Exploiting the Vascular Protective Effects of High-Density Lipoprotein and Its Apolipoproteins
An Idea Whose Time for Testing Is Coming, Part I

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“An invasion of armies can be resisted but not an idea whose time has come.” —Victor Hugo.

V aso-occlusive disease resulting from atherosclerosis and thrombosis is the leading cause of death and morbidity in the United States and other industrialized nations. Although the precise cause of atherosclerosis is unclear, an emerging paradigm suggests that atherosclerosis involves multiple pathways in which lipoprotein entry and retention, injury to the vessel wall from diverse stimuli, and an associated long-term inflammatory and immune response seem to play a key role.1–4 Dyslipidemia characterized predominantly by elevated levels of one or more circulating non-HDL cholesterol lipoproteins [LDL, VLDL, lipoprotein(a), triglycerides] and/or reduced HDL cholesterol is one of the key risk factors for atherosclerosis and cardiovascular disease.5–16 Over the past several years, a number of large, prospective, randomized, controlled clinical trials have demonstrated both angiographic and clinical benefits of lipid-lowering therapy, with a significant reduction in fatal and nonfatal cardiovascular events.17–23 These have primarily targeted LDL cholesterol through pharmacotherapy (mostly statins), with or without dietary counseling, lifestyle modification, or surgery (intestinal bypass in Program on the Surgical Control of the Hyperlipidemias [POCSH] trial). Overall, a significant and clinically worthwhile relative risk reduction ranging from 20% to 40% in major cardiovascular events has been achieved with these strategies, without significant adverse effects or increased noncardiovascular mortality. These remarkable results prompted Brown and Goldstein24 to predict that heart attacks will be gone with the century. This clearly reflects an overoptimistic point of view, because 60% to 70% of adverse cardiovascular events continue to occur despite LDL-lowering therapy.

Potential reasons why cardiovascular events may continue to occur despite low LDL levels or despite LDL-lowering therapy include the following: (1) we may not be lowering LDL cholesterol to optimal levels, because optimal levels are not clearly defined, and (2) there may be other risk factors that are more important in certain patients than simply elevated LDL cholesterol. These observations underscore a need for additional preventive and therapeutic interventions exploiting new targets to complement and augment the results of LDL lowering. One such potential target is HDL and its apolipoproteins. There is a large body of experimental evidence to suggest that augmenting HDL and/or its apolipoproteins can have major vascular protective effects ranging from prevention to stabilization and regression, independent of total or non-HDL cholesterol levels. Therefore, we think that the time is ripe for the development and clinical testing of this new frontier in antiatherogenic strategy.

Relationship of HDL/Apolipoprotein A-I to Atherosclerosis and Cardiovascular Disease

Since 1977, several case-control and prospective epidemiological studies have shown that there is an independent and inverse relationship between circulating HDL cholesterol levels and coronary heart disease.5,6,9–16,25 It has been calculated that every 1 mg/dL increase in HDL is associated with a 2% to 3% lower risk of coronary heart disease. Similarly, an inverse relationship has also been demonstrated between the levels of apolipoprotein A-I, the major structural protein component of HDL, and coronary heart disease.25–30 An inverse relationship between restenosis after percutaneous coronary transluminal angioplasty and restenosis after carotid endarterectomy has also been demonstrated in some, but not all, studies.31–34

There are, however, several exceptions to this general rule that should be mentioned. For example, some genetically determined low HDL states due to mutations in apolipoprotein A-I are not associated with an increased risk of atherosclerosis.35,36 Similarly, an elevated HDL level resulting from a deficiency of the cholesterol ester transfer protein (CETP) due to mutations in the CETP gene does not uniformly confer immunity against atherosclerosis.37–46 In experimental animals, transgenic overexpression of lecithin cholesterol acyl transferase (LCAT) increases atherosclerosis, despite an in-
crease in HDL, whereas hepatic overexpression of scavenger receptor (SR)-BI with gene transfer reduces atherosclerosis, despite a major reduction in HDL levels.\textsuperscript{45,46} Furthermore, apolipoprotein A-I deficiency does not promote atherosclerosis in mice in the absence of elevated LDL or the overexpression of apolipoprotein B.\textsuperscript{49,50} These observations highlight the complexity of the relationship between HDL levels and atherosclerosis. Steady-state HDL levels are also a static measure and may not fully reflect the actual efficiency of cholesterol fluxes between tissues and reverse cholesterol transport. Thus, the type of HDL (HDL size and composition) and the molecular mechanism by which HDL levels are altered may significantly influence the biological function and vascular protective effects of HDL, attesting to the structural and functional heterogeneity of HDL.

