

High-density lipoproteins: A consensus statement from the National Lipid Association

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Abstract: For >4 decades it has been recognized that elevated serum levels of high-density lipoprotein cholesterol (HDL-C) are associated with reduced risk of cardiovascular disease (CVD) and its sequelae. Many prospective observational studies performed around the world have confirmed an inverse relationship between HDL-C and cardiovascular risk in people irrespective of sex, race, or ethnicity. Consequently, it was assumed that, by extension, raising HDL-C through lifestyle modification and pharmacologic intervention would reduce risk of CVD. Animal studies are consistent with this assumption. Lipid treatment guidelines around the world promoted the recognition of HDL-C as a therapeutic target, especially in high-risk patients. Some post hoc analyses from randomized controlled trials also suggest that raising HDL-C beneficially affects the risk of CVD. However, a number of recent randomized studies putatively designed to test the “HDL hypothesis” have failed to show benefit. The results of these trials have caused many clinicians to question whether HDL-C is a legitimate therapeutic target. In response to the many questions and uncertainties raised by the results of these trials, the National Lipid Association convened an expert panel to evaluate the current status of HDL-C as a therapeutic target; to review the

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current state of knowledge of HDL particle structure, composition, and function; and to identify the salient questions yet to be answered about the role of HDL in either preventing or contributing to atherosclerotic disease. The expert panel's conclusions and clinical recommendations are summarized herein. The panel concludes that, although low HDL-C identifies patients at elevated risk, and much investigation suggests that HDL may play a variety of antiatherogenic roles, HDL-C is not a therapeutic target at the present time. Risk stratified atherogenic lipoprotein burden (low-density lipoprotein cholesterol and non-HDL-C) should remain the primary and secondary targets of therapy in patients at risk, as described by established guidelines. The National Lipid Association emphasizes that rigorous research into the biology and clinical significance of low HDL-C should continue. The development of novel drugs designed to modulate the serum levels and functionality of HDL particles should also continue. On the basis of an enormous amount of basic scientific and clinical investigation, a considerable number of reasons support the need to continue to investigate the therapeutic effect of modulating HDL structure and function.

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The proposition that high-density lipoproteins (HDLs) protect against the development of cardiovascular disease (CVD) is based on a number of robust and consistent observations. (1) Human population studies have shown consistently that plasma concentrations of both HDL cholesterol (HDL-C) and the major HDL apolipoprotein (apo), apoA-I, are statistically independent, inverse predictors of the risk of having a CVD event in multivariate models that adjust for established risk factor covariates.¹ (2) HDLs possess several properties with the potential to protect against CVD.^{2,3} (3) Interventions that increase the HDL concentration in a variety of animal models inhibit the development of atherosclerosis.⁴⁻⁷ (4) In proof-of-concept studies in humans, infusions of reconstituted HDLs (rHDL) and mutant forms of HDL (apoA-I_{Milano}) promote regression of coronary atheroma as assessed by intravascular ultrasound (IVUS).^{8,9}

However, interventions that increase the concentration of HDL-C in statin-treated humans have not yet been shown to translate into a reduction in clinical CVD events. Indeed, recent human clinical trials that investigated the effects of HDL-C-raising agents have failed to find any clinical CVD benefit,^{10,11} and in one case the treatment caused harm.¹²

The question arises: why has the robust evidence from the human population studies, the animal intervention studies, and the HDL functional studies not translated into a reduction in clinical CVD events in 4 recent trials with agents that increase the concentration of HDL-C? At this time it is not possible to provide a definitive answer to the question of whether it is too soon to abandon the HDL hypothesis. In this consensus statement, we advocate that much more research is needed to understand the reasons for the unexpected results in these failed clinical trials. This document reviews much of what we know about HDL particles and identifies many areas where more research is required.

Epidemiology

HDL-C as an independent risk factor for CVD

The epidemiologic evidence in support of HDL-C as an inverse predictor of CVD has been appreciated for >50

years. Gofman et al¹³ first reported an inverse association between HDL-C levels and risk of ischemic heart disease. Subsequently, an inverse association between HDL-C and CVD risk was found in the Norwegian Tromsø Heart Study,¹⁴ and this was soon followed by US longitudinal data available from the Honolulu Heart Study and the Framingham Heart Study (FHS).^{15,16} Both of those studies found low HDL-C to be either highly prevalent in patients with CVD or to increase the risk of myocardial infarction (MI), independent of other CVD risk factors. In fact, low HDL-C has been repeatedly found to be associated with increased CVD risk worldwide in both men and women. For example, observational studies in Germany (Fig. 1) and Israel (Fig. 2)¹⁷⁻²⁰ identified low HDL-C as the strongest predictor of incident MI, especially in men older than 50 years. Epidemiologic data are consistent with arteriographic studies that found low HDL-C to be prevalent in patients with left main coronary artery disease (CAD)²¹ as well as a dose-response relationship between HDL-C and extent of arteriographically defined CAD.²²

On the basis of the aforementioned studies, there was general acceptance by the mid-1980s that HDL-C was important to CVD risk factor assessment. It was, therefore, quite surprising, when the Adult Treatment Panel (ATP) of the National Cholesterol Education Program issued the inaugural guidelines for the identification and management

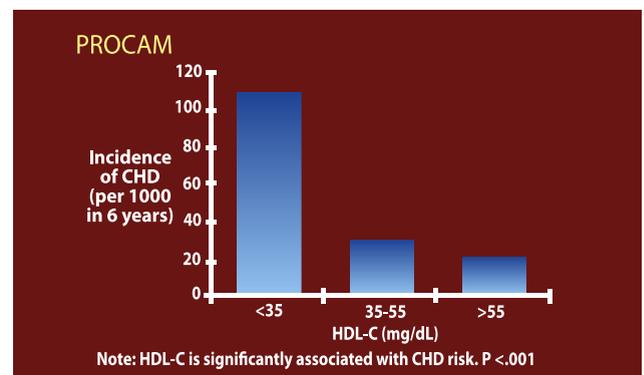


Figure 1 Incidence of CHD according to concentration of HDL-C in the Prospective Cardiovascular Münster study.²⁰ CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol.

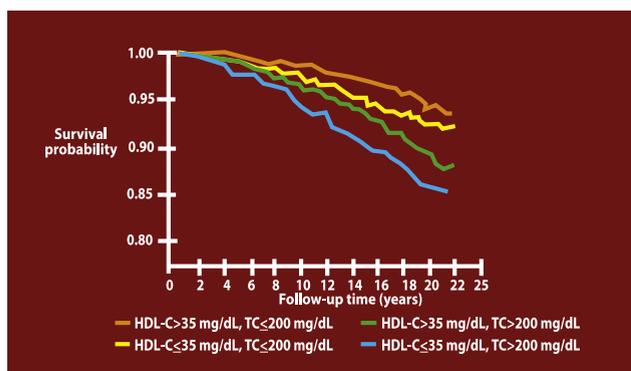


Figure 2 Kaplan–Meier survival curves by HDL-C and TC levels: 21-year CHD mortality from the Israeli Ischemic Heart Disease Study.¹⁹ HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol.

of hyperlipidemia in 1988,²³ that minimal emphasis was placed on screening for low HDL-C. Among the most controversial recommendations of ATP I was the recommendation not to perform a lipoprotein analysis if screening total cholesterol (total-C) was desirable, defined as <200 mg/dL. However, it was well recognized that at least 20% of MI survivors in the FHS had “desirable” total-C levels (Fig. 3).^{24,25} This finding raised the possibility that, in addition to cigarette smoking, hypertension, and diabetes mellitus, low HDL-C was an important contributor to MI risk in normocholesterolemic subjects. To further explore the role of HDL-C in subjects with desirable cholesterol concentrations, the FHS found incident CVD risk to be increased in subjects with low levels of HDL-C, independent of other cardiovascular risk factors.²⁶ This was followed by arteriographic evidence consistently showing that low HDL-C was the most common lipoprotein abnormality in patients with established CVD, irrespective of total-C levels^{27–30} and that a low HDL-C in the absence of elevated cholesterol was also predictive of future CVD events.³¹

The validity of the inverse association generated in observational studies extended to randomized clinical trials in which it was observed in both placebo- and drug-treated groups. The Lipid Research Clinics Coronary Primary Prevention Trial³² found that increases in HDL-C after

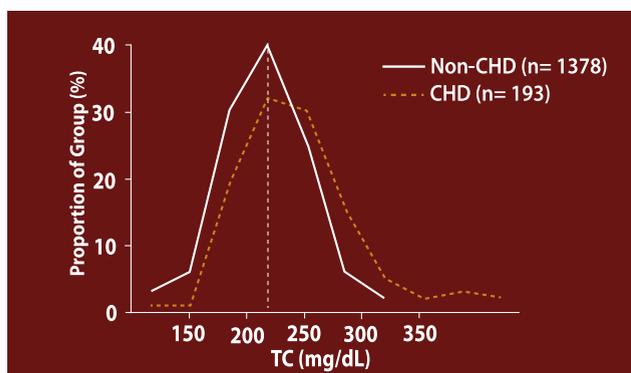


Figure 3 Distribution of TC concentration among men with and without CHD in the Framingham Heart Study, 16-year follow-up.^{24,25} CHD, coronary heart disease; TC, total cholesterol.

treatment with the bile acid resin, cholestyramine, correlated with reduced risk of initial MI or CVD death. In a follow-up analysis of men in the FHS, the Lipid Research Clinics Prevalence Mortality Follow-up Study, the Coronary Primary Prevention Trial, and the Multiple Risk Factor Intervention Trial it was concluded that that each 1-mg/dL rise in HDL-C was associated with a 3% to 4% reduction in CVD mortality rates (Fig. 4).³³ This result heightened interest in the possibility that increasing HDL-C would translate into clinical improvement in CVD risk. This possibility was further supported in the primary prevention Helsinki Heart Study (HHS) in which a 1% increase in HDL-C with gemfibrozil was associated with a 3% decrease in CVD events.³⁴ Another randomized controlled trial that tested the fibric acid gemfibrozil was the Veterans Affairs HDL Intervention Trial (VA-HIT), a secondary prevention study of male veterans with CVD who had a low HDL-C at baseline (<35 mg/dL).³⁵ Even though HDL-C levels rose modestly (6%), a significant proportion of the clinical benefit in CVD risk (22%) was attributable to HDL-C.³⁶

The suggestion that raising HDL-C contributed to reduced CVD risk in HHS and VA-HIT was subsequently evaluated in statin-based randomized controlled trials. A common theme emerged related to CVD risk in placebo-treated patients. Specifically, placebo-assigned subjects with low HDL-C (as defined by median or lowest tertile) levels in the Scandinavian Simvastatin Survival Study, West of Scotland Coronary Prevention Trial, and Air Force/Texas Coronary Atherosclerosis Prevention Study all exhibited higher event rates than patients with higher HDL-C levels at baseline. Moreover, arteriographic progression of CAD was also higher in patients with low vs higher HDL-C levels at baseline.³⁷ These data indicated that low HDL-C was both prevalent and predictive of CVD events, independent of other associated risk factors for major coronary heart disease (CHD). Further work by Brown et al³⁸ found

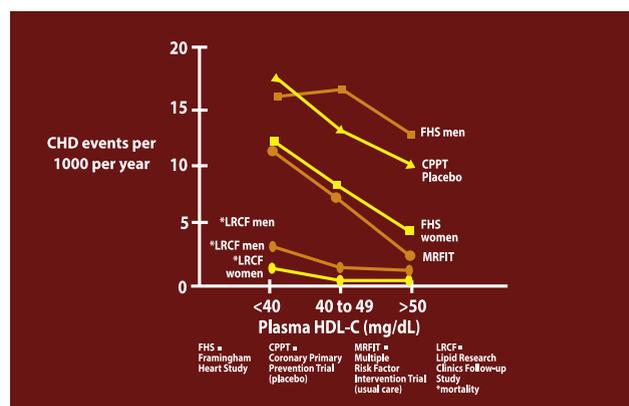


Figure 4 Correlation of HDL-C (low, middle, and high subgroups) with incidence of CHD in Framingham Heart Study, Lipid Research Clinics Follow-up, Coronary Primary Prevention Trial, and Multiple Risk Factor Intervention Trial.³³ CHD, coronary heart disease; H, HDL-C ≥ 50 mg/dL; HDL-C, high-density lipoprotein cholesterol; L, HDL-C < 40 mg/dL; M, HDL-C 40–49 mg/dL.

that the addition of the HDL-C-raising medication niacin when combined with a statin (with or without bile acid resins) improved CVD risk to a greater extent than observed with monotherapy to lower low-density lipoprotein cholesterol (LDL-C), thereby paving the way for hypothesis-generating trials to test whether raising HDL-C might provide incremental CVD risk reduction beyond statin monotherapy.

