levels of HDL cholesterol are an independent risk factor modulating CHD. Low HDL cholesterol is widely prevalent in the United States and Europe and is one of the defining features of the metabolic syndrome. Metabolic syndrome, by definition, is the presence of three of the following five abnormalities: (1) increased waist size (>40 inches in men, >35 inches in women), (2) elevated blood pressure (systolic pressure >130 mmHg), (3) elevated fasting blood glucose (>100 mg/dL), (4) elevated triglycerides (>150 mg/dL), and (5) decreased HDL cholesterol (<40 mg/dL in men, <50 mg/dL in women. These abnormalities are frequently associated with increased plasma levels of insulin and C-reactive protein (CRP) and with central obesity.
Weight Loss. The loss of excess body weight short-term not only reduces plasma levels of total cholesterol and triglyceride; it also has beneficial effects on lipoprotein metabolism. In obese men with metabolic syndrome, apoB plasma levels decreased significantly due to a marked decrease in the production rate of VLDL apoB which, in turn, was enhanced by marked increases in the catabolism of LDL apoB and in the rate of converting large VLDL apoB particles to smaller LDL particles. Reverse cholesterol transport was not altered, however, since the low-fat, low-caloric diet decreased both the catabolic and the production rates of apoA-I and had no effect on the HDL cholesterol levels.

Fish Oils. In patients with metabolic syndrome, capsules containing omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decreased the levels of plasma triglycerides and apoB by decreasing their production, while increasing significantly the clearance of LDL apoB-100 by the liver. They had no significant effect on HDL levels because a decrease in HDL apoA-I production was
offset by a decrease in apoA-I clearance. Low fish oil dosage is known to reduce sudden death in post-myocardial infarction patients, and 1800 mg of EPA has been shown to reduce CHD risk in a large Japanese study.

**Alcohol.** Epidemiological studies indicate that light to moderate alcohol consumption lowers the risk of premature coronary disease; and in some studies it has been associated with increases in HDL cholesterol levels. Human kinetic studies indicate that the alcohol-induced increases in HDL cholesterol and apoA-I levels associated with the decrease in CHD risk are due to an increase in the production rate of apoA-I. Alcohol also increased LCAT activity and lowered the activity of CETP and HL; but since the fractional catabolic rate of apoA-I did not change, the role of these plasma factors is uncertain.

**Estrogen.** While observational studies have shown the beneficial effects of estrogen replacement therapy for CHD risk, randomized clinical trials have resulted in a less positive outcome, with CHD risk being increased up to 29%, for example, in the Women’s Health Initiative study. When the outcomes were controlled for age and the time of replacement therapy, younger women were found to benefit; older women did not. Metabolically, estrogen replacement therapy in postmenopausal women increased HDL cholesterol and apoA-I levels by increasing the production rate of apoA-I. The plasma levels of small preβ1-migrating particles, large α-migrating HDL, and CRP were also increased.

**Niacin.** Either alone or in combination with other lipid-lowering medications, niacin reduces triglyceride levels and increases the plasma levels of HDL cholesterol and apoA-I. The increase in apoA-I has been shown in dyslipidemic men to be due to a significant increase in apoA-I production. Niacin also significantly affects HDL particle size and density distribution as well as the apoA-I levels of HDL subparticles, resulting in a shift towards large α1-, α2-, preα1-, and preα2-migrating particles enriched in cholesteryl esters. The redistribution of HDL subpopulations was not associated, however, with a change in CETP or LCAT mass. In this study extended release niacin at a dose of 2 grams/day also significantly lowered VLDL apoB-100 levels due to enhanced clearance.

Niacin is currently the most effective HDL-raising agent. In the Coronary Drug Project, it has been shown to reduce CHD morbidity and mortality significantly. It appears to be the most effective treatment for CHD risk reduction in patients with diabetes, despite a niacin-attributable increase in blood glucose levels. In combination with simvastatin, it has been found to promote the regression of coronary atherosclerosis, and one of the mechanisms for this regression is the niacin-induced increase in large α1-migrating HDL.

**PPAR agonists.** Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes involved in cellular differentiation, development, and metabolism, including the expression of genes involved in lipid and glucose homeostasis. PPARα regulates the genes encoding apoA-I, apoA-II, ABCA1, SR-BI, and LPL. PPARα agonists like gemfibrozil and fenofibrate improve the lipoprotein profile by increasing HDL cholesterol levels and decreasing plasma triglyceride levels.

