High-Density Lipoprotein and Atherosclerosis Regression Evidence From Preclinical and Clinical Studies

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Abstract: High-density lipoprotein (HDL) particles transport (among other molecules) cholesterol (HDL-C). In epidemiological studies, plasma HDL-C levels have an inverse relationship to the risk of atherosclerotic cardiovascular disease. It has been assumed that this reflects the protective functions of HDL, which include their ability to promote cholesterol efflux. Yet, several recent pharmacological and genetic studies have failed to demonstrate that increased plasma levels of HDL-C resulted in decreased cardiovascular disease risk, giving rise to a controversy regarding whether plasma levels of HDL-C reflect HDL function, or that HDL is even as protective as assumed. The evidence from preclinical and (limited) clinical studies shows that HDL can promote the regression of atherosclerosis when the levels of functional particles are increased from endogenous or exogenous sources. The data show that regression results from a combination of reduced plaque lipid and macrophage contents, as well as from a reduction in its inflammatory state. Although more research will be needed regarding basic mechanisms and to establish that these changes translate clinically to reduced cardiovascular disease events, that HDL can regress plaques suggests that the recent trial failures do not eliminate HDL from consideration as an atheroprotective agent but rather emphasizes the important distinction between HDL function and plasma levels of HDL-C. (Circ Res. 2014;114:205-213.)

Key Words: atherosclerosis ■ cholesterol, HDL ■ coronary artery disease ■ mice ■ regression

High-density lipoproteins (HDL) are typically defined as lipoprotein particles with buoyant densities from 1.063 to 1.21 g/mL and having apolipoprotein AI (apoAI) as the major apolipoprotein species. It has become increasingly appreciated that the HDL population consists of a collection of particles with diverse sizes, structures, and composition and functional properties that are thought to influence atheroprotectiveness. The compositional complexity reflects not only multiple species of proteins and lipids but also other macromolecules (eg, microRNA). Historically, the HDL
Nonstandard Abbreviations and Acronyms

- apoAI: apolipoprotein A1
- apoE–/–: apolipoprotein E–deficient
- CAD: coronary artery disease
- CCR7: C-C chemokine receptor type 7
- CVD: cardiovascular disease
- hAI: human apolipoprotein A1
- HDL-C: high-density lipoprotein cholesterol
- LDL-C: low-density lipoprotein cholesterol
- Ldrl: low-density lipoprotein receptor
- Ldrl–/–: low-density lipoprotein receptor–deficient
- RCT: reverse cholesterol transport
- WT: wild-type

The component of greatest clinical interest has been the cholesterol (HDL-C) content, which consists of both the free and the more predominant ester species because of the many epidemiological studies demonstrating a strong negative correlation between plasma HDL-C and the risk of cardiovascular disease (CVD).1–4

The mechanism for this association has been presumed to be that the plasma level of HDL-C reflects the availability of functional HDL particles with atheroprotective actions, particularly the stimulation of reverse cholesterol transport (RCT) from peripheral cells (including foam cells in coronary plaques) to the liver. On the basis of relatively small clinical trials or in vitro studies, we found that there is also support for the idea that HDL can protect the endothelium (by activation of the endothelial nitric oxide synthase pathway), inhibit low-density lipoprotein (LDL) oxidation, and exert anti-inflammatory and antithrombotic effects.5–8 However, the trend of the recently evolving data does not establish tight associations between plasma levels of HDL-C and either these functions or, more significantly from a clinical perspective, CVD risk. For example, the in vitro ability of plasma samples to promote cholesterol efflux was better than HDL-C as a predictor for angiographically proven coronary artery disease (CAD);9 for genetic polymorphisms that were associated with changes in HDL-C, there were no corresponding variations in CVD risk.10

These types of studies and the failure of several drugs that, among other effects, increase plasma HDL-C without reducing CVD risk11,12 have fueled the skepticism that plasma levels of HDL-C reflect any cardioprotective functions of HDL particles or that, more fundamentally, HDL has cardioprotective functions. However, as we13 and others (including in the present review series) have argued, it is important to appreciate the distinction between HDL functions and plasma levels of HDL-C, as well as to consider the evidence from several preclinical and clinical studies that support atheroprotection by HDL if the number of functional particles is increased. The focus of the present review is on these studies, although to put them in context we also discuss some clinical reports that have contributed to the HDL controversy.

