Elevated non-HDL and low HDL levels are key risk factors for the development of atherosclerotic vascular disease.\textsuperscript{1-5} Large-scale clinical trials have shown that reducing LDL levels confers a substantial relative risk reduction in major cardiac events.\textsuperscript{6-10} However, 60\% to 70\% of adverse cardiovascular events continue to occur despite LDL-lowering therapy. The next step in both prevention and therapy may center on HDL and its associated apolipoproteins. A large body of experimental evidence suggests that the HDL particle plays a crucial role in the mobilization of lipid from the arterial wall and exerts significant anti-oxidant and anti-inflammatory effects. In animal models of hypercholesterolemia and atherosclerosis, the manipulation of HDL, its associated protein apolipoprotein A-I, and their synthetic mimetics has resulted in lesion regression and lesion stabilization irrespective of non-HDL levels. Thus, an anti-atherogenic strategy focusing on HDL and its apolipoproteins represents a new frontier in the management of atherosclerosis.
HDL: potential anti-atherogenic properties

The development of atherosclerosis is a complex process whose central elements include the entrapment of LDL in the vessel wall, its subsequent oxidative modification, and the stimulation of pro-inflammatory gene expression leading to inflammatory cell recruitment, infiltration, and necrosis.\textsuperscript{11,12} HDL interacts with this process at multiple points, and these interactions could provide therapeutic targets to prevent, stabilize, or even promote the regression of atherosclerosis. A major physiologic function of HDL is reverse cholesterol transport, which involves the mobilization of free cholesterol from the arterial wall and its delivery to the liver and steroidogenic tissues.\textsuperscript{13} HDL may retard atherosclerotic progression by promoting cholesterol efflux from the arterial wall, thereby leeching the lipid content out of plaque as well as potentially inhibiting nascent fatty streak formation.\textsuperscript{14,15} The components that play a key role in reverse cholesterol transport are apolipoprotein A-I, ATP binding-cassette transporter protein, lecithin cholesterol acyl transferase (LCAT), cholesterol ester transfer protein (CETP), and phospholipid.\textsuperscript{16} \textbf{(Figure 1)} Paraoxonase and platelet activating factor acylhydrolase, two enzymes found within HDL, likely mediate in part HDL’s ability to inhibit LDL oxidation.\textsuperscript{17,18} Apolipoprotein A-I itself also removes seeding molecules for oxidation from the arterial wall.\textsuperscript{19,20} Since the oxidative modification of LDL initiates the inflammatory process that in turn stimulates the development of atherosclerosis, HDL’s ability to inhibit LDL oxidation may translate into an anti-inflammatory and anti-atherogenic effect. Indeed, HDL and/or its components have been shown to decrease tumor necrosis factor release, inhibit cytokine-induced monocyte adhesion receptor expression by endothelial cells,\textsuperscript{21-23} and reduce atherosclerotic plaque macrophage
content\textsuperscript{24,25} – i.e., HDL decreases inflammatory cell recruitment, adhesion, and infiltration. This may impede the monocyte’s ability to act as a driving force for atherogenesis.

**HDL: potential plaque stabilization properties**

HDL may not only retard (or reverse) the progression of atherosclerosis, but may also have a significant impact on plaque vulnerability. The relative stability of a given atherosclerotic plaque likely depends on the magnitude of its lipid core, the degree and activity of inflammatory infiltrate, and the thickness and smooth muscle content of its fibrous cap.\textsuperscript{26} HDL-mediated cholesterol efflux reduces plaque lipid content; HDL-mediated inhibition of lipid oxidation and the interrelated inflammatory process may reduce plaque macrophage content and activity; and HDL-associated phospholipid scavenging of the toxic byproducts of lipid oxidation decreases smooth muscle death,\textsuperscript{27,28} potentially stabilizing or strengthening the atherosclerotic plaque’s fibrous cap. Indeed, apolipoprotein A-I has been shown in animal models to decrease plaque cholesterol and macrophage content\textsuperscript{24,25,29} and to increase plaque smooth muscle content.\textsuperscript{25} All of these features may enable HDL to alter plaque composition to a more stable phenotype, preventing rupture and in turn decreasing the incidence of acute cardiovascular events.

**HDL: quality, not quantity**

Although HDL has been demonstrated to have physiologic effects that are potentially anti-atherogenic, the magnitude of these effects are determined in part by the qualitative function of the HDL species, not solely by its circulating levels. HDL is not always anti-
inflammatory: during the acute phase, HDL loses its paraoxonase and apolipoprotein A-I content, and becomes pro-oxidant and pro-inflammatory.\textsuperscript{12} Even outside of the acute phase, all HDL is not alike. Navab and colleagues found that the HDL from a group of patients with angiographically proven atherosclerosis, a normal lipid profile, and no other risk factors was dysfunctional in preventing the formation and inactivation of oxidized phospholipids in comparison to that of a control group.\textsuperscript{30} Thus the successful therapeutic use of HDL likely depends on specifically manipulating its anti-atherogenic components and the molecules with which they interact.