Lipoprotein kinetic studies have shown that fenofibrate treatment short-term in individuals with the metabolic syndrome decreased VLDL and IDL apoB-100 plasma levels by increasing significantly the clearance of apoB-100 and increased apoA-I plasma levels chiefly by enhancing the production rate of apoA-I. The catabolism of apoA-I was also accelerated, implying an overall enhancement of the reverse cholesterol transport pathway. The production of LpA-I:A-II particles...
increased significantly, but the kinetics of LpA-I were not altered. In a subanalysis from the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, fenofibrate treatment for 5 years resulted in a decrease in LpA-I and an increase in LpA-I:A-II, thereby, accounting for an increase in apoA-II levels without any effect on apoA-I. The contrasting results of short- and long-term treatment on plasma apoA-I may be associated with a fenofibrate-induced increase, long-term, in plasma homocysteine levels.

Fenofibrate did not significantly lower CHD risk in diabetic patients. Gemfibrozil, on the other hand, was found to lower CHD risk significantly in men with non-HDL cholesterol levels >200 mg/dL in the Helsinki Heart Study and in men with CHD and low HDL cholesterol in the VA-HIT Study. It also was found to increase the plasma levels of intermediate sized HDL particles containing both apoA-I and apoA-II, but not the levels of large atheroprotective HDL particles. Recent studies by Millar et al. have documented similar metabolic effects with a more potent fibrate.

The role of fibrates in CHD prevention remains controversial; yet, these agents are clearly beneficial. In severe hypertriglyceridemia they have been shown to lower plasma triglyceride levels and prevent pancreatitis. Gemfibrozil has been demonstrated to be very effective in preventing recurrent events in men with CHD, low levels of HDL cholesterol and elevated levels of insulin. Fenofibrate decreases the need for the treatment of retinopathy and leg amputations in patients with diabetes.

**Statins.** Statins have been shown to have a strikingly beneficial effect on reducing CHD, and the regression of coronary atherosclerosis induced by rosuvastatin monotherapy is thought to be associated with both the lowering of LDL cholesterol and CRP and the raising of HDL cholesterol levels.

In terms of lipoprotein metabolism, atorvastatin effectively reduces the levels of apoB-containing lipoproteins by enhancing their fractional catabolic rate; but it has little effect on HDL apoA-I kinetics. In individuals with metabolic syndrome, rosuvastatin, the most potent HDL-modulating statin, increased plasma HDL cholesterol and LpA-I concentrations in a dose-dependent manner by decreasing the catabolism of LpA-I. It also caused a dose-dependent increase in HDL particle size, but it did not alter the concentration or the kinetics of LpA-I:A-II particles. A more detailed examination of HDL subpopulations indicates that both atorvastatin, and rosuvastatin will decrease small preβ1-migrating HDL particles and increase large α1-migrating HDL significantly, with rosuvastatin being significantly more effective than atorvastatin in raising the levels of large HDL particles.

**CETP Inhibition.** Partial inhibition of CETP with the pharmacological agent torcetrapib markedly increased HDL cholesterol and apoA-I levels in hypoalphalipidemic patients in a dose-dependent manner. These changes were attributable to reductions in the fractional catabolic rate of apoA-I. The production rate of apoA-I was not affected. Decreased CETP activity led to an increase in the concentration of apoA-I in the large α1-migrating HDL and, in turn, normalized the apoA-I levels within these particles. There was no significant change in fecal sterol excretion.
When tested in large clinical trials, specifically, the ILLUMINATE Trial, torcetrapib was found not only to raise systolic blood pressure but also to increase the risk of total mortality and fatal infarctions; and the development of the drug was halted. It is now known that torcetrapib increased serum aldosterone and cortisol levels and altered serum electrolyte concentrations in some subjects, metabolic changes that could account for the adverse effects of the drug. Moreover, in the ILLUMINATE Trial, the subjects achieving the highest levels of HDL cholesterol appeared to get the most benefit in terms of CHD risk reduction, indicating that CETP inhibition does not necessarily promote dysfunctional HDL particles.