Our particular research interest has been in the area of the regression of atherosclerosis by HDL, which is highlighted. The Table summarizes several relevant preclinical and clinical studies on this point, but space does not allow for a comprehensive discussion of all of them and still others, so the interested reader is encouraged to consult the cited references and other sources for more comprehensive information.

**HDL and Atherosclerosis Regression: Preclinical Models**

That atheromata can regress at all is a concept that has met resistance for years. The reason for this may have been that advanced atherosclerotic lesions in humans and in animal models contain calcification and fibrosis, characteristics that seem irreversible.14,15 Nonetheless, several studies beginning >50 years ago argue that this is not the case. For example, the first interventional study demonstrating substantial shrinkage of atherosclerotic lesions was performed in cholesterol-fed rabbits.16 Animals received intravenous bolus injections of phospholipid. After less than 1 week and half of the treatment, the remaining plaques were fewer and smaller, with ≈75% of the arterial cholesterol stores being removed. The basis of this effect was explored in subsequent mechanistic studies17 that indicated that when intravenously injected at sufficient doses, initially cholesterol-free phospholipid vesicles remained intact in the bloodstream and were capable of extracting cholesterol from lipoproteins, particularly HDL. Thus, these circulating particles act as a sink for cholesterol, which is shuttled to them from tissues by HDL and lipopoor apoAI. Because the liver serves as the predominant organ for the clearance of phospholipid vesicles, the antiatherogenic effects of these particles likely result from their ability to act as synthetic mediators of RCT from peripheral tissues to the liver.

Using a variety of atherosclerotic animal models, including monkeys, other groups showed similar arterial benefits from a variety of interventions, including, again, the injection of dispersed phospholipids, as well as from dietary changes and treatment with hypolipidemic agents.16,18–21 Most relevant to the present review are studies of cholesterol-fed rabbits that demonstrated shrinkage or delayed progression of atheromata after injections of HDL22 or apoAI,23 respectively.

The availability of appropriate mouse models would allow more convenient and mechanistic investigations of atherosclerosis regression and the effects of HDL and apoAI. However, murine HDL metabolism has 3 major differences when compared with that in humans: HDL, not LDL, is the principal carrier of circulating cholesterol in mouse plasma; mouse LDL is a monodisperse population (ie, without HDL2 and HDL3 subfractions); and the activity of cholesterol-ester transfer protein (CETP), which in humans serves in the plasma to trade cholesteryl ester carried on HDL for triglycerides on the apoB-containing lipoproteins very LDL and LDL, is absent.7,8 To make mouse HDL metabolism more human-relevant, some investigators have introduced a human apoAI transgene that resulted in more disperse HDL particles from which mouse apoAI seemed to be displaced.24 Others have also genetically modified mice with a transgene for human CETP.25

Another limitation of the mouse for atherosclerosis research is that it is naturally resistant to the disease. To overcome this, mice with deficiency in either apoE (apoE–/–) or the LDL
Table. Selected HDL and ApoAI Regression Studies