Total-C/HDL-C ratio

The atherogenic component of serum total-C derives from the LDL-C fraction, and the HDL-C component was shown to be inversely related to the development of CHD.^{39,40} Although the strength of the relation of total-C to CHD declines after 60 years of age in men, the total-C/HDL-C ratio continues to predict events reliably in the elderly of both sexes (Table 1).⁴¹ This ratio has been found to be one of the most powerful lipid measures for predicting CVD events.^{42,43} Comparing age-adjusted fifth with first quintile lipid CVD risk ratios for the individual lipids and their ratios, it is evident that the total-C/HDL-C and LDL-C/HDL-C ratios are stronger predictors of CHD than the individual lipids that comprise them (Table 2).⁴⁴ However, knowledge of the individual (total-C and HDL-C) components is important, and in risk assessment and treatment recommendations both are examined as 2 separate, but related, risk factors.^{45–47} In addition, the joint consideration of HDL-C and non-HDL-C is now common.

Very high HDL-C levels (>100 mg/dL)

If low HDL-C levels predict elevated risk of adverse CVD outcomes, are high or very high HDL-C concentrations associated with diminished risk? In fact, ATP II guidelines added high levels of HDL-C, defined as ≥ 60 mg/dL, as a negative risk factor that might be used to counteract the presence of any traditional CVD risk factors.⁴⁸

The association between high HDL-C and longevity was initially suggested by Glueck et al⁴⁹ in 1975. However, it was not until the discovery that high HDL-C can be caused

by genetic variation in the cholesteryl ester transfer protein (CETP) that great interest emerged for this possible association.⁵⁰ Unfortunately, in contrast to epidemiologic and clinical evidence that support high LDL-C as highly correlated with increased CVD risk, subsequent studies to evaluate common genetic polymorphisms in *CETP* have been mixed, with some reports suggesting increased lifespan and others finding no evidence of CVD protection, despite the presence of very high HDL-C.^{51–54} In addition to *CETP*, genetic variation in scavenger receptor class B type 1 has also been suggested to influence HDL-C levels.⁵⁵ Genome-wide association studies have shown that multiple genetic loci are associated with serum levels of HDL-C, but most are also associated with levels of triglycerides (TGs) or LDL-C or both, making it difficult to assess how the modulation of HDL-C levels by these genes affects CVD risk.³ However, a recent study that used the principle of Mendelian randomization evaluated the effect of multiple single nucleotide polymorphisms in the gene for endothelial lipase on the risk of CVD.⁵⁶ No association between increased serum levels of HDL-C and risk of CVD could be discerned. Taken together, these data challenge the long-held notion that raising levels of plasma HDL-C reduces risk of MI and other CVD events.

Finally, the recent isolation and characterization of the HDL proteome and the 80 to 100 associated proteins involved in biologic processes that include lipoprotein transport and immune function have yet to identify novel cargo-related proteins that either quantitatively raise HDL-C levels, presumably through increased stabilization in the circulation and reduced degradation, or enhance its functionality.⁵⁷ Thus, from an epidemiologic viewpoint, although high HDL-C (>60 mg/dL) and very high HDL-C (>100 mg/dL) levels are uncommon in the US population (16% and 0.7% of men; 32% and 1% of women, respectively),⁵⁸ the clinical significance of elevated HDL-C vis-à-vis relative CVD protection remains unestablished.

HDL-C as a risk factor or biomarker of risk

The epidemiologic inconsistency in the dose-response relationship between HDL-C and CVD risk raises the

Table 1 Development of CHD by total-C/HDL-C ratio vs total-C according to age: 16-year follow-up of the Framingham Heart Study⁴¹

	Total-C/HDL-C			Total-C	
	Quintile 5/Quintile 1*			(>240/<200 mg/dL)	
Age, year, range	49–59	60–69	70–81	35–64	65–94
Men	3.4 [†]	2.9 [†]	2.3 [†]	1.9 [‡]	1.2 [§]
Women	3.7 [†]	6.7 [†]	3.3 [†]	1.8 [¶]	2.0 [‡]

CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; total-C, total cholesterol.

*Quintile 5/Quintile 1, ratio fifth risk quintile to first risk quintile for total-C/HDL-C.

[†] $P < .05$.

[‡] $P < .001$.

[§]Not significant.

[¶] $P < .01$.

Table 2 Efficiency of blood lipids and ratios in predicting CHD: Framingham Heart Study subjects, ages 50–80 years²⁶⁸

	Age-adjusted Q5/Q1 risk ratios	
	Men	Women
Total-C	1.9	2.5
LDL-C	1.9	2.5
HDL-C	0.4	0.5
Total-C/HDL-C	2.5	3.1
LDL-C/HDL-C	2.5	2.8

CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Q, quintiles of blood lipid distribution; total-C, total cholesterol.

possibility that HDL-C may be more of a biomarker of risk rather than directly causative in this process. Specifically, HDL-C levels are inversely correlated with obesity and waist circumference, TGs and small LDL particles, insulin resistance, and cigarette smoking, making it difficult, if not impossible, to adequately assess, even after adjustment for these covariates. Perhaps a “purer” manner to evaluate HDL-C as a causative factor in CVD would be to study men and women who have isolated low HDL-C (<40 mg/dL), who have never smoked, with a body mass index (measured by both height and weight; kg/m²) <25, normal TG levels (eg, <100 mg/dL), and normal fasting glucose levels (<100 mg/dL). Unfortunately, the likelihood of finding sufficient numbers of middle-aged men and women who meet this criteria is low; in the National Health and Nutrition Examination Survey III, only 4.8% of men and 8.7% of women met the HDL-C and TG criteria.⁵⁹ Because HDL-C is so closely intertwined with other factors that influence CVD risk, it is unlikely that epidemiologic studies will ever be able to adequately resolve this issue.

Epidemiology conclusions

For the past 50 years, observational studies have identified a solid inverse relationship between HDL-C and CVD risk. Early studies found that each 1% rise in HDL-C correlated with an approximate 3% reduction in CVD events that appeared to be more robust than the corresponding association with LDL-C lowering. However, in the statin era, although low HDL-C continued to portend unfavorable outcomes, statin treatment in subjects with low HDL-C was associated with reduced arteriographic progression of CAD or CVD events, even though mean levels of HDL-C rose modestly or not at all. Epidemiologic inconsistency has also arisen between levels of HDL-C and CVD events, and monogenic abnormalities that result in extremely low or high HDL-C levels have not reliably suggested premature CVD or longevity, respectively. Complicating this paradigm are associated metabolic features such as obesity, hypertriglyceridemia, small LDL particles, and insulin resistance that tend to cluster with low HDL-C. Therefore, from an epidemiologic perspective, HDL-C

levels per se may not be the proper parameter to adequately assess the contribution of HDL to CVD risk.

Analytical methods

A need is increasing to characterize and quantify the diverse roles of HDL particles in atherogenesis to improve the diagnosis, prevention, and treatment of CVD.^{60,61} This information provides a foundation for fostering improved understanding of the pathophysiology of atherosclerosis, direct the future course of research, and design interventions that effectively reduce CVD risk in various patient populations. This section describes features of the major analytic procedures used to assess HDL heterogeneity and reviews evidence for the associations of HDL measurements on the basis of these procedures with CVD risk. Finally, a classification scheme for 5 major HDL subfractions is presented that can serve as a framework for comparing results obtained with different methodologies.

Analyses of HDL subfractions as a function of size, density, or both

Analytical ultracentrifugation

The earliest method used for quantifying HDL involved analytical ultracentrifugation with the use of Schlieren optics. In the late 1940s, Gofman et al¹³ identified HDL subclasses as a function of size and density on the basis of their ultracentrifugal flotation rate ($F_{1,2}$) in a high salt solution. These studies established that most HDL particles have buoyant density between 1.063 and 1.21 g/mL. This provided the basis for standard preparative ultracentrifugal isolation of HDL^{1,62} and for the designation of large buoyant HDL₂ and smaller more dense HDL₃ (Fig. 5). Subsequently, a curve deconvolution method was developed to refine this analysis and to define further heterogeneity within HDL.⁶³ This “gold standard” method was the first to be used in a prospective study to show an inverse relation of plasma HDL-C concentration to CHD risk.⁶² Recently, long-term follow-up (29 years) of 1905 men in this study has found that both HDL₂ and HDL₃ are independently related to CHD risk.⁶⁴

Non-denaturing gradient gel electrophoresis

Gradient gel electrophoresis in conjunction with automated densitometry was used⁶⁵ to identify the following 5 HDL subspecies separable on the basis of particle diameter: HDL_{3c} (7.2–7.8 nm), HDL_{3b} (7.8–8.2 nm), HDL_{3a} (8.2–8.8 nm), HDL_{2a} (8.8–9.7 nm), and HDL_{2b} (9.7–12.9 nm) (Fig. 6). Subsequent studies reported that HDL_{2b} (very large HDL [HDL-VL]), which is strongly correlated with total HDL-C, was most strongly inversely related to CHD risk⁶⁰ and that increased HDL_{3b} (small HDL [HDL-S]) was associated with an atherogenic lipoprotein phenotype characterized by increased TGs and small, dense LDL, together with reduced HDL_{2b} (HDL-VL).⁶¹ As described

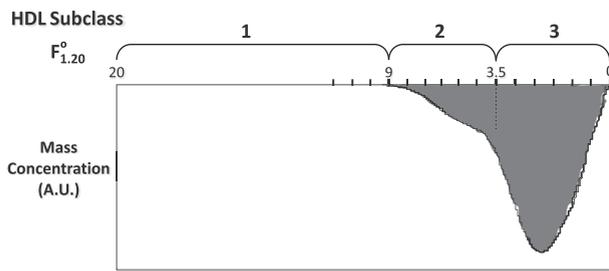


Figure 5 Analytical ultracentrifugation. Measurement of HDL by analytical ultracentrifugation. Major HDL particle subclasses are distinguished by flotation rate in a salt solution of density 1.2 g/mL ($F_{1.2}$), and total mass, represented by the area under the curve, which is determined by first principles of physics from Schlieren optics. Initially, 3 major HDL subclasses were identified. HDL₁, with the highest flotation rate, is generally not detectable in significant concentrations in human plasma. A curve-fitting procedure was later developed to resolve 2 subclasses of HDL₂ (HDL_{2a} and HDL_{2b}). From Rosenson et al.⁸⁷ A.U., arbitrary units; HDL, high-density lipoprotein.

below, the use of two-dimensional electrophoresis has found that particles corresponding to HDL_{2b} (large HDL [HDL-L]) are independently and inversely related to CHD risk.⁶⁶

Density gradient fractionation

Precise and reproducible fractionation of the major HDL particle subpopulations (HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}) in human plasma is based on an isopycnic

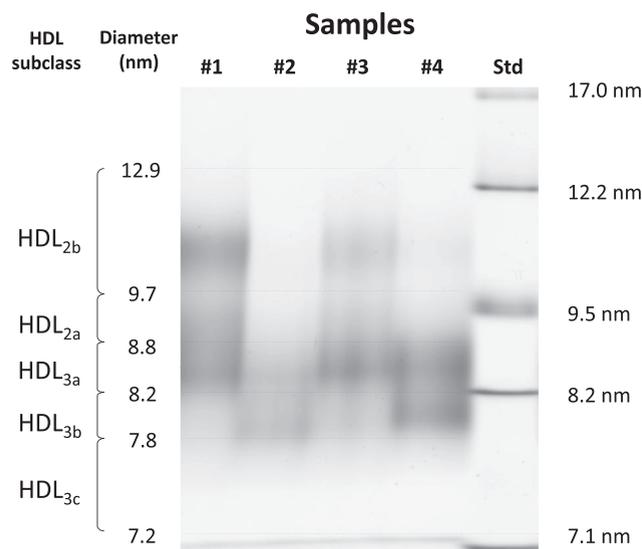


Figure 6 Nondenaturing gel electrophoresis. Separation of HDL subclasses from 4 representative plasma samples by gradient gel electrophoresis. HDL is isolated from plasma by ultracentrifugation at density of 1.21 g/mL and electrophoresed in a 4% to 30% nondenaturing gradient gel. After staining the protein with Coomassie blue and scanning the gels by densitometry, the size distribution is determined by calibration with the use of protein standards (right lane). This procedure resolves 5 distinct subclasses, although the smallest, HDL_{3c}, is generally present at low concentrations. From Rosenson et al.⁸⁷ HDL, high-density lipoprotein; Std, standard.

equilibrium methodology (Fig. 7).⁶⁷ The plasma or serum sample (3 mL) is layered on the surface of a NaCl-KBr solution of density 1.24 g/mL, and the density is then adjusted to 1.21 g/mL. The procedure involves a single ultracentrifugal step, which allows almost quantitative recovery of highly resolved HDL fractions of defined hydrated density and physicochemical properties. This method avoids major contamination with plasma proteins and facilitates HDL isolation in a nondenatured, nonoxidized state. Gradients are fractionated with a precision pipette from the meniscus downward to avoid contamination with plasma proteins of density >1.25 g/mL present in the residue at the base of the tube.