**HDL and Apolipoprotein A-I Have Direct Antiatherogenic and Vascular Protective Effects**

Because low HDL cholesterol levels are often associated with other metabolic abnormalities, such as elevated triglyceride levels, insulin resistance, and small dense LDL, it has been argued that the inverse relationship between coronary heart disease and HDL may not reflect a cause and effect. However, experimental observations make a compelling argument that HDL and apolipoprotein A-I have direct antiatherogenic and vascular protective effects.

**Preclinical Studies**

Repeated intravenous injection of a crude preparation of homologous HDL, derived from plasmapheresis, inhibited the progression and induced the regression of early aortic fatty streaks in cholesterol-fed rabbits.\textsuperscript{51,52} Similar results were achieved in cholesterol-fed rabbits receiving intravenous apolipoprotein A-I injections.\textsuperscript{53} In another study, intravenous reconstituted HDL containing recombinant apolipoprotein A-I\textsubscript{Milano} was also shown to prevent progression or promote regression of aortic atherosclerosis in apolipoprotein E–null mice, while reducing the lipid and macrophage content in plaques without changing the high circulating total cholesterol levels.\textsuperscript{54} Similarly, the overexpression of the human apolipoprotein A-I gene increases HDL cholesterol levels and markedly attenuates atherosclerosis in transgenic atherosclerosis-prone mice and rabbits, despite profound hypercholesterolemia.\textsuperscript{55–59} These results are further supported by experiments in which adenovirus-mediated apolipoprotein A-I gene transfer inhibited the progression and promoted the regression of atherosclerosis in genetically hyperlipidemic apolipoprotein E–null mice.\textsuperscript{59,60} In addition to favorable effects in models of atherosclerosis, intravenous wild-type HDL, reconstituted HDL containing recombinant apolipoprotein A-I\textsubscript{Milano} (a mutant form of apolipoprotein A-I), or the gene transfer of apolipoprotein A-I also reduce the neointimal response to arterial injury.\textsuperscript{61–64}

**Clinical Studies**

The first hint that HDL increase could be beneficial in patients was provided by the Helsinki Heart Study.\textsuperscript{65} In this study involving 4000 men with elevated cholesterol, gemfibrozil treatment resulted in an 11% increase in HDL, and a multivariate analysis identified an increase in HDL as an independent predictor of a reduction in clinical events.\textsuperscript{66} The patients with high triglyceride levels and low HDL experienced a 78% relative risk reduction with gemfibrozil. The Scandinavian Simvastatin Survival Study (4S) trial using simvastatin demonstrated a relationship between increases in HDL and clinical event reduction,\textsuperscript{19} whereas a National Heart, Lung, and Blood Institute (NHLBI) type II intervention trial discovered that the delayed progression of angiographic atherosclerosis was related to both a reduction in LDL and an increase in HDL.\textsuperscript{67} However, the Cholesterol And Recurrent Events (CARE) trial and the West of Scotland Coronary Prevention Study (WOSCAPS), both of which used pravastatin, failed to demonstrate an independent relationship between HDL increase and reduction in clinical events,\textsuperscript{20,21} These trials were designed primarily to examine the effects of LDL lowering and were not specifically designed to test the effects of raising HDL.

Recently, several clinical trials were completed that examined the effects of increasing HDL and reducing triglycerides, using fibrates, on angiographic or clinical end points in patients with coronary artery disease. In the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT),\textsuperscript{67} bezafibrate reduced the angiographic progression of coronary artery disease ($P=0.049$ compared with placebo), without a significant reduction in LDL cholesterol. Because bezafibrate reduced triglycerides by 31% and raised HDL by 9%, reduced progression was attributed to these effects. In the Lopid Coronary Angiography Trial (LOCAT), gemfibrozil treatment did not alter the primary angiographic outcome (progression of disease in unby-passed arteries or segments distal to the graft), but it did reduce disease progression when all native vessel segments were analyzed.\textsuperscript{68} There was a 40% reduction in triglycerides and a 14% increase in HDL cholesterol levels with gemfibrozil treatment, with only minimal decreases in LDL cholesterol. However, an increase in total HDL cholesterol was not predictive of reduced disease progression whereas, on therapy, triglyceride-rich lipoprotein levels were strong predictors of disease progression.