Based on the current information, two separate conceptual approaches - acute and chronic HDL therapy - are under development to raise HDL cholesterol and potentially decrease clinical events. Acute HDL therapy is directed toward acutely increasing lipid-poor, nascent, preβ HDL by infusion into acute coronary syndrome patients at high risk for repeated clinical events. The underlying hope is that the HDL infusions will accelerate the efflux of cholesterol from macrophages and foam cells in the arterial wall and reduce the number of vulnerable plaques that could rupture and result in acute cardiac events.
The first clinical data to suggest that infusions of lipid-poor apoA-I would be effective in decreasing atherosclerosis were provided by the apoA-I Milano infusion study. In patients with acute coronary syndrome, five weekly infusions of apoA-I Milano/phospholipid complexes resulted in significant regression in coronary artery atherosclerosis, as assessed by intravascular ultrasound (IVUS). The decrease in plaque volume was greater than that obtained with 80 mg/day of atorvastatin therapy. Infusions of autologous selectively delipidated HDL have also shown reduction in atherosclerosis as quantitated by IVUS, similar to the apoA-I Milano results.

Under development at present are synthetic apoA-I mimetic peptides based on the amphipathic structure of apoA-I. These include D-4F, which increases the levels of preβ HDL particles; Esperion ETC 642, which is designed to elevate LCAT activity; and NIH-5A, which selectively removes cholesterol via the ABCA1 transporter. The aim is a peptide that, at the least, increases plasma levels of total cholesterol and HDL cholesterol, selectively improves ABCA1-mediated cholesterol efflux, converts HDL from proinflammatory to anti-inflammatory particles, and reduces the concentration of cellular adhesion molecules and other inflammatory factors.

The chronic approach involves the development of oral agents that significantly increase plasma HDL cholesterol and can be used for a long or indefinite period of time. Currently available are fibrates and niacin. Niacin co-administered with a PGD2 inhibitor shown to decrease flushing markedly and facilitate patient compliance is in development. The most promising HDL-raising strategy, until recently, was CETP inhibition. CETP inhibitors both increase HDL and reduce LDL. As mentioned previously, evidence to support the concept that the failure of torcetrapib was due to an off-target, compound-specific, rather than a class, effect is accumulating. Experiments in preclinical models have revealed that the increased blood pressure caused by torcetrapib is independent of CETP inhibition and is accompanied by increased circulating levels of aldosterone. Taken together with the apparent lack of this side effect with other CETP inhibitors, such as anacetrapib and dalcetrapib, there is renewed optimism for CETP inhibition as a therapeutic target; and second generation CETP inhibitors are now being evaluated as potential approaches to increasing HDL and reducing cardiovascular risk.

In the end, however, it is very likely that no individual therapy will be completely effective in reducing the residual cardiac events present in statin-treated patients. The most beneficial treatment may be a combination of HDL-raising and LDL-lowering agents, having effects that are complementary and additive, that improve both the absolute plasma cholesterol levels and the functional quality of the lipoprotein particles.
CONCLUSIONS

Low levels of HDL cholesterol (<40 mg/dL or 1 mmol/L) have clearly been shown to be an independent risk factor for premature heart disease, even at low levels of LDL cholesterol. HDL formation depends upon the synthesis and secretion of apoA-I, the major protein of HDL. In the absence of apoA-I gene expression in humans, there is pronounced HDL deficiency, normal plasma levels of triglycerides and LDL, and marked premature heart disease. Genetic variants of apoA-I, however, are not necessarily associated with premature heart disease. Those affecting LCAT activity possibly are not associated; and the apoA-I Milano variant appears to decrease the risk of heart disease.

HDL is clearly important for cellular cholesterol efflux. After the formation of apoAI and its combination with a small amount of phospholipid, preβ1-migrating HDL particles are formed which interact with the ABCA1 transporter, resulting in small discoidal HDL particles of α4-mobility. The free cholesterol on these particles is esterified to form small spherical particles known as α3 HDL. It is known that these particles can interact with the transporter ABCG1 for promoting more cholesterol efflux and the further enlargement of the particles, forming α2- and α1-migrating particles, as the free cholesterol is esterified.