The main disadvantage of this method is the same as that of other ultracentrifugal separations, because lipoproteins are subject to high ionic strength and centrifugal force (57×10^7 g/min); shear forces are reduced by use of a swing-out rotor.

Vertical rotor ultracentrifugation

Vertical Auto Profile (VAP) is another HDL subfractionation method that is based on ultracentrifugation.⁶⁸ Unlike

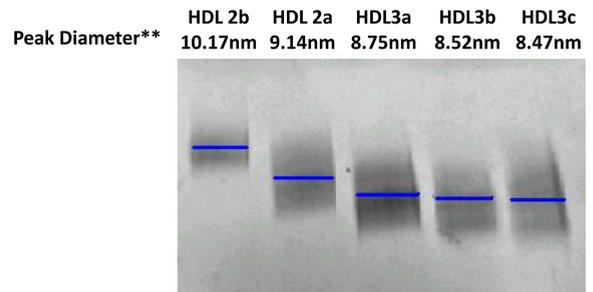


Figure 7 Density gradient ultracentrifugation. Representative electrophoresis profiles and mean particle sizes of HDL subfractions (HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}) from normolipidemic human plasma separated by isopycnic, single-spin, density gradient ultracentrifugation. Human plasma is separated by isopycnic, single-spin, density gradient ultracentrifugation. The plasma or serum sample (3 mL) is layered on a cushion of NaCl-KBr solution of density 1.24 g/mL to the surface of the gradient, and the density is then adjusted to 1.21 g/mL. The procedure involves a single ultracentrifugal step, allows almost quantitative recovery of highly resolved HDL fractions of defined hydrated density and physicochemical properties, avoids major contamination with plasma proteins, and facilitates HDL isolation in a nondenatured, nonoxidized state. Gradients are fractionated with a precision pipette from the meniscus downward to avoid contamination with plasma proteins of density > 1.25 g/mL present in the residue at the base of the tube. One-dimensional electrophoresis was performed in nondenaturing gradient polyacrylamide gel (4%–20%). **Peak diameter was determined at the maximum absorption intensity of each band with the use of Kodak 1D software filters after staining with Coomassie Brilliant Blue. Size by negative stain electron microscopy provided smaller estimates (HDL_{2b} + HDL_{2a}: mean diameter 9.6 nm and range 10.8–7.2 nm; HDL_{3a} + HDL_{3b} + HDL_{3c}: mean diameter 7.3 nm and range 9.0–5.4 nm), reflecting the nonhydrated state. From Rosenson et al.⁸⁷ HDL, high-density lipoprotein.

most other ultracentrifugation methods, VAP is done in a vertical rotor, which makes the method relatively fast and more practical for the analysis of routine clinical specimens. For HDL, it measures the cholesterol content of its 2 main size or density subfractions, namely HDL₂ (HDL-VL, HDL-L) and HDL₃ (medium HDL [HDL-M], HDL-S, and very small HDL [HDL-VS]).⁶⁹ VAP is relatively precise, with intra-assay coefficients that range from 4% to 10%.⁷⁰

Two-dimensional gel electrophoresis

This procedure separates HDL subfractions on the basis of both their size and charge (Figs. 8 and 9).⁷¹ Concentrations of these subfractions are expressed in milligrams per deciliter (mg/dL) of apoA-I and as a percentage of total plasma apoA-I concentration. Five main HDL particles are identified: (1) very small discoidal precursor HDL of pre β mobility (known as pre β -1 HDL or HDL-VS; diameter approximately 5.6 nm) which contains apoA-I and phospholipid; (2) very small discoidal HDL of α mobility (known as α -4 HDL or HDL-S; diameter approximately 7.4 nm) which contains apoA-I, phospholipid, and free

cholesterol; (3) small spherical HDL of α mobility (known as α -3 HDL or HDL-M; diameter approximately 8.2 nm), which contains apoA-I, apoA-II, phospholipid, free cholesterol, cholesteryl ester, and TGs; (4) larger-sized spherical HDL of α mobility (known as α -2 HDL or HDL-L; diameter approximately 9.2 nm) which contains the same constituents as α -3 HDL; and (5) very large spherical HDL of α mobility (known as α -1 HDL or HDL-VL) which contains the same constituents as α -3 and α -2 HDL, except for the near absence of apoA-II (Fig. 8). Adjacent to the α particles are pre α particles that are of similar size, but present in lower amounts, and do not contain apoA-II. In addition, there are large pre β -migrating HDL known as pre β -2 HDL, which are classified as HDL-VS.⁷² The ability of HDL particles to promote cellular efflux, a property thought to contribute to their antiatherogenic role, is shared by pre β -1 HDL (HDL-VS), which are most efficient in interacting with the adenosine triphosphate-binding cassette transporter A1 (ABCA1), and spherical HDL (HDL-M, HDL-L, and HDL-VL), which preferentially interact with ABCG1 to promote cellular cholesterol efflux onto spherical HDL particles that contain both apoA-I and apoA-II.⁷³

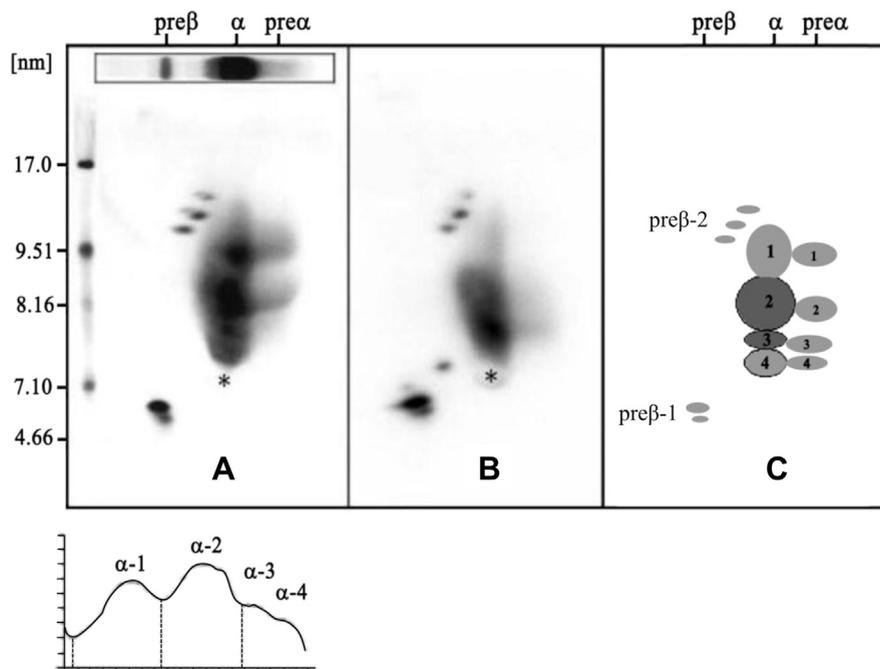


Figure 8 Two-dimensional gel electrophoresis. The apoA-I-containing HDL subpopulation profiles of a healthy subject (a) and a patient with CHD (b), with a schematic diagram of all of the apoA-I-containing HDL particles shown on the right (c). Below panel a is a plot of a densitometric scan across the α -migrating HDL particle region, indicating the presence of 4 α -migrating HDL particles that range in mean particle diameter from very large α -1 HDL (11.0 nm diameter) to very small pre β -1 HDL (5.6 nm diameter). In the schematic diagram α -migrating apoA-I-containing particles in the α -2 region (9.2 nm in diameter) and in the α -3 region (8.1 nm in diameter) contain both apoA-I and apoA-II (more heavily shaded), whereas all other particles that contain apoA-I, including small α -4 HDL (7.4 nm in diameter), do not contain appreciable amounts of apoA-II (less heavily shaded). The asterisk marks the serum albumin or α front. On the basis of their composition, very small pre β -1 HDL and small α -4 HDL are discoidal particles that do not contain cholesteryl ester or TGs, whereas medium, large, and very large α -3, -2, and -1 HDL are spherical and contain cholesteryl ester and TGs in their cores. Patients with CHD in general in the untreated state tend to have significant decreases in the levels of apoA-I in very large and large α -migrating HDL particles and modest increases in apoA-I in very small pre β -1 HDL and small α -4 HDL particles. From Rosenson et al.⁸⁷ Apo, apolipoprotein; CHD, coronary heart disease; HDL, high-density lipoprotein; TG, triglyceride.

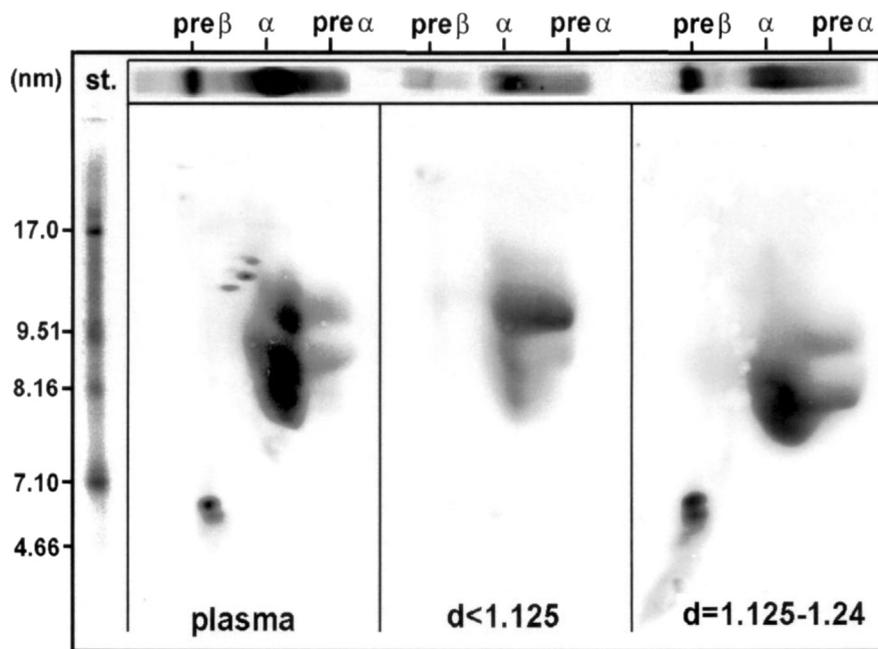


Figure 9 The 2-dimensional gel electrophoresis. The 2-dimensional gel electrophoresis patterns after apoA-I immunoblotting observed for whole plasma are shown in the left panel, for lipoproteins of density <1.125 g/mL as separated by ultracentrifugation are shown in the center panel, and for lipoproteins of density 1.125 to 1.24 g/mL are shown in the right panel. These data indicate that apoA-I-containing HDL of density <1.125 g/dL mainly comprise very large and large α -migrating HDL, whereas apoA-I-containing HDL particles of density 1.125 to 1.24 g/mL contain mainly medium and small α -migrating HDL and very small pre β -1 HDL. From Rosenson et al.⁸⁷ Apo, apolipoprotein; d, density; HDL, high-density lipoprotein.

Spherical HDLs also contribute to delivery of cholesterol to the liver via SR-B1.⁷³ Other HDL particles contain apoE without apoA-I (very large pre β -migrating HDL) and small HDL particles that contain apoA-IV without apoA-I.⁷³ The functions of these latter particles have not been well defined. Patients with CAD also often have small discoidal HDL particles and decreased large α -1 (HDL-L) and α -2 (HDL-VL) HDL particles. Levels of these subfractions have been reported to be superior to HDL-C in risk prediction.^{74,75}

NMR spectroscopy

Measurements of HDL subfractions by nuclear magnetic resonance (NMR) spectroscopy are based on the principle that protons (the nuclei of hydrogen atoms) within lipoprotein particles of different size have a *natural* magnetic distinctness arising from their unique physical structure.⁷⁶ As a result, lipoprotein particles of different size in unfractionated plasma or serum give rise to distinguishable lipid NMR signals that have characteristically different frequencies (Fig. 10, left panel).^{77,78} The NMR signal frequencies (chemical shifts) of HDL subfractions, compared with LDL and very low-density lipoprotein (VLDL) subfractions, are particularly well differentiated (Fig. 10, right panel).

Lipoprotein subclass reference standards used in the fitting model were determined from preparative isolation of pure lipoprotein subclass standards. For the purposes of generating highly purified lipoprotein subfractions with narrow size distributions, a combination of

ultracentrifugation and agarose gel filtration chromatography were used.^{79,80} After removal of VLDL and plasma lipoproteins by ultracentrifugation, LDL fractions were purified on an agarose gel filtration A-15 column, and HDL fractions were further purified on an A-1.5 column. The mean particle diameters of the purified subcomponents of the 38 lipoprotein subclasses were measured by electron microscopy. Average lipoprotein diameters were derived by measuring the diameters of at least 200 lipoprotein particles quantified from 2 or more grids. A total of 30 lipoprotein subcomponents have been used to provide a better representation of the continuum of particle subspecies present in plasma. By appropriate grouping and summation of the levels of the expanded subcomponent set, 10 subclasses of lipoproteins can be quantified with acceptable precision into large, medium, and small VLDL; intermediate-density lipoprotein (IDL); large, medium-small, and very-small LDL; and large, medium, and small HDL. The diameter ranges for the HDL subclasses are as follow: large HDL 8.8 to 13 nm, medium HDL 8.2 to 8.8 nm, and small HDL 7.3 to 8.2 nm. The coefficients of variation for large, medium, and small HDL subclasses are $<10\%$, 15% , and 10% , respectively.