<table>
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<tr>
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<td>rHDL, V156K-rHDL (V156K point mutant of apoAI), R173C-rHDL (apoAI&lt;sub&gt;mouse&lt;/sub&gt;)</td>
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<td>Feig et al</td>
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<tr>
<td>Shaw et al</td>
<td>Human (PAD)</td>
<td>rHDL (CSL-111)</td>
<td>80 mg/kg</td>
<td>IV</td>
<td>SFA (after atherecemy 5–7 d after infusion)</td>
<td>Lipid content ↓ Macrophages cell size ↓ VCAM-1 expression ↓</td>
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ACS indicates acute coronary syndrome; anti-miR, anti-microRNA; apoAI, apolipoprotein AI; apoE, apolipoprotein E; CCR7, C-C chemokine receptor type 7; ERASE, Effect of rHDL on Atherosclerosis-Safety and Efficacy; FC, free cholesterol; HDL, high-density lipoprotein; IV, intravenous; IVUS, intravascular ultrasound; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; NS, nonsignificant; PAD, peripheral artery disease; PAV, percent atheroma volume; PL, phospholipids; rHDL, reconstituted high-density lipoprotein; s.c., subcutaneous; SFA, superficial femoral artery; TC, total cholesterol; VCAM-1, vascular cell adhesion molecule 1; and VHDL, very-high-density lipoprotein.

receptor (Ldlr<sup>-/-</sup>) were created, providing robust models of hypercholesterolemia and atherosclerosis. Notably, there are several similarities between atherosclerosis pathology in humans and mice, although the latter do not exhibit the plaque rupture. Early studies of atherosclerosis in mice included the demonstration of the delayed progression of plaques on the increased hepatic expression of apoAI (using a human apoAI transgene [hAI]) in apoE<sup>-/-</sup> mice. More recently, mouse models of atherosclerosis have been used for regression studies as well. In the present review, we emphasize these preclinical studies with a focus on apoAI or HDL particles and not on HDL-C per se. Hepatic production of hAI was increased by adeno viral vectors after plaques began to form in several studies. In 1999, Tangirala et al<sup>29</sup> reported that in Ldlr<sup>-/-</sup> mice, this resulted in 3-times increase in plasma levels of apoAI (versus control virus injected mice) and 70% and 46% reductions in atherosclerosis lesion area measured by aortic en face analysis or by aortic root plaque cross-sectional area, respectively. However, the mice had been fed the atherogenic diet for only 5 weeks before the viral treatments began, meaning that the regression induced by increasing the production of hAI was of early fatty streak–like plaques. Using a similar approach, Li et al<sup>29</sup> used an adeno viral vector expressing hAI in Ldlr<sup>-/-</sup> mice fed an atherogenic diet, but for a considerably longer period than the previous study (36 weeks). In contrast to the early lesions in Ldlr<sup>-/-</sup> mice, hAI expression was no longer
sufficient to produce regression of lesion size. One factor underlying the differences between the effects of hAI expression in the 2 studies is that as plaques advance in mouse models or in humans, the percentage occupied by macrophages decreases. Thus, if these cells are major beneficiaries of increasing several functional HDL particles, then the effect on lesion size would be attenuated in advanced plaques. Note that there could still be an improvement in plaque stability if an advanced plaque had its composition altered to become macrophage-poor and collagen-rich, as we have found in apoE−/− mice after increasing plasma hAI levels.30,31

In another approach to increasing the several functional HDL particles, Shah et al32 reported in 2001 the results of studies in which a high dose of recombinant hAI (the Milano variant; complexed to PC) was administered to apoE−/− mice fed an atherogenic diet for 26 weeks to develop advanced plaques. Dramatically, a single intravenous bolus of the hAI preparation resulted in reductions by 48 hours in plaque lipid and macrophage contents of ≤50% and 36%, respectively.

In 2001, we reported a new mouse model33 in which rapid changes in the plasma lipoprotein profile could be made and sustained indefinitely. The initial approach was to transplant either an atherosclerotic thoracic34 or an aortic arch segment34 from hypercholesterolemic apoE−/− donor mice to normolipidemic wild-type (WT) recipient mice. As in the study of Shah et al, regression was rapidly apparent (as judged by plaque content of CD68+ monocyte-derived cells, which are primarily macrophages), with a 50% reduction by 3 days after transfer.34-37 Notably, the quantitative change in macrophage content was associated with emigration of CD68+ cells from plaques to regional and systemic lymph nodes under regression, but not progression, conditions.35,37 By analyzing RNA obtained from laser-captured plaque CD68+ cells, we found an increase in the gene expression of the C-C chemokine receptor type 7 (CCR7), a factor previously shown to be required for the migration of dendritic cells (which, like macrophages, are primarily monocyte-derived)38 and only in the regression environment.37 Furthermore, we went on to show a substantial functional contribution of CCR7 for regression in this model.37