A further meta-analysis of 14 angiographic trials in which the primary goal was to reduce total cholesterol suggested a weakly positive relationship between an increase in HDL cholesterol and disease regression. In addition to these angiographic trials, 2 additional trials, the BIP trial and the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), which examined the effects of fibrates on clinical outcomes, have also been reported.\textsuperscript{69,70} In the BIP trial, bezafibrate reduced triglycerides by 18% and LDL cholesterol by 6% and increased HDL cholesterol by 15%. However, there was only a nonsignificant 9% relative reduction in the risk of the primary end point (nonfatal myocardial infarction and cardiac death), although a post hoc analysis showed a 40% risk reduction in the subgroup with triglycerides $>200$ mg/dL.\textsuperscript{69} In VA-HIT, gemfibrozil treatment reduced triglycerides by 24% and increased HDL by 7.5%, without a significant reduction in LDL cholesterol.\textsuperscript{70}
Gemfibrozil-treated patients experienced a 22% reduction ($P=0.006$) in the primary clinical event. Thus, a summary of the clinical data to date demonstrates inconsistency in the relationship between HDL cholesterol changes and angiographic or clinical events. These inconsistencies may result from the inclusion of different subsets of patients in different trials, differences in the biological effects of various fibrates, and relatively small sample sizes in some of the studies. Furthermore, it is difficult to determine which of the fibrate effects is responsible for benefit in coronary artery disease because fibrates can reduce triglycerides and increase HDL, and they have nonlipid effects, such as a reduction in fibrinogen. Finally, the effects of currently available drugs on HDL cholesterol levels are relatively modest, with average increases ranging from 5% to 10% with statins, 15% to 20% with fibrates, and 20% to 40% with niacin.

**Mechanisms of Vascular and Atheroprotective Effects of HDL and Apolipoprotein A-I**

The beneficial vascular effects of HDL and apolipoprotein A-I may be attributed to one or more of the following biological actions (Figures 1 and 2).

**Stimulation of Reverse Cholesterol Transport**

HDL is considered an important mediator of reverse cholesterol transport, a process that involves the transfer and uptake of free cholesterol from the peripheral tissues, such as the arterial wall, with subsequent delivery to the liver and other steroidogenic tissues, such as the gonads and adrenals.$^{71-83}$ It has been suggested that apolipoprotein A-I is critical for...
stimulating cholesterol transfer from the cells of the vessel wall, whereas the phospholipid in HDL acts as a sink for the transferred cholesterol. Cell culture studies have shown that HDL and apolipoprotein A-I stimulate reverse cholesterol transport from cholesterol-loaded macrophages, fibroblasts, and hepatic cell lines. The small lipid-free pre-β-HDL discoidal particle has been shown to be an efficient stimulator of reverse cholesterol transport.

In 1999, the discovery of the genetic defect responsible for Tangier’s disease, a condition characterized by very low or absent HDL cholesterol, was a major step forward in the understanding of a critical step in reverse cholesterol transport. Studies involving patients with Tangier’s disease and other non-Tangier’s forms of low HDL states have identified mutations in the ABC-A1 (ATP binding cassette transporter) gene as the basis for the hypoalphalipoproteinemia in these disorders. This gene codes transmembrane proteins that seem to be critical for the transfer of free cholesterol and phospholipids from cells to HDL particles. When free cholesterol is mobilized from the arterial wall and transferred to the lipid-poor apolipoprotein A-I containing HDL, it is esterified by lecithin cholesterol acyl transferase (LCAT), an enzyme whose activation is critically dependent on apolipoprotein A-I. After esterification, the esterified cholesterol is disposed off in the following 3 ways: (1) delivered to the liver and other steroidogenic tissues (adrenals and gonads) through selective uptake by a recently discovered member of the scavenger receptor family (the SR-BI receptor); (2) through the action of cholesterol ester transfer protein (CETP), HDL-cholesterol ester is also transferred to apolipoprotein B-containing LDL/VLDL particles in exchange for triglycerides for eventual delivery to the liver via the classic LDL receptor pathway; and (3) holoparticle uptake of the entire HDL particle through a receptor called Cubilin and its coreceptor Megalin, which are located on the surface of renal tubular cells. Experimental studies from our laboratory and those reported by Rodríguez et al have demonstrated that phospholipid is critical for the efficient stimulation of reverse cholesterol transport by apolipoprotein A-I, because phospholipid-free apolipoprotein A-I does not stimulate cholesterol efflux in cell culture.

Experimental studies have suggested that apolipoprotein A-I_{null} is a more effective stimulator of reverse cholesterol transport than wild-type apolipoprotein A-I. Although opposite results were reported by Bielicki et al using a different experimental model. The experimental observation that apolipoprotein A-I_{null} reduced lipid content in aortic atherosclerotic lesions in apolipoprotein E-deficient mice is consistent with its stimulatory effect on reverse cholesterol transport.