The lipoprotein NMR signals used for lipoprotein quantification are those of the terminal lipid methyl group protons, because they are unresponsive to, and therefore unaffected by, fatty acid or other chemical compositional differences.⁷⁸ Furthermore, to a close approximation, the number of methyl protons in a lipoprotein particle of given

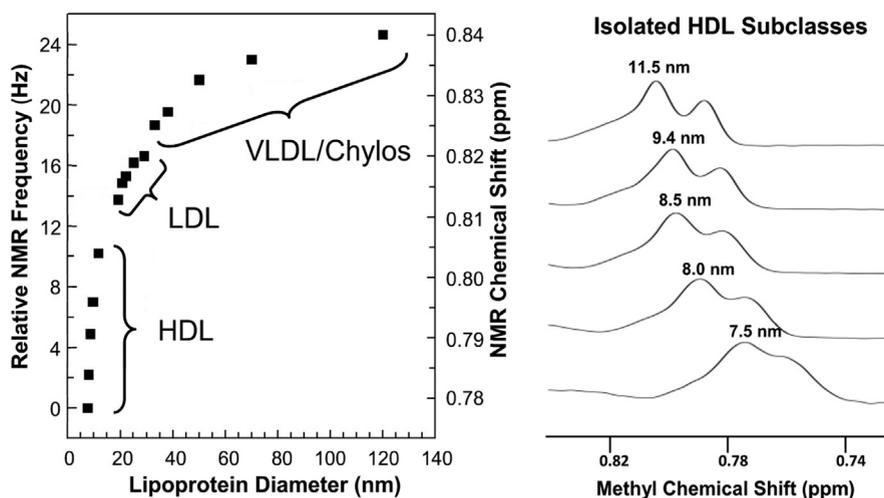


Figure 10 NMR spectroscopy. Relationship of lipoprotein subclass diameter to lipid methyl group NMR chemical shift and frequency (left panel) and methyl signal line shape and chemical shift of 5 isolated HDL subclasses of differing diameter (right panel). The natural magnetic distinctness of lipoprotein particles of different size makes it possible in theory to use any NMR instrument in any laboratory for lipoprotein analysis, but in practice it requires dedicated instrumentation. The subclass NMR signals highly overlap, making it necessary to computationally “deconvolute” the plasma NMR signal envelope to extract the amplitudes of the subclass signals that are used to calculate subclass concentrations. Accurate and reproducible deconvolution is only possible if the NMR conditions (magnetic field strength, temperature, etc) used to generate the subpopulation reference signal library exactly match the conditions used subsequently to measure (in approximately 1 minute) each patient plasma sample. The NMR LipoProfile-3 method currently used by LipoScience (Raleigh, NC) models the plasma signal as the sum of the contributing signals of 26 subpopulations of HDL as well as 47 subpopulations of LDL, VLDL, and chylomicrons. Given the limited measurement precision of the derived concentrations of each of the many subpopulations, they are grouped for routine reporting purposes into large, medium, and small subclass categories. From Rosenson et al.⁸⁷ Chylos, chylomicrons; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; VLDL, very low-density lipoprotein.

diameter is constant, even in the face of significant variations in core cholesterol ester and TG content. These properties render the detected subclass methyl signal amplitudes directly proportional to subclass particle number and enable NMR-derived concentrations of HDL to be given in particle number units (μmoles of particles per liter; $\mu\text{mol/L}$).⁷⁸

Investigations are ongoing to establish the relationship between CVD and NMR-determined concentrations of HDL particle subclasses.^{81,82} An important consideration in interpreting the clinical significance of observed univariate disease associations with individual HDL subclasses or HDL size is the confounding that arises from the strong inverse correlation between large and small HDL subclasses and the even stronger inverse associations of large HDL particles (and HDL size) with total (and small) LDL particle concentrations.^{78,79} Without conducting regression analyses that eliminate the confounding caused by these correlations, misleading conclusions may be reached about the clinical importance and potential functional differences among HDL subclasses.^{81,82} Thus, published studies that report associations between HDL subclasses and CHD risk by using NMR spectroscopy may differ from other methods cited above because of the more robust statistical modeling.

Ion mobility

Ion mobility is a gas-phase differential electrophoresis macromolecular mobility-based method for lipoprotein

separation.⁸³ In this high-throughput procedure, after charge neutralization, lipoprotein particles are separated by size on the basis of the mobility of the particle passing through a voltage gradient, and the isolated particles are counted directly. The initial ion mobility method involves the reduction of albumin contamination of the HDL size region by incubation of plasma with blue dextran and a short ultracentrifugation in the absence of salt. Recently, to optimize recovery of small HDL particles, a simplified procedure for lipoprotein isolation was developed by using incubation of a small plasma aliquot with dextran sulfate attached to magnetic beads. An automated curve deconvolution method was used to resolve 5 HDL subfractions that correspond to those measured by gradient gel electrophoresis (Fig. 11).

In samples derived from the prospective Malmö Diet and Cancer Study,⁸⁴ very large HDL particles (HDL_{2b}, HDL-VL) were inversely correlated with risk of MI. This association was based on the inclusion of HDL_{2b} (HDL-VL) in 2 independent principal components analyses, which were determined from ion mobility measurements that included all lipoprotein fractions. One of the component analyses corresponds to the atherogenic lipoprotein phenotype that includes increased levels of TGs and smaller LDL particles, and the second component includes smaller HDL particles. Genetic analyses indicated that these components have differing underlying determinants, thus suggesting 2 different mechanisms for the cardioprotective effects of HDL.⁸⁴

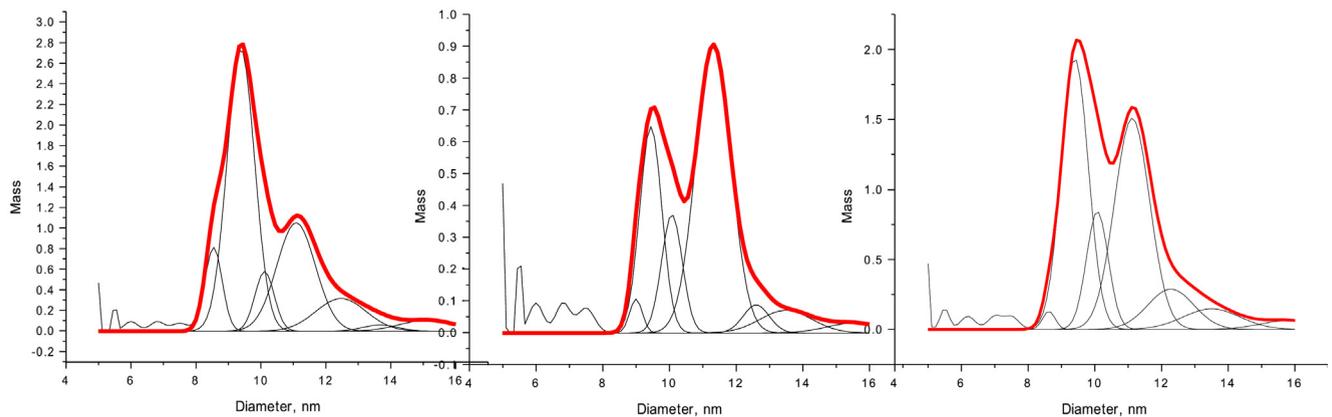


Figure 11 Ion mobility. Representative results for ion mobility analysis of HDL particle subclasses performed on 3 representative plasma samples. The y-axis shows mass concentration (arbitrary units) derived from transformation of particle concentration, which is measured directly by this procedure. Shown from left to right are deconvoluted curves that delineate the 5 major HDL subclasses (HDL-VS, HDL-S, HDL-M, HDL-L, and HDL-VL) as described in Table 3. This method also indicates small peaks larger than HDL-VL, which have not yet been characterized. Particle concentration for each subclass is determined from the area under the curve. HDL, high-density lipoprotein; HDL-L, large high-density lipoprotein; HDL-M, medium high-density lipoprotein; HDL-S, small high-density lipoprotein; HDL-VL, very large high-density lipoprotein; HDL-VS, very small high-density lipoprotein.

HDL measurements based on apolipoprotein content

ApoA-I and apoA-I:A-II particles

A system initially devised by Aloupovic⁸⁵ used an immunochemical method to distinguish HDL particles, as well as other lipoproteins, by their apo content. The main HDL species, lipoprotein (Lp) A-I and LpA-I:A-II, contain approximately 35% and 65% of plasma apoA-I, respectively.⁸⁶ In plasma, both LpA-I and LpA-I:A-II are heterogeneous and can be separated into subfractions according to lipid composition, density, size, and charge. As reviewed extensively elsewhere,⁸⁷ the cardioprotective roles of LpA-I and LpA-I:A-II have been controversial, as has the utility of measuring total apoA-I level, compared with HDL-C, in assessing CHD risk.

Proposed nomenclature

As discussed before, use of different techniques and procedures has led to different terms in defining HDL species. To provide guidelines for future studies and to compare and contrast published data that have used different methods, new HDL nomenclature and classification on the basis of size (and corresponding density) of the major HDL particle subclasses has been proposed (Table 3).⁸⁷ The upper limit of the HDL density range for HDL is now defined as 1.25 g/mL to incorporate pre β HDL (HDL-VS). In addition, these terms are compared with other designations available in the literature. In this nomenclature, HDL particles are termed very large (VL), large (L), medium (M), small (S), and very small (VS). In addition, pre β HDL is included as a subspecies within HDL-VS.

HDL-targeted intervention studies in animals

Many studies have investigated the effects of increasing HDL concentration on atherosclerosis animal models. It should be emphasized, however, that all of these models have their limitations and that none is a true model for human disease. However, with this reservation, the animal studies have, with very few exceptions, provided powerful evidence that increasing HDL-C concentration does protect against atherosclerosis.

Badimon et al⁴ were the first to report direct antiatherogenic effects of HDLs. With the use of a model of experimental atherosclerosis in rabbits, they showed that weekly infusions of HDLs significantly reduced the development of aortic fatty streaks.⁴ A similar beneficial effect was observed in rabbits infused with rHDL that contained complexes of phospholipids and apoA-I.⁸⁸ But the most compelling evidence has come from studies in a range of genetically modified animals, with overexpression of the human *APOA1* gene in transgenic mice, resulting in an increased concentration of HDL-C and a protection against atherosclerosis.⁵⁻⁷

Another example relates to the inhibition of CETP in rabbits. Rabbits (like humans) have a high natural level of activity of CETP, the plasma protein that promotes transfers of cholesteryl esters from the nonatherogenic HDL fraction to particles in the atherogenic VLDL and LDL fractions. The existence of a high level of activity of CETP in rabbits may be one reason for the susceptibility of this species to development of atherosclerosis. As outlined below, this suggestion is supported by the observation that inhibiting CETP in rabbits reduces the development of atherosclerosis.

Table 3 Classification of HDL by physical properties

Proposed term	Very large HDL (HDL-VL)	Large HDL (HDL-L)	Medium HDL (HDL-M)	Small HDL (HDL-S)	Very small HDL (HDL-VS)
Density range, g/mL	1.063–1.087	1.088–1.110	1.110–1.129	1.129–1.154	1.154–1.25
Size range, nm	12.9–9.7	9.7–8.8	8.8–8.2	8.2–7.8	7.8–7.2
Density gradient ultracentrifugation	HDL _{2b}	HDL _{2a}	HDL _{3a}	HDL _{3b}	HDL _{3c}
Density range, g/mL	1.063–1.087	1.088–1.110	1.110–1.129	1.129–1.154	1.154–1.170
Gradient gel electrophoresis	HDL _{2b}	HDL _{2a}	HDL _{3a}	HDL _{3b}	HDL _{3c}
Size range, nm	12.9–9.7	9.7–8.8	8.8–8.2	8.2–7.8	7.8–7.2
2D gel electrophoresis	α -1	α -2	α -3	α -4	Pre β -1 HDL
Size range, nm	11.2–10.8	9.4–9.0	8.5–7.5	7.5–7.0	6.0–5.0
NMR	Large HDL-P	Medium HDL-P		Small HDL-P	
Size range, nm	12.9–9.7	9.7–8.8	8.8–8.2	8.2–7.8	7.8–7.2
Ion mobility	HDL _{2b}	HDL _{2a} + HDL ₃			
Size range, nm	14.5–10.5	10.5–7.65			

HDL, high-density lipoprotein; HDL-P, high-density lipoprotein particles; NMR, nuclear magnetic resonance; 2D, 2-dimensional. Modified from Rosenson et al.⁸⁷

Feeding rabbits a diet rich in cholesterol leads to the development of extensive atherosclerosis. However, in cholesterol-fed rabbits in which the *CETP* gene had been inhibited by injection of antisense oligodeoxynucleotides against *CETP*, there was a reduction in hepatic *CETP* mRNA and mass, a reduction in non-HDL-C, and an increased concentration of HDL-C.⁸⁹ These changes were accompanied by a substantial reduction in aortic atherosclerosis.