In the WT recipients, relative to the donor mice, non–HDL-C levels decreased and HDL-C levels were restored from ≈33% of normal to WT levels. Importantly, the differences in HDL-C levels between WT and apoE−/− mice reflect their respective plasma levels of apoAI and, hence, several HDL and lipid-poor apoAI particles. To test the effects of the changes in these levels on plaque regression selectively, we now used as recipients hAI transgenic/apoA1poliprotein E knockout mice,30,39 which, as noted, have increased hepatic production of apoAI and suppressed atherosclerosis progression, despite persistent non-HDL hypercholesterolemia.30,37

Remarkably, despite the persistent non-HDL hypercholesterolemia in hAI/apoA1poliprotein E knockout recipients, plaque CD68+ cell content decreased by >50% by 1 week after transplantation, whereas there was little change in apoAI−/− recipients, despite hypolipidemia, indicating a need for functional HDL particles. As in the WT recipient study, the decreased content of plaque CD68+ cells in the hAI/apoA1poliprotein E knockout recipients was associated with their emigration and induction of their CCR7.31 One molecular mechanism for the induction of CCR7 in plaque macrophages by increasing functional HDL is that after cholesterol depletion of the plaque,31 there is stimulation of CCR7 transcription in the macrophages through the sterol response element in the murine and human gene promoters.40 Because HDL can also decrease the circulating pool of monocytes41 and the expression of monocyte adhesion factors expressed by endothelial cells in hypercholesterolemic mice,42 it is likely that ongoing monocyte recruitment is also decreased, which would be expected to contribute to plaque regression.43

An increasingly recognized goal of atherosclerosis treatment is the resolution of the plaque inflammatory state,44 which can be accomplished not only by a decrease in the content of activated macrophages but also by an enrichment in macrophages with anti-inflammatory properties. On the basis of primarily studies in vitro, activated and anti-inflammatory/tissue-repairing macrophages have been characterized by their differential expression levels of several molecules and have been broadly classified as M1 and M2, respectively.45 Using such markers, macrophages with characteristics of the M1 or M2 state have been found in both human and murine atherosclerotic plaques.46,47

As noted, HDL is thought to contribute to atheroprotection as an anti-inflammatory through, for example, antioxidant properties of its enzymatic and nonenzymatic components, the ability to remove normal and toxic lipid species from cells, and the dampening of Toll-like receptor signaling by regulating plasma membrane cholesterol content.7,48,49 To probe whether the anti-inflammatory effects of HDL included changes in the balance between M1 and M2 macrophages, markers of these states were assessed in plaques in donor (apoE−/− mice after 16 weeks of an atherogenic diet) and in recipient (hAI/apoA1poliprotein E knockout) mice. The increases in plasma levels of apoAI and HDL particles were associated with decreased expression of inflammatory factors and enrichment of M2 markers.31,50 Furthermore, using an inhibitor of miR-33, which increases lipid-poor apoAI production by the liver and enhances ABCA1/G1-mediated cholesterol efflux from macrophages,31 in collaboration with Moore,51 we found similar results as in the transplant model (ie, decreased macropage content in plaques in Ldlr−/− mice as well as reduced and increased M1 and M2 marker expression, respectively).

Recently, the balance between the 2 macrophage states has been therapeutically manipulated and effects on atherosclerosis progression have been measured. Ldlr−/− mice were treated with M2 polarizing factors, which, consistent with the favorable association of the M2 state in regression, attenuated atherosclerosis progression.33,34 How HDL contributes to a reduction in M1 or an enhancement in M2 polarization may depend on a number of its properties. As noted, there is growing evidence that it or apoAI may limit Toll-like receptor responsiveness to inflammatory stimuli (by regulating plasma membrane cholesterol content and microenvironments), and there is more recent evidence from our group indicating that it can promote the phosphorylation of STAT6, an integral signaling component of the M2 polarization pathway.51,48,49,55-57

In summary, the preclinical data convincingly demonstrate the ability of functional HDL and lipid-poor apoAI particles to promote the regression of atherosclerosis by effects on the
number and the inflammatory state of plaque macrophages. A diagrammatic summary of the current preclinical understanding of how HDL accomplishes this is given in the Figure. The availability of an expanding variety of mouse models of regression will allow deeper studies of the associated mechanisms and will likely inform the human biology.