**From potential to practice: therapeutic strategies to exploit HDL**

Circulating HDL tends to increase with regular exercise, weight loss, moderate alcohol consumption, smoking cessation, and a high fat diet. Pharmacological intervention with statins, niacin, fibrates, dilantin, chromium picolonate, and female hormone replacement can also raise plasma levels.\textsuperscript{31} These types of interventions have therapeutic limitations because of the modest degree of elevation in HDL levels and the functional heterogeneity of the HDL itself. One alternative is to directly administer functionally competent HDL, its associated protein apo A-I, or synthetic mimetics. Experimental evidence supports the contention that this may provide significant therapeutic benefit. Repeated parenteral administration of recombinant apo A-I\textsuperscript{milano}-phospholipid complex prevented the progression of aortic atherosclerosis in apo E deficient mice despite severe hypercholesterolemia.\textsuperscript{24,32} Injection of discs containing apo A-I and phosphatidylcholine increased in-vivo production of small pre-\(\beta\)-HDL particles and
increased the efflux and esterification of tissue-derived unesterified cholesterol in healthy male volunteers, and injection in humans of liposomes containing a precursor of apolipoprotein A-I increased fecal excretion of cholesterol. This data supports the hypothesis that direct administration of HDL compounds can stimulate reverse cholesterol transport and potentially affect atherosclerotic progression. This potential was demonstrated recently by Navab and colleagues, who showed that treatment with an oral apolipoprotein A-I mimetic peptide reduced atherosclerotic lesion area by 79% in LDL-receptor null mice on a Western diet and by 75% in apo E null mice.

Direct administration of HDL components also favorably alters atherosclerotic plaque composition to a more stable phenotype, as demonstrated by the observation that parenteral recombinant apo A-I_milano-phospholipid-complex reduced plaque cholesterol by 40% and reduced macrophage content by 46% in apo E knockout mice. Even acutely, a single high dose administration resulted in a 40% to 50% lower lipid content (P<0.01) and 29% to 36% lower macrophage content in aortic root plaques at 48 hours. One could speculate that HDL administration could “cool” off “hot” plaques during acute coronary syndromes.

Other novel anti-atherogenic therapies may be directed at key points in HDL metabolic pathways, either through pharmacological manipulation, or in the more distant future, via gene therapy. (Table 1) Candidate targets are the HDL-associated proteins involved in reverse cholesterol transport. CETP inhibition may be potentially anti-atherogenic by raising HDL concentrations, since CETP catalyzes the transfer of esterified cholesterol.
from the HDL particle to LDL, and thus lowers circulating HDL levels and raises LDL levels. A vaccine against an epitope of CETP has been shown to inhibit CETP, increase HDL cholesterol levels, and reduce fatty streaks in cholesterol-fed rabbits.\textsuperscript{37} However, CETP inhibition may also prove to be pro-atherogenic, since CETP facilitates the production of pre-\(\beta\)-HDL particles, which are efficient stimulators of reverse cholesterol transport.\textsuperscript{38} This complex relationship between CETP activity and atherosclerosis makes CETP a less attractive candidate for therapeutic manipulation in humans. ATP binding cassette transporter, a transmembrane protein encoded by the gene ABC-A1, mediates the initial step of reverse cholesterol transport, the cholesterol efflux from cells to HDL particles.\textsuperscript{39} ABC-A1 transcription is regulated by nuclear hormone receptors such as PPAR\(\alpha\), PPAR\(\delta\), PPAR\(\gamma\), liver X receptor (LXR) and the retinoid X receptor.\textsuperscript{40-42} One strategy to enhance reverse cholesterol transport is to modulate ABC-A1 transcriptional regulation through ligands of these nuclear hormone receptors. Indeed, an orally administered selective agonist of PPAR\(\delta\) increases expression of the ATP binding cassette transporter and induces apolipoprotein A1-specific cholesterol efflux, as well as dramatically increases HDL levels, reduces small dense LDL, and corrects hyperinsulinemia in primates.\textsuperscript{43} Ligands of LXR play a broad role in the regulation of lipid metabolism in the bile, peripheral tissues, and gut. PPAR\(\alpha\) and PPAR\(\gamma\) activators, through the enhanced expression of LXR, induce the expression of ABC-A1 and increase cholesterol efflux from macrophages.\textsuperscript{44} Oral administration of an RXR agonist dramatically inhibited atherogenesis in apo E-null mice in an LXR dependent fashion.\textsuperscript{45}
A novel hepatic receptor, SRB-1, has been identified that is responsible for hepatic HDL-associated cholesterol uptake independent of the classic LDL pathway. Adenovirus-mediated, hepatic over-expression of SRB-1 in mice resulted in the virtual disappearance of plasma HDL and dramatically increased bile cholesterol; transient adenovirus-mediated hepatic overexpression of SRB-1 in LDL-receptor null mice led to regression of both early and advanced atherosclerotic lesions and a marked reduction in circulating HDL levels. Thus the enhancement of SRB-1 activity and/or its expression represents a potential strategy to significantly promote reverse cholesterol transport and inhibit atherosclerosis.