A vaccine approach has been used to generate autoantibodies against CETP in vivo in rabbits.⁹⁰ In a cholesterol-fed rabbit model, animals that were immunized against CETP had a reduced plasma activity of CETP, an increase in the concentration of HDL-C, a modest decrease in LDL-C concentration, and a significant reduction in aortic atherosclerotic lesions.⁹⁰

Chemical inhibitors of CETP were shown in cholesterol-fed rabbits to reduce CETP activity by >90% and to almost double the level of HDL-C.⁹¹ These changes were accompanied by a substantial reduction in atherosclerotic lesions in the aortas of these animals.⁹¹ The increase in HDL-C that resulted from CETP inhibition in rabbits is generally accompanied by a decrease in non-HDL-C, although in one study the antiatherogenic effects of inhibiting CETP were apparent even when the increase in HDL-C was not accompanied by changes in other lipoprotein fractions.⁹² Thus, evidence from animal studies that HDL-raising interventions are antiatherogenic is robust.

Clinical trials of pharmacologic interventions to raise low levels of HDL-C

Elevated LDL-C has long been known as a major predictor of CHD risk. Importantly, during the past decade,

the introduction of statins has unquestionably revolutionized treatment of dyslipidemia by reducing significantly major vascular events. In primary and secondary prevention trials that used a statin, plasma LDL-C was reduced 25% to 55% and CHD event rates by 24% to 45%, compared with placebo.^{93–96} Growing numbers of patients with low HDL-C and normal LDL-C levels (as many as 40%–45% of all patients with CHD),^{97,98} as well as patients with diabetes, metabolic syndrome, or multiple lipid abnormalities, comprise an expanding reservoir of patients for whom statin monotherapy may *not* be the best therapeutic option. Statin therapy, unfortunately, raises HDL-C levels modestly by 5% to 10% and by as much as 10% to 15% in persons with the metabolic syndrome.⁹⁹ Yet it is unclear to what extent these HDL-C effects contribute to the clinical benefits of statins.¹⁰⁰ Lack of a clinically meaningful effect of statins on HDL-C has focused interest in use of other therapeutic dyslipidemic interventions such as nicotinic acid or niacin, fibrates, omega-3 fatty acids, or CETP inhibitors. Niacin is presently the most effective clinically available therapeutic agent for raising low HDL-C levels. In addition, niacin also produces moderate reductions in TGs, Lp(a), and, at higher doses, LDL-C levels.⁹⁶ This section discusses the findings from clinical trials that have evaluated these interventions on surrogate outcome measures (such as B-mode ultrasonographic assessment of carotid intimal media thickness [cIMT], coronary angiographic studies, and IVUS), as well as placebo-controlled randomized trials that have assessed clinical event reduction with HDL-C-raising therapies.

Clinical effects of raising HDL-C

Low HDL-C is also frequently associated with elevated TGs and variable levels of small, dense LDL-C, an

atherogenic lipid triad typically found in persons with type 2 diabetes and metabolic syndrome.¹⁰¹ More than 23 million Americans have type 2 diabetes,¹⁰² whereas an estimated 68 million have the metabolic syndrome,¹⁰³ with >65% of diabetes-related deaths due to late-stage effects of cardiac or blood vessel disease.^{102,104} These numbers are expected to increase in the next 10 years, because of the alarming rise in obesity across the globe.¹⁰² HDL-C levels can also be increased by adoption of certain healthy lifestyle factors. Modifiable lifestyle changes such as smoking cessation, increased physical activity, and weight control can produce small increases in HDL-C (2–7 mg/dL), although results vary and are frequently disappointing in persons with low HDL-C.¹⁰⁵

Studies with fibrates

Fibrates are also recommended for treatment of persons with low HDL-C or high TG levels, but outcome trials with fibrates have shown mixed results. Positive studies for gemfibrozil were reported for trials in participants with low HDL-C or high cholesterol and TG levels (eg, HHS in primary prevention and VA-HIT).^{34–36} In VA-HIT, 2531 male veterans with established CHD and low levels of baseline HDL-C were randomly assigned to gemfibrozil 1200 mg daily vs matching placebo and were followed for a mean of 5.1 years. Statins were not used in this trial. LDL-C at baseline was 111 mg/dL and was 113 mg/dL at 5.1 years. Gemfibrozil raised HDL-C only modestly (from 32 mg/dL to 34 mg/dL) compared with placebo; this 6% relative increase in HDL-C, combined with a relative reduction in TGs of 31%, was associated with a significant 22% reduction in the cumulative rate of CHD death or nonfatal MI, which was the trial primary end point.

In another fibrate trial, the Bezafibrate Infarction Prevention study, 3090 patients with CHD were randomly assigned to bezafibrate 400 mg daily vs placebo and followed for 6.2 years. HDL-C increased 18%, TGs decreased 21%, and LDL-C decreased 6.5%, but these beneficial lipid changes were associated with only a 10% reduction in CHD events ($P = \text{NS}$)¹⁰⁶; however, a post hoc analysis of patients with a baseline TG >200 mg/dL showed a significant 40% reduction of CHD events.¹⁰⁶

By contrast, fenofibrate failed to show CHD benefits in recent trials conducted in diabetic participants in whom most were without overt dyslipidemia (Fenofibrate Intervention and Event Lowering in Diabetes and Action to Control Cardiovascular Risk in Diabetes).^{107,108} Post hoc analyses of all these trials (HHS, Bezafibrate Infarction Prevention, VA-HIT, Fenofibrate Intervention and Event Lowering in Diabetes, and Action to Control Cardiovascular Risk in Diabetes) showed that fibrates significantly reduced CHD events in subgroups of patients with reduced HDL-C and elevated TGs, whereas no benefits were observed in the subgroups without these characteristics.^{108–110}

Cardiovascular outcome studies with niacin as monotherapy and combination dyslipidemic therapy

In the first large clinical trial of lipid-lowering pharmacotherapy, the Coronary Drug Project (CDP),¹¹¹ 8341 men with CHD were randomly assigned to 5 lipid-altering regimens (low- and high-dose estrogen, thyroxine, clofibrate, and niacin) for 6 years. Only niacin reduced significantly the incidence of nonfatal MI (26%) and stroke (24%) compared with placebo (Table 4). A 15-year follow-up to the CDP found an 11% reduction in total mortality in the original niacin cohort compared with the placebo group.¹¹² Unfortunately, HDL-C was measured only in a small subgroup; therefore data were not sufficient to evaluate the effect of HDL-C change and reduction of CHD end points during the trial.

The Stockholm Ischaemic Heart Disease Secondary Prevention Study randomly assigned men and women ($n = 555$) to treatment with niacin plus clofibrate or placebo.¹¹³ After 5 years, nonfatal CV events were reduced by 33% and total mortality by 26% overall in the drug therapy group compared with the placebo group. The Cholesterol-Lowering Atherosclerosis Studies (CLAS, CLAS II) were the first angiographic studies to find a clear treatment effect on atherosclerotic lesions.^{114,115} After 2 years, 162 men randomly assigned to niacin plus colestipol had significant increases in stenosis regression and decreases in progression compared with those on placebo.¹¹⁴ After 4 years, regression and nonprogression rates continued to improve with active treatment.¹¹⁵

In the single-site Familial Atherosclerosis Treatment Study, men ($n = 146$) were randomly assigned to treatment with colestipol and niacin, colestipol and lovastatin, or usual care (43% received colestipol).¹¹⁶ After 2.5 years, combination treatment was associated with significant decreases in coronary stenosis, assessed by quantitative coronary arteriography, as well as a substantial 73% relative reduction in death, MI, or refractory ischemic symptoms that required surgical or interventional treatment. Moreover, during a 10-year follow-up, patients who continued on triple therapy experienced sustained reductions in outcomes and a 93% reduction in total mortality compared with patients who had returned to usual care.¹¹⁷

Similar findings were reported for the patients in the HDL Atherosclerosis Treatment Study (HATS), which included 160 men and women who had an apoB lipoprotein level ≥ 125 mg/dL at baseline.¹¹⁸ After 3 years, treatment with simvastatin (mean dose 13 mg/d) plus niacin (mean dose 2400 mg/d) was associated with a significant regression by quantitative coronary angiography and a 90% reduction in the composite end point of death, MI, stroke, or revascularization ($P = .03$) compared with placebo. Of note, the addition of antioxidants tended to mitigate the angiographic and clinical efficacy of combination therapy. Both the Familial Atherosclerosis Treatment Study and HATS show that powerful LDL-C lowering and HDL-C raising can substantially reduce CV morbidity

Table 4 Review of niacin clinical trials on atherosclerosis, clinical events, or both

Study (year)	No. (% F)	Treatment	Entry requirement	Duration, y	Baseline			Change in lipids, %			Atherosclerosis change by angiography or carotid U/S (treatment vs control)			Reduction in clinical events %		
					Age, y	HDL-C, mg/dL*	LDL-C, mg/dL†	TG, mg/dL	HDL-C	LDL-C‡	TG	Regression, %	Progression, %	Stenosis	CHD death + nonfatal MI	PTCA or CABG
CDP (1975) ¹¹¹	3908 (0)	Niacin 3 g vs placebo	MI	5	45	(252)	7 meq/L	(-10)	-26.1					15‡	67‡	-4 (NS)
CDP follow-up (1986) ¹¹²	3467		MI	15	51									-12.4		-11.9§
SIHD Secondary Prevention (1988) ¹¹³	555 (20)		MI	5	61	48	161 (251)	208	(-13)	-19				36‡		26¶
CLAS (1987) ¹¹⁴	162 (0)	Niacin 4 g + colestipol 30 g vs placebo	CABG	2	54	45	171	151	37	-43	-22	16.2 vs 2.4‡	39 vs 61			
CLAS II (1990) ¹¹⁵	103 (0)		CABG	2	54	44	171	154	37	-40	-18	18 vs 6¶	48 vs 85			
FATS (1990) ¹¹⁶	146 (0)	Niacin 4 g + colestipol 230 g vs usual care	Angiographic CHD	2.5	46	39	190	194	43	-32	-29	39 vs 11‡	25 vs 46‡	-0.9 vs 2.1‡	2 (NS)	75
FATS follow-up (1998) ¹¹⁷	176 (0)		Angiographic CHD	10		43	202	210	23	-48	-36				72¶	93§
HATS (2001) ¹¹⁸	160 (13)	Niacin 1-4 g + simvastatin 10-20 mg vs placebo	Angiographic CHD	3	53	31	125	213	26	-42	-36			-0.4 vs 3.9§	60	57
ARBITER 2 (2004) ¹¹⁹	167 (7)	ERN 0.5-1 g + statin vs placebo + statin	CHD on statin and HDL-C < 45 mg/dL	1	67	39	87	154	21	-2.3	-13				cIMT 1.6 vs 5.1§	
ARBITER 3 (2006) ¹²⁰	130 (8)		Open-label ARBITER 2 patients switched to ERN	2	67	40	88	165	23	-9.5	-20	-4.6% decrease in cIMT (P = .008)				
ARBITER 6 (2009) ¹²¹	208 (22)	ERN 2 g/d vs ezetimibe 10 mg	CHD and HDL-C < 50 for men and < 55 mg/dL for women	1.17	64	43	81	126	18	-20	-18	-1.58% decrease in cIMT (P = .001 vs baseline)				
Oxford (2009) ¹²³	63 (94)	ERN 2 g statin vs placebo + statin	T2DM with CHD or carotid/peripheral atherosclerosis HDL-C < 40 mg/dL niacin 2 g/d	1	65	39	85	168	23	19	11	-1.1 mm ² for ERN vs 1.2 mm ² for placebo		-1.64 mm ² in carotid wall area by MRI (P = .03)	9/165 in ezetimibe vs 2/160 in ERN-treated patients	

ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; CABG, coronary artery bypass grafting; CDP, Coronary Drug Project; CHD, coronary heart disease; cIMT, carotid intimal medial thickness; CLAS, Cholesterol Lowering Atherosclerosis Study; ERN, extended-release niacin; F, female; FATS, Familial Atherosclerosis Treatment Study; HATS, HDL-Atherosclerosis Treatment Study; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; MRI, magnetic resonance imaging; NS, not significant; PTCA, percutaneous coronary angioplasty; SIHD, Stockholm Ischemic Heart Disease; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; U/S, ultrasound.