**HDL and Atherosclerosis Regression: Clinical Studies**

In addition to preclinical studies, there are a limited number of clinical studies in which plasma levels of HDL particles have been manipulated and in which the effects on plaques have been assessed (a partial summary is in the Table). For example, patients at high risk for CVD were infused with either an artificial form of HDL (apoAl milano/phospholipid complexes) or saline (placebo) once per week for 5 weeks. By intravascular ultrasound, there was a significant reduction in atheroma volume (≈4.2%) in the combined (high-dose and low-dose) treatment group, although no enhancement by the higher dose was observed. In the second infusion study (Effect of HDL on Atherosclerosis-Safety and Efficacy [ERASE]), high-risk patients received 4 weekly infusions with reconstituted HDL (containing WT apoAI) or saline (placebo). Similar to the previous study, there was a significant decrease in atheroma volume (≈3.4%; as assessed by intravascular ultrasound) after treatment with reconstituted HDL when compared with baseline but not when compared with placebo (a comparison for which the study was not powered). However, the reconstituted HDL group had statistically significant improvements in a plaque characterization index and in a coronary stenosis score on quantitative coronary angiography when compared with the placebo group. In the third infusion trial, a single dose of reconstituted human HDL was infused into patients undergoing femoral atherectomies, with the procedure performed 5 to 7 days later. When compared with the control group (receiving saline solution), in the excised plaque samples in the HDL infusion group, macrophage activation state (eg, diminished vascular cell adhesion molecule 1 expression) and cell size (because of diminished lipid content) were reduced, consistent with the results from the preclinical studies reviewed.

**HDL-C and Atherosclerosis Regression: The Clinical Controversy**

In contrast to the small number of clinical studies of increasing several HDL particles, there is more literature on the effects on plaques and CVD risk of pharmacological manipulations that increase HDL-C. There have been 2 major such strategies, niacin and, more recently, CETP inhibition. The presumption has been that the increases in HDL-C would reflect the actions of an increased supply of functional HDL and lipid-poor apoAI particles, which would be expected to benefit plaque size, composition, and CVD risk. We consider each agent in turn.

Niacin modestly increases plasma levels of HDL-C and lowers those of LDL- cholesterol (LDL-C), triglycerides and lipoprotein(a) through mechanisms that remain largely undefined. Its effect on CVD risk has been studied for decades, but we confine our survey to those most relevant to atherosclerosis regression. However, the pleiotropic effects of niacin on plasma lipids/lipoproteins and its frequent use with other lipid-lowering agents make it difficult to attribute its effects in most clinical studies solely to changes in HDL metabolism.

The major clinical studies of niacin that have included effects on plaques are (oldest first): the Familial Atherosclerosis Treatment Study (FATS); the Cholesterol-Lowering Atherosclerosis Study (CLAS); the HDL-Atherosclerosis Treatment Study (HATS); and the Arterial Biology for the Treatment of Risk Factors in Ischemia (ARBITER) series of studies. The patient population sizes in all were small (120–162 patients). In FATS, patients with documented CAD were randomly assigned to treatment groups: niacin and the bile acid resin colestipol; the statin lovastatin alone; colestipol alone; or placebo. After 2.5 years, HDL-C in the niacin–colestipol group increased by 43% and was associated with angiographic atherosclerotic regression in 39%. There was also an associated significant outcome benefit with a 73% reduction in clinical events (death, myocardial infarction, or revascularization). In CLAS, placebo or niacin and colestipol was administered to patients with known CAD. Repeat angiography after 4 years showed significantly more patients with nonprogression (52% versus 15%) and regression