### Antioxidant Effect of HDL (Role of Paraoxonase and Platelet Activating Factor-Acetylhydrolase)

There is substantial evidence to suggest that oxidative modification of LDL trapped in the vessel wall is critical for the stimulation of proinflammatory genes that are critical for inflammatory cell recruitment and the initiation and progression of atherosclerosis. Therefore, the ability to inhibit LDL oxidation in the vessel wall may translate into an anti-inflammatory and antiatherogenic effect. Several experimental studies have suggested that HDL and apolipoprotein A-I protect LDL from cell and transition metal-mediated oxidation. These antioxidant effects of HDL have been attributed to the binding of transition metals by HDL and to the presence of paraoxonase, an arylesterase enzyme carried predominantly by apolipoprotein A-I and apolipoprotein J (clusterin)—containing HDL particles, which has powerful antioxidant effects. Several genetic polymorphisms of the human paraoxonase gene have been identified, some of which seem to be associated with an increased risk of coronary heart disease. Recent studies have shown that the introduction of a paraoxonase-null genotype in LDL receptor–null mice increases atherosclerosis, suggesting that paraoxonase may contribute to the antiatherogenic effects of HDL.

Similarly, the antioxidant effects of HDL have also been attributed to another enzyme, platelet-activating factor (PAF) acetylhydrolase. Recently, a PAF-acetylhydrolase gene polymorphism was described and linked to an increased risk for acute myocardial infarction, and a PAF receptor antagonist was shown to reduce fatty streak formation in LDL receptor–null mice. Recent studies by Navab et al have shown that HDL cholesterol from normal subjects (but not from patients with coronary artery disease), apolipoprotein A-I (but not apolipoprotein A-II), and apolipoprotein A-I mimetic peptides scavenge the seeding molecules 13-hydroperoxyoctadecadienoic acid (HPODE) and 15-hydroperoxyeicosatetraenoic acid (HETE), which are both products of 12-lipoxygenase, from LDL and endothelial cells, thereby protecting LDL from oxidation by cells of the vessel wall.

### Anti-Inflammatory Effects of HDL and Apolipoprotein A-I

HDL and apolipoprotein A-I have been shown to (1) bind and neutralize lipopolysaccharide and endotoxin, thereby preventing lipopolysaccharide-induced tumor necrosis factor release, (2) inhibit complement activation, and (3) reduce cytokine-induced endothelial vascular cell adhesion molecule induction and reduce macrophage infiltration in rabbits and mice. Some of the anti-inflammatory effects of HDL/apolipoprotein A-I may result from the inhibition of LDL oxidation and the scavening of oxidized lipids that trigger pro-inflammatory responses, although other mechanisms may also be involved. It is interesting to note that acute-phase HDL binds ceruloplasmin and serum amyloid A, loses its paraoxonase and apolipoprotein A-I content, and becomes pro-oxidant and proinflammatory, highlighting the dynamic nature of HDL composition and function. Recent studies have shown that an increase in intracellular ceramide through the inhibition of sphingosine kinase may contribute to the anti-inflammatory effects of HDL.

### Scavenging of Toxic Phospholipids Such as Lysophosphatidylcholine

HDL has been shown to reduce the endothelial incorporation of lysophosphatidylcholine (Lyso-PC), a toxic component generated during LDL oxidation, and to promote the extrusion of endothelial Lyso-PC. Our laboratory has demon-
strated that apolipoprotein A-I reduces the smooth muscle cell cytotoxicity induced by Lyso-PC and PAF and protects smooth muscle cells from the apoptotic effects of 25-hydroxycholesterol, an oyster component of oxidized LDL.145

Attenuation of Endothelial Dysfunction by HDL/Apolipoprotein A-I

HDL and apolipoprotein A-I have been shown to attenuate significantly the reduced vasodilator capacity resulting from the endothelial dysfunction associated with atherosclerosis or induced by dyslipidemia or exposure to oxidized LDL or Lyso-PC.144–148 In clinical studies, HDL cholesterol levels have been shown to be positively related to endothelium-dependent vasodilator responses in coronary arteries.148,149

Antithrombotic and Profibrinolytic Effects of HDL and Apolipoprotein A-I

HDL and apolipoprotein A-I reduce platelet activation/aggregation and promote protein C–mediated anticoagulant effects.150–152 Furthermore, stimulation of fibrinolysis by HDL and apolipoprotein A-I has also been demonstrated.153

Reduced Lipoprotein Retention

Apolipoprotein E–enriched HDL, a subpopulation of HDL particles, may prevent the binding and retention of atherogenic apolipoprotein B–containing lipoproteins to lipoprotein lipase–bound proteoglycans in the arterial wall. Reduced retention of LDL may confer antiatherogenic effects.154,155

Conclusions

The pleotropic biological effects of HDL and some of its constituents, as discussed in this section, provide an excellent rationale for enhancing the levels and/or biological function of HDL in an attempt to influence favorably the course of atherothrombotic vascular disease. In part II of this article,156 we will review the structure of HDL and explore various approaches to enhancing the levels and function of HDL for vascular protection.

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