*Lipids in mg/dL, except TG for the CVD (1975) study in which TG were meq/L.

†If LDL-C value is not available, TC value is given in parentheses.

‡P < .01.

§P ≤ .001.

¶P ≤ .05.

||P < .0001.

and mortality, although these studies do not directly address whether niacin provides additive benefit to statins, or whether an increase in HDL-C adds to the benefit of lowering LDL-C levels.^{116,118}

In the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2 trial, 167 patients with known CAD and low HDL-C (<45 mg/dL) were randomly assigned to extended-release niacin (ERN) 1000 mg at bedtime or placebo.¹¹⁹ All subjects were required to be on statin therapy with an LDL-C entry criterion of <130 mg/dL. At 12 months, the ERN-treated patients had almost no change in cIMT (0.014 ± -0.104 mm; $P = .23$), whereas the placebo group on statin therapy alone exhibited a significant increase in cIMT (0.044 ± -0.100 mm; $P < .001$), indicating significant progression of atherosclerosis.¹¹⁹ It should be noted, however, the differences between ERN and placebo did not achieve statistical significance.

After the completion of ARBITER 2, patients were continued on an open-label, 12-month follow-up (ARBITER 3).¹²⁰ Follow-up was completed in 104 subjects for an additional year: 47 that were crossed-over from placebo and started on ERN and 57 that continued ERN for a total of 24 months. At the completion of the 2-year treatment, patients converted from placebo to ERN experienced significant regression of cIMT (-0.095 ± 0.019 mm; $P < .001$ vs placebo phase). The ARBITER 2 and 3 studies were the first to indicate the potential for stabilization and subsequent regression of atherosclerosis with the addition of a second antidyplidemic agent, in this case ERN, to stable statin therapy.

In the ARBITER 6 trial, 363 participants with CHD or CHD equivalent on statin treatment with 80 mg/dL LDL-C were randomly assigned to ezetimibe (10 mg/d) or ERN (2000 mg/d). This trial compared the effectiveness of combination therapy directed at lowering LDL-C (ezetimibe) with combination therapy directed at raising HDL-C (niacin) on atherosclerosis measured by cIMT.¹²¹ The study was terminated early after 14 months; ezetimibe reduced LDL-C more than niacin (-17.6 ± 20.1 mg/dL vs -10 ± 24.5 mg/dL; $P = .01$), whereas niacin increased HDL-C more than ezetimibe (7.5 ± 9.2 mg/dL vs -2.8 ± 5.7 mg/dL; $P < .001$). At 8 and 14 months, ezetimibe treatment did not show significant changes in mean cIMT from baseline (0.0014 ± 0.0020 mm [$P = .38$], 0.0007 ± 0.0035 mm [$P = .84$], respectively). By comparison, niacin significantly reduced the mean cIMT from baseline at both 8 and 14 months (-0.0102 ± 0.0030 mm [$P = .004$] and -0.0142 ± 0.0041 mm [$P < .001$], respectively). Superiority of niacin over ezetimibe was maintained after inclusion of data from an additional 107 subjects who completed a close-out assessment.¹²² The ARBITER 6 trial shows potential superiority of niacin and its effects on both HDL-C and LDL-C in regression of atherosclerosis monitored by cIMT, compared with ezetimibe, which primarily modulates LDL-C, in patients already on a statin with relatively low levels of LDL-C.

The Oxford Niaspan Study evaluated effects of ERN 2000 mg/d on atherosclerosis measured by magnetic resonance imaging in patients ($n = 71$) with type 2 diabetes mellitus and CHD or carotid or peripheral artery disease who achieved LDL-C lowering with statin but had low HDL-C levels (<40 mg/dL).¹²³ After 1 year, ERN increased HDL-C 23% and decreased LDL-C 19%. After 12 months, magnetic resonance imaging showed ERN significantly reduced plaque in the carotid wall area compared with placebo (-1.64 mm² [95% CI -3.12 to -0.16]; $P = .03$). Mean changes in carotid wall area were -1.1 ± 2.6 mm² for ERN compared with 1.2 ± 3.0 mm² for placebo. The Oxford Niaspan and ARBITER 6 studies were conducted with participants on statin therapy and well-managed LDL-C levels at baseline. Each found that niacin treatment could reduce atherosclerosis.

Analysis from HATS showed that niacin/simvastatin combination therapy produced nearly a 50% relative reduction in major clinical events in the subset of participants with diabetes or impaired fasting glucose,¹²⁴ with no significant difference in glycemic control between active treatment or placebo groups. Absolute risk reduction was similar among participants with and without diabetes. Similarly, in a post hoc analysis of the CDP, participants with diabetes treated with niacin experienced significantly lower overall CV events at 6 years¹²⁵ and lower 15-year total mortality than participants treated with placebo.¹²⁵ Another post hoc analysis of 6- and 15-year event rates in patients with metabolic syndrome found significant reductions in the niacin group over placebo.¹²⁶

The AIM-HIGH trial

The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health (AIM-HIGH) trial was a randomized, placebo-controlled clinical trial in patients with a history of atherosclerotic CVD and atherogenic dyslipidemia (low HDL-C, high TGs).¹²⁷ The AIM-HIGH investigators hypothesized that raising HDL-C with ERN would reduce the risk of CV events among patients who had achieved target levels of LDL-C (40–80 mg/dL) with intensive simvastatin \pm ezetimibe therapy 10 mg daily, as needed, in either arm. After a 4- to 8-week open-label run-in with simvastatin 40 mg daily and rapid up-titration of progressively increasing dosages of ERN (500 mg daily during week 1; 1000 mg daily during week 2; 1500 mg daily during week 3; 2000 mg daily during week 4), participants tolerating at least 1500 mg daily were randomly assigned in a double-blind 1:1 randomization scheme to ERN or matching active placebo (50 mg niacin per tablet). In this event-driven trial, it was projected that 800 adjudicated primary events during a 2.5- to 7-year (mean 4.6 year) follow-up would provide 85% power to detect a relative 25% treatment difference between the ERN and placebo groups. Follow-up was scheduled to conclude in December 2012. A total of 3414 men and women, mean age of 64 years, were recruited from 92 enrolling centers across the United

States and Canada and followed for an average of 36 months. At entry, 3196 patients (94%) were taking a statin, with a mean baseline LDL-C of 71 mg/dL, HDL-C was 35 mg/dL, and TG level was 161 mg/dL; by contrast, only 218 patients (6%) were statin-naïve at trial entry. In this small subset, mean baseline LDL-C was 125 mg/dL, HDL-C was 33 mg/dL, and TGs were 215 mg/dL.

During a 36-month follow-up, compared with placebo, ERN raised mean HDL-C by 25% (to 42 mg/dL), lowered TGs by 29% (to 122 mg/dL), whereas LDL-C further declined from 74 mg/dL to 62 mg/dL. As noted earlier, the trial was stopped at a formal interim analysis because of lack of efficacy of ERN. The primary end point (time to first event for the composite of CHD death, nonfatal MI, ischemic stroke, hospitalization for acute coronary syndrome [ACS], or symptom-driven coronary or cerebral revascularization) occurred in 282 ERN-treated subjects (16.4%) compared with 274 placebo-treated patients (16.2%) (hazard ratio [HR] 1.02; 95% CI 0.87–1.21; $P = .80$).

Second Heart Protection Study

A much larger secondary prevention trial similar in design to AIM-HIGH outside North America (the Second Heart Protection Study, Treatment of HDL to Reduce the Incidence of Vascular Events [HPS2-THRIVE]) that compared simvastatin plus ERN/laropirant with simvastatin alone in 25,673 patients has recently concluded.¹²⁸ Study participants between 50 and 80 years of age with a history of MI, cerebrovascular atherosclerotic disease, peripheral artery disease, or diabetes with other evidence of symptomatic CHD were enrolled from China, Scandinavia, and the United Kingdom. All subjects received at baseline either simvastatin 40 mg daily (with a total-C <130 mg/dL) or ezetimibe/simvastatin 10/40 mg daily. The proprietary ERN preparation used in HPS2-THRIVE was fundamentally different from that used in AIM-HIGH in that this agent was combined with laropirant, a prostaglandin-D₂ receptor-1 antagonist, to mitigate niacin-induced flushing. Unlike AIM-HIGH, participants were enrolled regardless of their baseline (entry) HDL-C levels. The primary outcome measure was major vascular events, defined as the first occurrence of major coronary event (nonfatal MI or coronary death) or stroke (any nonfatal or fatal stroke, including subarachnoid hemorrhage) or revascularization (coronary or noncoronary artery surgery or angioplasty [including amputation]).

Demographics indicated that 83% of enrolled subjects were men, the mean age was 64.9 years, 78% had a history of prior CHD, and 32% were diabetic. The baseline lipid values showed a remarkably stable and exceedingly well-treated population with a mean total-C of 128 mg/dL, direct LDL-C of 63 mg/dL, HDL-C of 44 mg/dL, and TGs of 125 mg/dL. During an average 4-year follow-up, the ERN/laropirant patients compared with placebo patients showed an average 10 mg/dL further decrease in LDL-C, 6 mg/dL increase in HDL-C, and a 33 mg/dL decrease in TG

levels—directional changes that were virtually identical to those observed in AIM-HIGH. Among patients randomly assigned to the ERN/laropirant combination, compared with simvastatin plus placebo, the HR for major vascular events was 0.96 (95% CI 0.90–1.03; $P = .29$). No differences were observed in the components of the primary end point or in any of the secondary end points as a function of treatment assignment. Similarly, no treatment differences were observed among enrolled subjects who were <65 years of age, those between 65 and 75 years of age, or those >75 years of age by treatment assignment. A borderline interaction ($P = .06$) was observed for the region from which subjects were enrolled with a better response to ERN/laropirant among European study participants than among participants from China, whereas the mean changes in lipids over time (especially LDL-C) were notably less among Chinese (−7 mg/dL) than among Europeans (−12 mg/dL). Importantly, subgroup analysis also showed a statistically significant interaction between baseline LDL-C levels and treatment effects such that participants with LDL-C levels <78 mg/dL showed no benefit with ERN/laropirant, whereas participants with LDL-C levels above this level did have benefit.

Notably, several serious adverse events were reported among patients randomly assigned to ERN/laropirant compared with simvastatin plus placebo. In particular, there were significant excess rates of any diabetic complication (HR 1.55; 95% CI 1.34–1.78), serious bleeding (HR 1.38; 95% CI 1.17–1.62), and serious infection (HR 1.22; 95% CI 1.12–1.34). As expected, the incidence of statin-related myopathy was significantly higher among Chinese enrollees. The reasons for the excess serious adverse events observed in HPS2-THRIVE remain unknown. The Oxford Trialists who conducted the study indicated that niacin was the most likely cause for the observed findings. Yet, study participants were randomly assigned to the combination of niacin and laropirant, so it is not possible in this trial to determine which agent, if either, is responsible for these unexpected adverse events. Furthermore, in AIM-HIGH, in which patients were treated with niacin alone, no such pattern of excess adverse events was observed for serious infections or bleeding. As expected, niacin worsens glycemic control in approximately 10% of patients and is associated with cutaneous side effects. Nevertheless, because the ERN combination used in HPS2-THRIVE included a prostaglandin inhibitor, it is certainly plausible that some (or most) of the off-target effects observed in this trial may be related to laropirant as opposed to niacin. A critical issue is whether the prostaglandin D₂ receptor-1 antagonist, laropirant, which was used in HPS2-THRIVE to reduce niacin-induced flushing, is really biologically inert with respect to atherosclerosis and thrombosis, as the Oxford Trialists have maintained in their public commentary after the presentation of the trial preliminary results in March 2013. In particular, there is a paucity of scientific information that relates to the known pathobiologic effects of prostaglandin D₂. Of note, experimental data suggest that

prostaglandin D₂ receptor-1 deletion in mice augmented aneurysm formation and accelerated atherogenesis and thrombogenesis, implicating the possibility that niacin-induced prostaglandin D₂ release may function as a constraint on platelets during niacin therapy.¹²⁹ Landmesser¹³⁰ suggested that the effects of inhibition of the prostaglandin D₂ receptor-1 by laropiprant on thrombosis and atherosclerosis in humans in vivo may be complex and difficult to predict, because it has been observed that, on the one hand, laropiprant at low concentrations may prevent the inhibitory effects of prostaglandin D₂ on platelet function, including effects on platelet aggregation and thrombus formation, whereas, on the other hand, laropiprant at higher concentrations may attenuate platelet activation induced by thromboxane and inhibited thrombus formation.¹³⁰ These observations raise uncertainty as to whether the prostaglandin D₂ receptor-1 antagonist laropiprant used to reduce niacin-induced flushing is really biologically inert with respect to atherosclerosis and thrombosis and certainly raise the possibility that the unexpected serious adverse effects observed in HPS2-THRIVE may have, in part, been related to the use of this agent when combined with ERN.