![Figure](https://example.com/figure.png)

**Figure.** The promotion of atherosclerosis regression by high-density lipoprotein (HDL) in an aortic transplantation mouse model. Monocytes are recruited into plaques and become macrophages. These macrophages become activated, cholesterol-laden foam cells, as a result of ingesting normal and modified apolipoprotein B-containing lipoproteins and are retained in the plaque. On the basis of in vitro and preclinical studies, we found that the recently recognized ways in which HDL can contribute to plaque regression include reduced monocyte recruitment because of reduced leukocytosis or endothelial cell adhesion molecule expression, the stimulation of C-C chemokine receptor type 7 (CCR7) expression by the promotion of cholesterol efflux from foam cells, which results in emigration of macrophages to lymphoid tissue and to the systemic circulation, and the stimulation of the STAT6 pathway to polarize macrophages to the M2 state, as indicated by the increase in the markers mannose receptor (MR), interleukin (IL)-10, and arginase I (Arg I). As tissue repairs cells, M2 macrophages also exhibit enhanced efferocytosis (disposal) of apoptotic cells. See text for details and for additional mechanisms. LDL indicates low-density lipoprotein.
(18% versus 6%) in the treatment versus the placebo group. In HATS, niacin–simvastatin alone or together with antioxidant vitamin therapy or placebo was administered to patients with CAD. At 3-year follow-up, niacin–simvastatin was associated with significant regression of coronary stenosis and a combined 90% reduction in major clinical events (including death from coronary causes, nonfatal myocardial infarction, stroke, or revascularization for worsening angina). Finally, in ARBITER 2, once-daily extended-release niacin with and without statin therapy was administered to patients with CAD. At 1 year, mean carotid intimal-medial thickness, a controversial surrogate marker of coronary plaque burden, increased significantly in the statin-alone group but was unchanged in the niacin–statin group. Because of these successes, the lack of efficacy of niacin to reduce cardiovascular events in the much larger Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides (AIM-HIGH) (3414 subjects) and Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-Thrive) (25 673 patients) was an unexpected surprise.

There have been several speculations for these disappointing results, but perhaps the most commonly voiced explanation has been that in both studies the concurrent use of statins to lower LDL-C aggressively (to the range of 60 mg/dL) made an additional benefit of increasing HDL-C a challenge. Furthermore, the increase in HDL-C was small in both studies (6–7 mg/dL). Another interpretation is that improvements in plaque size or characteristics may not translate into reduced event rates, although the few small-scale studies that have included imaging do not supply sufficient data to assess this possibility definitively.

Alternatively, increases in plasma HDL-C may not necessarily affect plaque biology if the functions of HDL, such as its effectiveness to mediate RCT, are not consistently reflected by plasma levels of HDL-C. Several preclinical studies have shown disconnections between plasma HDL-C and the level of RCT and atheroprotection (eg, in scavenger receptor class B, member 1 transgenic mice). This alternative also has been borne out in 1 relatively small clinical study in which the in vitro efficacy of apoB-lipoprotein–depleted serum (ie, enriched in HDL) was correlated with CVD risk but not plasma levels of HDL-C. The mechanistic bases for the disconnect between plasma levels of HDL-C and HDL function are discussed in the Dysfunctional apoAI and HDL: Mechanistic Considerations section of this article.

For CETP, its activity to transfer cholesterol from HDL to apoB-lipoproteins in plasma means that the route back to the liver of cholesterol effluxed to HDL has 2 pathways: one direct with HDL docking with hepatic scavenger receptor class B, member 1 and unloading its cholesteryl ester and one indirect via apoB-lipoprotein uptake by the hepatic LDL receptor. If CETP is blocked, then the direct pathway is favored and there is accumulation of HDL particles in the plasma compartment because they tend to be larger, thereby delaying their clearance, which is inverse to HDL size. This is in contrast to the infusion studies in mice, rabbits, and humans, as well as in the hAI transgenic mice, in which increased HDL-C is a result of supplying more HDL particles by exogenous or endogenous means. Still, as proposed by Tall et al, CETP inhibition may still lead to the entry of increased numbers of HDL particles into the artery wall, where they can act as acceptors of macrophage cholesterol.