HDL remodeling is modulated by not only by the enzymes LCAT and HL but also by CETP and the HDL receptor, SR-BI. CETP facilitates the transfer of cholesteryl esters to triglyceride-rich, apoB-containing lipoproteins in exchange for triglyceride. In the absence of CETP, HDL cholesterol levels are very high, there is no clear evidence of heart disease, and longevity is enhanced. SR-BI allows cholesterol to be taken up by the liver for excretion from the body.

HDL particles also appear to have important anti-inflammatory and anti-oxidative properties. Both apoA-I and apoA-II inhibit acute vascular inflammation and do so, in part, by inhibiting the expression of chemokines and their receptors. Large HDL2 particles that promote sterol efflux via ABCG1 may have a particular role in protecting endothelial cells and macrophages from the adverse effects of oxysterols consumed in the diet or formed on LDL. HDL3-enriched protein clusters have been found in vitro to attenuate LDL oxidation in human endothelial cells. Such observations substantiate the existence of HDL subpopulations with atheroprotective functions other than reverse cholesterol transport.

Both genetic and nutritional factors regulate HDL levels. Effective ways to increase plasma levels of HDL cholesterol and large atheroprotective HDL particles include weight loss, exercise, alcohol consumption, restriction of sugars, estrogen, and pharmacological agents like niacin, statins, fish oil, fibrates, and CETP inhibitors. Of these modalities, the ones that are currently used widely include statins, fibrates, and niacin. Large scale studies are now underway testing whether statin-niacin and statin-CETP inhibitor combinations are more effective in CHD risk reduction than statin alone. Infusion therapy is also under development, and preliminary clinical trials using apoA-I Milano as well as reconstituted HDL have been very promising. In the next 5 years many clinical trials concentrating on HDL metabolism will be completed. This will provide us with significant information and data to support incorporating HDL cholesterol into the guidelines for therapy with specific targets for CHD prevention.
SUGGESTED READINGS

Assmann G, Schulte H, Cullen P, Seedorf U.
Assessing risk of myocardial infarction and stroke; new data from the Prospective Cardiovascular Muenster (PROCAM) Study.

Asztalos BF, de la Llera-Moya M, Dallal GE, Horvath KV, Schaefer EJ, Rothblat GH.
Differential effects of HDL subpopulations on cellular ABCA1- and SRB1-mediated cholesterol efflux.

Role of LCAT in HDL remodeling: investigation in LCAT deficiency states.

Effects of torcetrapib in patients at high risk for coronary events.

Brewer, HB Jr.
HDL metabolism and the role of HDL in the treatment of high-risk patients with cardiovascular disease.

Brown BG, Stukovsky KH, Zhao XQ.
Simultaneous low-density lipoprotein-C lowering and high-density lipoprotein-C elevation for optimum cardiovascular disease prevention with various drug classes, and their combinations: a meta-analysis of 23 randomized lipid trials.
Curr Opin Lipidol 2006; 17:631-36.

Calabresi L, Franceschini G.
High density lipoprotein and coronary heart disease: insights from mutations leading to low high density lipoprotein.

Functional lecithin:cholesterol acyltransferase is not required for efficient atheroprotection in humans.
Circulation 2009; 120:628-35.

Canner PL, Furberg CD, Terrin ML, McGovern ME.
Benefits of niacin by glycemic status in patients with healed myocardial infarction (from the Coronary Drug Project).

Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function.
**SUGGESTED READINGS**

GISSI Prevenzione Investigators.  
Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico.  

Holleboom, AG, Vergeer M, Hovingh GK, Kastelein JJ, Kuivenhoven JA.  
The value of HDL genetics.  

Keech AC, Mitchell P, Summanen PA, O’Day J, Davis TM, Moffitt MS, et al., FIELD study investigators.  
Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial.  

Kocher O, Krieger M.  
Role of the adaptor protein PDZK1 in controlling the HDL receptor SR-BI.  

Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia.  
*Atherosclerosis* 1985; 58:175-86.

Kontush A, Chapman MJ.  
Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis.  

Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and ApoB-containing lipoproteins.  

Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study.  

Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis.  

Pencina MJ, D’Agostino RB Sr, Larson MG, Massaro JM, Vasan RS.  
Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study.  
*Circulation* 2009; 119:3078-84.

Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH.  
The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis.  

Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR.  
C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men.  
SUGGESTED READINGS