In the aftermath of the neutral findings of both AIM-HIGH and HPS2-THRIVE, uncertainties have abounded in terms of how the trial results should be interpreted and incorporated into clinical practice. Accordingly, the following questions have been posed. Was the fundamental “HDL cholesterol hypothesis” as configured in AIM-HIGH and HPS2-THRIVE wrong? Was the therapeutic intervention in HPS2-THRIVE complicated by adverse effects on the vasculature? Were the expectations of clinical benefit for ERN and simvastatin incorrect?

For >4 decades, an abundance of robust epidemiologic evidence supports the observation that low levels of HDL-C and elevated levels of LDL-C are independently predictive of the risk of developing CHD in both men and women. As already noted, in the original placebo-controlled CDP, high-dose immediate-release niacin (3000 mg/d) was associated with a significant 14% reduction in CHD death or MI, a 26% reduction in nonfatal MI alone, and a 21% reduction in stroke or transient ischemic attacks—event rate reductions that are comparable with reductions achieved in contemporary placebo-controlled statin trials. In addition, VA-HIT found a 22% reduction in CHD death or nonfatal MI during a 5.1-year mean follow-up, whereas the combined incidence of CHD death, MI, or stroke was reduced significantly by 24%. Seemingly, these data confirmed the so-called HDL hypothesis that raising low levels of HDL-C (by 6%, or 2 mg/dL) from 32 mg/dL at baseline to 34 mg/dL at follow-up and lowering TG levels (by 31%) from 160 mg/dL at baseline to 115 mg/dL at follow-up was associated with significant clinical event reduction. Importantly, however, baseline LDL-C in VA-HIT, which predated widespread statin use, was 111 mg/dL, compared with 71 mg/dL in the present study among those receiving a statin at trial entry. This 40 mg/dL between-trial difference in baseline LDL-C is consistent with the significant effect

statins have made on both reducing elevated LDL-C levels and CV risk.

In addition to the anticipated effects of ERN on raising HDL-C and lowering both TGs and LDL-C, AIM-HIGH was designed with an aggressive on-treatment LDL-C target of 40 to 80 mg/dL, in part because of the continued evolution in clinical practice that has supported lower levels of LDL-C in high-risk patients with metabolic syndrome and atherogenic dyslipidemia, who were targeted for enrollment in the trial. As noted earlier, 94% of patients who were randomly assigned to AIM-HIGH had a mean baseline LDL-C level of 71 mg/dL, in contrast to the 6% of statin-naïve patients, whose mean baseline LDL-C level was 125 mg/dL—an almost 45-mg/dL difference. Again, by comparison with VA-HIT in which the baseline LDL-C was 111 mg/dL and patients were not receiving a statin, the baseline LDL-C difference was 40 mg/dL. In addition to the well-controlled LDL-C levels in patients at baseline in AIM-HIGH, the levels of baseline non-HDL-C (mean 108 mg/dL) and apoB (mean 81 mg/dL) were likewise very low at baseline. Hence, the patients enrolled in AIM-HIGH exhibited *excellent* lipid control at baseline, which reflected the proficiency and dedication of the trial investigators in optimizing lipid treatment and secondary prevention. Similarly, in HPS2-THRIVE, one might dispute the characterization that this was a high-risk study population, because these patients were so well treated and had a mean baseline LDL-C in the mid-60-mg/dL range with a mean baseline HDL-C in the mid-40-mg/dL range. Given a baseline lipid profile that is well within existing ATP III clinical practice guidelines for the optional, “optimal” LDL-C target, why would one expect a dyslipidemic intervention such as niacin to provide incremental clinical benefit? In retrospect, it may well be that the inclusion of very well-treated patients with such low levels of baseline LDL-C, non-HDL-C, and apoB played an important role in mitigating much of the long-term residual risk the investigators sought to find with ERN. Importantly, 75% of the statin-treated patients in AIM-HIGH at baseline had been taking a statin for at least 1 year, and 40% of patients had been taking a statin for 5 or more years. Because of the long-standing treatment with statins and the concomitant use of aggressive secondary prevention (or disease-modifying therapies), it may have been difficult to discern incremental clinical benefit with ERN.

Finally, what could explain the lack of clinical benefit viewed from the perspective of the practicing clinician, who is often faced with patients who have low levels of HDL-C but varying levels of LDL-C? As hypothesized earlier, it is possible that long-standing, aggressive LDL-C reduction therapy with statins may deplete the soluble constituents of the large eccentric lipid cores of vulnerable coronary plaques and, by so doing, may convert vulnerable plaques destined for rupture (with associated sudden cardiac death, MI, or ACS) to stable, quiescent plaques, where the risk of such plaque ruptures is significantly reduced. This remains speculation at

present, but it could provide a plausible explanation for the observed findings.

In summary, how should clinicians interpret the results of AIM-HIGH and HPS2-THRIVE and, as a corollary, are there subsets of patients for whom niacin should (or should not) continue to be administered? Recognizing that the study, by design, included only those patients with established, stable, nonacute atherosclerotic CVD, the results of these trials only apply to the types of patients enrolled and should not be generalized to the broader subpopulations that were excluded (such as patients with acute MI or ACS or patients likely to require myocardial revascularization in the subsequent 4–8 weeks after trial enrollment). By contrast, for those patients with stable, nonacute CHD with residually low levels of HDL-C who are able to achieve and maintain very low levels of optimal LDL-C on a statin, the results of AIM-HIGH and HPS2-THRIVE do not support the use of ERN to further reduce clinical risk and to improve CVD outcomes. Data derived from several, prospective, observational registries suggest that only approximately 15% to 20% of all patients with treated high-risk CHD are able to achieve and maintain the kinds of very low LDL-C that were achieved in AIM-HIGH and HPS2-THRIVE; as such, these trial results may directly apply only to this subset of more unselected patients with CHD and dyslipidemia.

In a recently updated meta-analysis, including the results of AIM-HIGH, Lavigne and Karas¹³¹ examined whether niacin reduced CVD events in previously published clinical trials of niacin. (Note that the HPS2-THRIVE trial was not published in full and was not included in their report.) This analysis included 11 studies, with 9959 subjects who were followed for 2.7 years on average. In this analysis, niacin significantly reduced the composite end points of any CVD event (OR 0.66; 95% CI 0.49–0.89; $P = .007$; Fig. 12) and major CHD event (OR 0.75; 95% CI 0.59–

0.96; $P = .02$; Fig. 13). In a provocative finding, meta-regression analysis found no significant association between the extent of HDL-C-raising observed in the trials and the observed reduction in CVD events.

CETP inhibitor trials

Apart from fibrates and niacin, CETP inhibition has likewise been a therapeutic target for HDL-raising therapy. However, the cardioprotective role of raising HDL through its surrogate marker HDL-C has become clouded after early termination of the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial with the CETP inhibitor torcetrapib in patients with high risk of CHD¹² and the more recent termination of the dal-OUTCOMES trial with dalcetrapib.¹¹ In combination with atorvastatin, torcetrapib increased HDL-C by 72% and further lowered LDL-C by 25%. However, it caused a significant 60% higher rate of CV events and mortality than did atorvastatin monotherapy. Whether this excess mortality signal was attributed to so-called “off-target” effects (increased activation of the renin-angiotensin system and increased blood pressure) of torcetrapib or due to other factors (eg, large “dysfunctional” HDL molecules) remains uncertain, but the ILLUMINATE trial indicates that increasing the quantity of HDL-C may be inadequate for cardioprotection.

More recently, the large dal-OUTCOMES trial in 15,871 patients with ACS was terminated earlier than planned. Patients received dalcetrapib 600 mg daily or placebo in addition to best available evidence-based care. The primary efficacy end point was a composite of CHD death, nonfatal MI, ischemic stroke, unstable angina, or resuscitated cardiac arrest. Despite raising HDL-C levels by 31% to 40% compared with placebo, no differences were observed in the rate of the primary end point at a median 31 months of follow-up after 1135 primary events had been adjudicated.

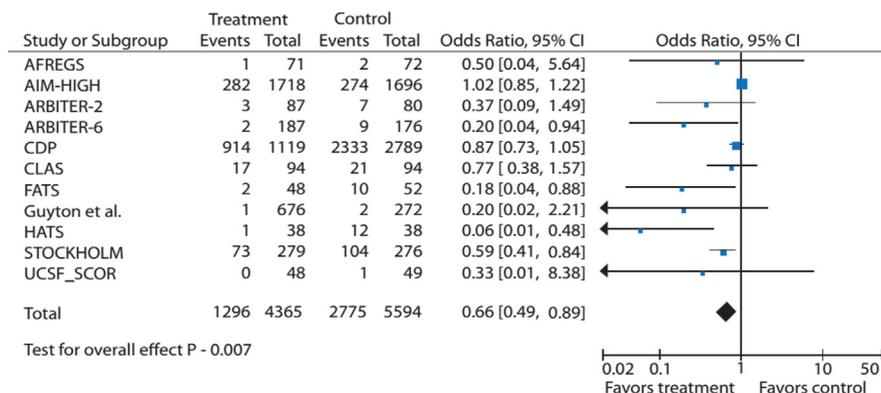


Figure 12 Effect of niacin therapy on the occurrence of any CVD event.¹³¹ AFREGS, Armed Forces Regression Study; AIM-HIGH, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health; ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; CDP, Coronary Drug Project; CLAS, Cholesterol-Lowering Atherosclerosis Study; CVD, cardiovascular disease; FATS, Familial Atherosclerosis Treatment Study; HATS, HDL-Atherosclerosis Treatment Study; HDL, high-density lipoprotein; STOCKHOLM, Stockholm Ischemic Heart Disease; UCSF_SCOR, University of California, San Francisco, Atherosclerosis Specialized Center of Research.

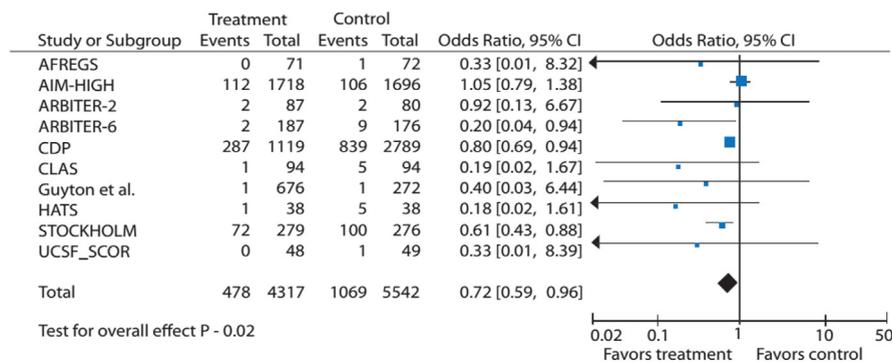


Figure 13 Effect of niacin therapy on the occurrence of major CHD events.¹³¹ AFREGS, Armed Forces Regression Study; AIM-HIGH, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health; ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; CDP, Coronary Drug Project; CHD, coronary heart disease; CLAS, Cholesterol-Lowering Atherosclerosis Study; FATS, Familial Atherosclerosis Treatment Study; HATS, HDL-Atherosclerosis Treatment Study; HDL, high-density lipoprotein; STOCKHOLM, Stockholm Ischemic Heart Disease; UCSF_SCOR, University of California, San Francisco, Atherosclerosis Specialized Center of Research.

Compared with placebo, dalcetrapib did not alter the risk of the primary end point (cumulative event rate 8.0% and 8.3%, respectively; the HR for dalcetrapib was 1.04; 95% CI 0.93–1.16; $P = .52$). Thus, evidence from clinical trials supports HDL-raising therapy and CV event reduction with niacin or gemfibrozil in the absence of statin therapy (CDP, VA-HIT) but not in the setting of statin coadministration (AIM-HIGH, HPS2-THRIVE, ILLUMINATE, dal-OUTCOMES).

Ongoing studies

Clinical end point–driven, large-scale, randomized controlled trials of 2 additional CETP inhibitors (anacetrapib and evacetrapib) are currently ongoing, and results of these studies will likely provide further insight into the role of pharmacologic inhibition of CETP to further reduce CV risk. In addition, other therapeutic strategies for manipulating the HDL axis are also ongoing, including, for example, infusion of peptides that mimic the functional aspects of apoA-I. Finally, subgroup analysis after publication from the recently completed AIM-HIGH and HPS2-THRIVE studies may also provide additional insight and may help to formulate new hypotheses about the role of targeting HDL-C as a therapeutic intervention to reduce CVD risk.