In the Japanese population, loss-of-function mutations of CETP have been associated in some families with high levels of HDL-C (>60 mg/dL) and reduced rates of CVD. However, in other families, particularly those with lower levels of HDL-C, the risk was elevated. The potential requirement for high levels of HDL-C to be achieved (presumably reflecting a sufficiently expanded plasma pool of efflux-competent HDL particles) may explain the recent failure of the CETP inhibitor dalcetrapib to reduce cardiovascular events in the dal-OUTCOMES trial.

Another CETP inhibitor, torcetrapib, which potently increases plasma levels of HDL-C and apoAI and significantly lowers that of LDL-C, was also judged a failure in the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial. It has been presumed that this involved off-target actions of the compound, because there were elevations in blood pressure and serum potassium, presumably from aldosterone stimulation. Nevertheless, CETP inhibitor partisans were encouraged by the post hoc analysis that showed that treated subjects achieving the greatest increases of plasma levels of HDL-C or apoAI had evidence of atherosclerosis regression on intravascular ultrasound and also a lower rate of major cardiovascular events.

There are 2 other CETP inhibitors in clinical trials, anacetrapib and evacetrapib. Like torcetrapib, both significantly increase plasma levels of HDL-C and lower LDL-C. Unlike torcetrapib, neither has exhibited adverse effects on blood pressure or serum potassium. Completion of the phase III studies (anacetrapib, REVEAL; evacetrapib, ACCELERATE) is expected to provide definitive answers to the question of whether CETP inhibition is an effective strategy to reduce cardiovascular events. Whether the increase in the type of HDL particles produced by CETP inhibition will promote the regression of atherosclerosis is not to be addressed, however, because there are no imaging studies included in these trials. Even if there were, the effects of the significant reduction in LDL-C, which itself can lead to regression, will be difficult to separate from those of the increase in HDL-C. In fact, it is generally challenging to establish the clinical relationships among HDL function, HDL-C levels, plaque size/composition, and cardiovascular risk, given that all of these are not simultaneously assessed in large-scale intervention or observational studies, and that detecting CVD risk change is not in the time frame of short-term intervention studies, such as those with apoAI or HDL infusions.

**Dysfunctional apoAI and HDL: Mechanistic Considerations**

Although apoAI is the major protein constituent of HDL particles, they are not functionally equivalent, as exemplified by the preferences of the cholesterol efflux factors for one or the other (ABCA1, lipid-poor apoAI; scavenger receptor class B, member 1 and ABCG1, HDL). Lipid-poor apoAI (pre-β HDL on gels) constitutes ≈5% of plasma apoAI and may be derived either from its primary secretion by liver or intestine or released from chylomicrons, very LDL, or HDL on lipoprotein remodeling. The majority of cholesterol released
from macrophages in mouse models is apoAI-dependent, indicating that lipid-poor apoAI may have an exceptionally important role in RCT. In contrast, lipid-poor apoAI does not possess several of the endothelial cell–protective and anti-inflammatory activities of HDL that are mediated by binding to scavenger receptor class B, member 1.79–81

Multiple studies have shown that apoAI recovered from the human artery wall exhibits extensive post translational modifications through oxidative processes, particularly those mediated by myeloperoxidase and nitric oxide–derived oxidants.82 Furthermore, ex vivo modification of apoAI, to a comparable extent, by the myeloperoxidase pathway markedly inhibits cholesterol efflux and lecithin–cholesterol acyltransferase activity of the lipoprotein. Myeloperoxidase oxidation of HDL not only causes it to lose its endothelial cell protective effects but also gains a proinflammatory activity, inducing endothelial cell adhesion molecule expression.81