**Conclusion**

A wealth of experimental data has demonstrated that HDL and apo A-I have significant anti-oxidant and anti-inflammatory properties, scavenge toxic products from the endothelium, ameliorate endothelial dysfunction, and mobilize cholesterol from the vessel wall. Qualitative HDL function may be crucial in addition to circulating HDL levels in exerting these effects. Elevations in HDL levels through statin, fibrate, or niacin therapy may not be adequate to exploit the anti-atherogenic and vasculo-protective potential of HDL and apo A-I. Direct administration of plasma-derived or recombinant HDL/apo A-I or their synthetic mimetics as a drug may provide significant clinical benefit. Activation of specific subtypes of nuclear hormone receptors, particularly the rexinoids, and pharmacological or gene therapy to enhance the expression and/or activity of the specific hepatic HDL-cholesterol scavenger receptor represent novel strategies to augment reverse cholesterol transport. This may inhibit the initiation and progression of
atherosclerosis, and promote its regression. The 1990’s were the decade of statins and LDL lowering -- perhaps the coming years will prove to be the decade of HDL.
### Table 1: High density lipoprotein, anti-atherogenic properties, and therapeutic strategies

<table>
<thead>
<tr>
<th>HDL component/associated protein</th>
<th>Antiatherogenic/vasculoprotective effect</th>
<th>Therapeutic strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apolipoprotein A-I</strong></td>
<td>Facilitates reverse cholesterol transport</td>
<td>Direct IV/PO administration of plasma-derived wild-type apo A-I, apo A-I_milano, whole functional HDL, or synthetic mimetic peptides</td>
</tr>
<tr>
<td></td>
<td>Removes oxidative seeding molecules from endothelium</td>
<td>Apo A-I gene transfer</td>
</tr>
<tr>
<td></td>
<td>Scavenges toxic products from arterial wall</td>
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<tr>
<td></td>
<td>Reduces smooth muscle cell apoptosis/necrosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduces plaque lipid content</td>
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</tr>
<tr>
<td></td>
<td>Reduces plaque macrophage content</td>
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<tr>
<td></td>
<td>Improves endothelial dysfunction</td>
<td></td>
</tr>
<tr>
<td><strong>Phospholipid</strong></td>
<td>Facilitates reverse cholesterol transport</td>
<td>Synthetic discs/liposomes</td>
</tr>
<tr>
<td><strong>Paraoxonase, PAF-acetylhydrolase</strong></td>
<td>Inhibits LDL oxidation</td>
<td>Increased activity by pharmacological/gene therapy?</td>
</tr>
<tr>
<td><strong>CETP</strong></td>
<td>Facilitates reverse cholesterol transport</td>
<td>Vaccination, pharmacological inhibitor</td>
</tr>
<tr>
<td><strong>ATP binding cassette transporter</strong></td>
<td>Facilitates reverse cholesterol transport</td>
<td>Rexinoids, PPAR receptor family agonists, LXR agonists</td>
</tr>
<tr>
<td><strong>SRB-1 receptor</strong></td>
<td>Facilitates reverse cholesterol transport (hepatic HDL uptake)</td>
<td>Increased activity/expression by gene therapy, pharmacological therapy?</td>
</tr>
</tbody>
</table>
Figure 1. The reverse cholesterol transport pathway. The ABC-A1 gene encodes transmembrane proteins which are critical, in addition to apolipoprotein A-I, for mobilization of free cholesterol from the macrophage to the apolipoprotein A-I containing HDL. Activation of nuclear hormone receptors enhance the expression of ABC-A1. LCAT catalyzes the esterification of free cholesterol. The cholesterol ester of HDL has three possible fates: transfer to LDL by CETP for hepatic uptake by the classic LDL pathway; selective SRB-1 mediated CE uptake in the liver and steroidogenic tissue; or holoparticle uptake in the kidney via the Cubilin and Megalin receptors. CE indicates cholesterol ester; FC, free cholesterol; TG, triglycerides; ABC, ATP-binding cassette transporter; LCAT, lecithin cholesterol acyl transferase; LDL-R, LDL receptor; RXR, retinoid X receptor; LXR, liver X receptor; FXR, farnesoid X receptor; and CETP, cholesterol ester transfer protein. See text for details.
Figure 2. Schematic of intestinal regulation of cholesterol transport. Ligand-mediated nuclear hormone receptor activation leads to activation of the ABC transporter family, leading to inhibition of cholesterol uptake in the gut and increased cholesterol efflux from peripheral tissues. Nuclear hormone receptor activation also stimulates biliary excretion of bile acids. C, cholesterol; RXR, retinoid X receptor; LXR, liver X receptor; and FXR, farnesoid X receptor.