In a recent study,83 we examined the function and distribution of apoAI recovered from normal and atherosclerotic human arterial tissues. Remarkably, the function and distribution (HDL particle association) of apoAI recovered from normal human arterial tissues were markedly different from those observed in plasma. Specifically, in contrast to what is observed in plasma, the overwhelming majority of apoAI (>95%) within normal and atherosclerotic human arterial tissue was found to be predominantly lipid-poor and to not to reside on an HDL particle. Furthermore, the majority of apoAI within arterial tissues was found to be extensively oxidized and cross-linked. In addition, apoAI recovered from human aorta was found to be dysfunctional, with 80% to 90% reductions in cholesterol efflux activity and ability to activate lecithin–cholesterol acyltransferase when incorporated into reconstituted HDL particles. Finally, examination of the relatively lipid-poor fraction of apoAI in the circulation was found to be substantially more oxidatively cross-linked than the apoAI recovered in circulating HDL. These results collectively suggest that in addition to the plasma level of HDL-C not necessarily being functionally relevant, even studies that focus on biological activities of apoAI recovered from plasma or serum HDL may not reflect the biology of apoAI within the artery wall.

Conclusions
The cardioprotective effects of HDL were initially suggested by the strong inverse relationship between plasma HDL-C levels and CVD risk in observational studies. It was assumed that the levels reflected the efficacy of HDL particles to efflux cholesterol from macrophage foam cells in atherosclerotic plaques, as well as other atheroprotective functions. More recently, several pharmacological and genetic studies have raised the questions of whether HDL-C is a reliable biomarker of HDL functionality and, in a further erosion of the HDL hypothesis, whether HDL function itself is important, especially once plaques advance significantly or LDL-C is sufficiently lowered. This controversy has obscured the preclinical and human studies to date that have generally shown that when the levels of functional HDL particles are increased, either by stimulating endogenous production of (lipid-poor) apoAI or by providing HDL or apoAI exogenously, regressive changes in plaques result that would be expected to translate to the reduction in CVD risk.

Going forward, this clinical translation remains to be rigorously established by incorporating outcome and imaging data within large-scale sufficiently powered studies. Also remaining is the unraveling at progressively deeper levels the mechanistic bases for the beneficial effects of apoAI and HDL on plaque size, composition, and inflammatory state, and how their modifications can impair these effects. Despite the incompleteness of our current clinical and preclinical knowledge, if further investigations continue to support the power of HDL to favorably modify plaque biology, rather than abandon the HDL hypothesis entirely as a therapeutic strategy, then a more prudent approach would be to shift the target of simply increasing HDL-C to that of increasing the supply of functional HDL particles or the intrinsic functions through other means.

Acknowledgments
We thank Kathryn Moore for helpful discussions.

Sources of Funding
High-density lipoprotein–related research was supported by National Institutes of Health (NIH) grants HL-084312, HL-098055 (Drs Fisher, Smith, and Hazen), NIH fellowship AG-029748 (J.E. Feig), and fellowship from the German Research Foundation (DFG: HE 6092/1–1; B. Hewing).

Disclosures
Drs Hazen and Smith report being listed as coinventors of pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics. Dr Hazen reports having been paid as a consultant for the following companies: AstraZeneca Pharmaceuticals LP; Cleveland Heart Laboratory; Esperion; Lilly; Liposcience Inc; Merck & Co, Inc; Pfizer Inc; and Takeda. Dr Hazen reports receiving research funds from Abbott, Cleveland Heart Laboratory, and Liposcience Inc. Dr Smith reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics from Cleveland Heart Laboratory and being paid as a consultant for Esperion. Dr Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and the following companies: Cleveland Heart Laboratory; Frantz Biomarkers, LLC; Liposcience Inc; and Siemens. Dr Fisher reports that he is a member of the Merck Atherosclerosis Global Advisory Board. The other authors report no conflicts.

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High-Density Lipoprotein and Atherosclerosis Regression: Evidence From Preclinical and Clinical Studies
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Circ Res. 2014;114:205-213
doi: 10.1161/CIRCRESAHA.114.300760

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