Summary of clinical trials

Advances in dyslipidemic therapy have contributed to the sustained decline in CHD mortality observed over the past 4 decades; however, despite these impressive gains, CHD remains the most frequent cause of death worldwide. Although large-scale clinical trials with statins have found that reducing LDL-C decreases mortality and CVD events by 25% to 35% compared with placebo, these rates remain unacceptably high, in the range of 65% to 75% of the rates observed in placebo-treated patients. Although evidence suggests that low HDL-C levels should also be considered a target for therapy, particularly in patients with multiple risk

factors, established CHD, or its equivalents, results from multiple placebo-controlled trials for clinical event reduction with fibrates, niacin, and CETP inhibitors have failed to provide convincing clinical benefit to date. Thus, it remains unclear whether HDL-C is a treatment target to further mitigate CVD risk.

Challenges posed by a sequence of negative studies and rationale for not abandoning further study prematurely

Despite the well-documented inverse relationship between levels of high HDL-C and CV risk in human population studies and despite the robust evidence in animals that HDL raising translates into a reduction in atherosclerosis, the hypothesis that HDL raising in humans will reduce clinical CVD events has still not been substantiated. In fact, as outlined earlier, 4 recent human trials with HDL-raising agents did not show a reduction in CVD events, and, in 1 trial, the treatment was associated with serious harm. The ILLUMINATE trial with the CETP inhibitor, torcetrapib, caused harm,¹² whereas the AIM-HIGH trial with niacin¹⁰ and the dal-OUTCOMES trial with the CETP inhibitor, dalcetrapib,¹¹ did not cause harm but were both stopped early for futility. Finally, it has now been reported that the HPS2-THRIVE study with ERN/lanopirant failed to meet its primary end point and showed evidence of an excess of serious adverse events.

These 4 trials have undermined the HDL hypothesis and have led to suggestions that it is time to stop further efforts to target HDL as a cardioprotective strategy in humans. However, there is a highly plausible case to be made that these negative trials have not, in fact, tested the hypothesis that HDL raising is cardioprotective, whether because of using a flawed drug (torcetrapib), because of problems with trial design (AIM-HIGH), or because of the possibility that the hypothesis was tested in the wrong population (dal-OUTCOMES). Lessons learned from these trials have

informed the design of new trials that use agents that raise the level of HDL-C.

ILLUMINATE trial

This trial tested the hypothesis that inhibition of CETP by treatment with torcetrapib would reduce CVD events in humans. The rationale for conducting this study was based on several observations.

- (1) CETP promotes the transfer of cholesterol from the protective HDL fraction to potentially proatherogenic particles in the VLDL/LDL fractions. Its inhibition results in an increase in the concentration of HDL-C and (usually) a decrease in the concentration of cholesterol in the VLDL/LDL fraction.
- (2) Inhibition of CETP in rabbits, whether by genetic manipulation, by the use of an anti-CETP vaccine, or by the use of small molecule inhibitors of CETP, greatly reduces the susceptibility of rabbits to the development of atherosclerosis.
- (3) In a large meta-analysis of 92 studies that involved 113,833 participants, it was concluded that participants with *CETP* polymorphisms that are associated with decreased CETP activity and mass have an elevated concentration of HDL-C and a decreased risk of having a coronary event.¹³² A similar conclusion was drawn from an analysis of a cohort of 18,245 healthy Americans in the Women's Genome Health Study.¹³³ This conclusion was further supported by another recent meta-analysis in which it was concluded that a common genetic variation of the *CETP* gene reduces the risk of MI to the same extent as reported in the earlier meta-analysis. In this analysis it was found that the apparently protective *CETP* gene variant was accompanied not only by higher levels of HDL-C but also by lower levels of LDL-C.⁵⁶

However, use of the CETP inhibitor, torcetrapib, did not reduce atherosclerosis in 3 imaging trials and caused serious harm in a large clinical outcome trial, the ILLUMINATE trial. The trial was conducted in 15,067 people with manifest CVD or type 2 diabetes mellitus. Participants, all of whom were receiving atorvastatin at a dose required to reduce the level of LDL-C to <100 mg/dL, were randomly assigned to receive either torcetrapib or matching placebo, with an estimated follow-up of 4.5 years. Despite a 72% increase in HDL-C and a 25% decrease in LDL-C in the group receiving torcetrapib, the trial was terminated early because of statistically significant excesses of both CVD events (464 vs 373; $P < .001$) and total mortality (93 vs 59; $P = .006$) in the torcetrapib-treated group.¹² Treatment with torcetrapib increased the number of deaths from both CVD and non-CVD causes. More patients in the torcetrapib group than in the control group died of cancer (24 vs 14) and infection (9 vs 0), although there was no evidence that torcetrapib increased the total (fatal plus nonfatal) numbers of neoplasms and infections.

Why did torcetrapib cause harm in the ILLUMINATE trial?

It is possible that torcetrapib generates HDLs that do not function normally, although this is not supported by the observation that HDLs isolated from torcetrapib-treated patients have an increased (not decreased) ability to promote the efflux of cholesterol.¹³⁴ Nor is it supported by the observation in the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation study in which those torcetrapib-treated patients who achieved HDL-C levels in the upper quartile showed significant regression of coronary atherosclerosis.¹³⁵

It is also possible that the harm caused by torcetrapib was the result of an off-target effect unrelated to CETP inhibition.¹³⁶ Treatment with torcetrapib in the ILLUMINATE trial was associated with a 5-mm Hg increase in systolic blood pressure, an increase in serum aldosterone, a reduction in serum potassium, and an increase in serum concentrations of bicarbonate and sodium. Furthermore, preclinical studies conducted since termination of the torcetrapib program have shown that treatment with torcetrapib also increases blood pressure in animals that lack CETP. Torcetrapib has been shown to increase the synthesis of both aldosterone and cortisol in adrenal cortical cells growing in tissue culture. Torcetrapib also impairs endothelial function in a process that is independent of either CETP inhibition or changes in HDL-C levels. It has also been shown that compounds structurally related to torcetrapib (but lacking CETP inhibitory activity) raise blood pressure in animals and induce synthesis of aldosterone by adrenal cortical cells. Other CETP inhibitors, including dalcetrapib, anacetrapib, and evacetrapib, have minimal effects on blood pressure or serum aldosterone levels in either animals or humans nor do they induce synthesis of aldosterone in studies of adrenal cortical cells.

However, although consistent with a proposition that off-target effects of torcetrapib may have been responsible for the harm observed in the ILLUMINATE trial and the absence of an effect on atherosclerosis in the imaging trials, these post hoc and preclinical studies cannot be regarded as definitive, and it cannot be concluded that torcetrapib would have reduced CV events if these off-target effects had not occurred.

Dal-OUTCOMES

This was a multicenter, randomized, double-blind, placebo-controlled trial designed to test the hypothesis that CETP inhibition with dalcetrapib reduces CVD morbidity and mortality in patients with recent ACS. More than 15,000 patients, all of whom were being treated with statins to achieve recommended levels of LDL-C, were randomly assigned to receive dalcetrapib or matching placebo. The primary outcome was time to first occurrence of a

composite CV end point. The trial was planned to continue until 1600 primary end point events had occurred.

The trial was terminated early on the basis of futility after the Data and Safety Monitoring Committee concluded that further continuation of the study had virtually no chance of yielding a positive result.¹¹ It is important to stress that the dal-OUTCOMES trial was not terminated on the basis of safety.

The explanation for the failure of dalcetrapib is not known, although several possibilities should be considered.

- (1) The increase in HDL-C concentration induced by dalcetrapib may not have been accompanied by an enhancement of the protective properties of HDL.
- (2) It is also possible that CETP inhibition is not effective in patients treated soon after an acute coronary event as was the case with dal-OUTCOMES. This possibility is supported by the unexpected observation in dal-OUTCOMES that the level of HDL-C in the placebo group did not predict the risk of having a CVD event.¹¹

Whether CETP inhibitors have a future will depend on the results of ongoing large-scale CVD clinical outcome trials being conducted with anacetrapib and evacetrapib.

AIM-HIGH

This trial tested the hypothesis that treatment with niacin would reduce CVD events in statin-treated humans who had low levels of HDL-C. The rationale for AIM-HIGH was that niacin increases the level of HDL-C by up to 30% and also reduces the level of LDL-C by approximately 15%. When given as monotherapy, niacin has been shown to reduce clinical CVD events. Furthermore, when given in combination with a statin, niacin promotes regression of atherosclerosis as assessed by measuring cIMT.

AIM-HIGH was designed on the assumption that niacin increases the concentration of HDL-C by approximately 25%. It was an event-driven trial designed to have an 85% power to detect a 25% reduction in CVD events. It was calculated that a sample size of 3400 participants followed for 2.5 to 7 years would generate the required 800 primary events. However, AIM-HIGH was terminated early on the basis of futility at which time there had been approximately 550 primary events.¹⁰

Treatment with niacin increased the level of HDL-C by 25% (as predicted) to an on-treatment level of 42 mg/dL. However, the level of HDL-C also increased substantially in the placebo group to an on-treatment level of 38 mg/dL. As a consequence, the on-treatment difference in HDL-C between the 2 groups was only 4 mg/dL. The median on-treatment levels of LDL-C were 68 and 63 mg/dL, respectively, in the placebo and niacin groups, a difference of 5 mg/dL. If the inverse relationship between HDL-C and vascular disease risk in observational studies is causal and if one-half of this risk is reversible within a few years (as observed with statin therapy), it would then follow that an observed 4-mg/dL increase in HDL-C would translate into

a 3% to 4% reduction in risk. On the basis of meta-analyses of statin clinical trials, a difference in LDL-C of 5 mg/dL predicts a 2.5% difference in event rates between treatment groups. Thus, the observed differences in the 2 groups would predict a CVD event rate in the niacin group of at most 6.5% lower than in the placebo group, only one-quarter of the predicted 25% reduction on which the power calculations were based.

Put simply, in no way did this trial have the power to detect a 6.5% reduction in events. Thus, whatever conclusions are drawn from AIM-HIGH, it has not tested the HDL hypothesis, nor was it in any way powered to test the potential benefits of niacin. The use of low-dose niacin in the placebo group, choosing patients without high TGs and low HDL-C such as those commonly treated in practice, and very aggressive LDL-C lowering beyond the common application of current guidelines also reduced the likelihood of being able to detect a significant difference in the primary end point between treatment groups.

HPS2-THRIVE

The value of adding niacin to effective statin therapy was also investigated in the much larger HPS2-THRIVE study that randomly assigned 25,000 participants. As described earlier, this trial failed to achieve its primary efficacy outcome. Patients treated with ERN/laropiprant had LDL-C levels 10 mg/dL lower and HDL-C levels 6 mg/dL higher than in patients taking placebo. Thus, as was the case with AIM-HIGH, this trial did not test the HDL hypothesis. The observed 10-mg/dL decrease in LDL-C in HPS2-THRIVE would be expected (on the basis of statin trial meta-analyses) to produce a 5% to 6% proportional reduction in major vascular event risk. If the inverse relationship between the concentration of HDL-C and the risk of having a vascular event observed in population studies is causal and if half of this risk is reversible (as observed in LDL-C-lowering intervention studies), it may follow that the observed 6-mg/dL increase in HDL-C would produce a 4% to 5% reduction in CV event rates. Consequently, the combined lipid changes in HPS2-THRIVE might have been expected to reduce the risk of having a major vascular event by approximately 10%, which is compatible with the 4% reduction that was observed.

In other words, the observed effect of the treatment on the primary end point was compatible with the observed changes in concentrations of LDL-C and HDL-C. If this were not the case, one interpretation of the results of HPS2-THRIVE is that the changes in LDL-C and HDL-C induced by ERN/laropiprant were not sufficient to translate into a significant effect on major vascular events. Thus, although it is reasonable to conclude that in statin-treated patients whose serum LDL-C concentration is very low, the addition of ERN/laropiprant does not result in a significant reduction in major vascular events. It should be made clear that the result does *not* invalidate the hypothesis that there may have been significant beneficial effects had there been

greater reductions in LDL-C or greater elevations in HDL-C or both.

Emerging HDL-targeted therapies

Intravenous infusion of reconstituted HDLs

rHDL-like particles consist of complexes of phospholipids with the main HDL apolipoprotein, apoA-I. Intravenous infusions of rHDLs have been shown consistently in a variety of animal models to inhibit experimental atherosclerosis.^{5,88} Two proof-of-concept studies suggest a similar antiatherogenic effect of infusing rHDLs into humans.^{9,10}

ApoA-I_{Milano} is a mutant form of apoA-I that was discovered in Italian families with low HDL-C but apparently decreased CV risk. In a small study in humans who received 5 once-weekly intravenous infusions of rHDLs containing apoA-I_{Milano}, a significant reduction was observed in coronary atheroma, as assessed by IVUS.⁸

In another human study (Effect of rHDL on Atherosclerosis – Safety and Efficacy trial), participants received 4 once-weekly intravenous infusions of rHDLs containing apoA-I isolated from healthy humans (CSL-111).⁹ These infusions resulted in statistically significant improvements in plaque characterization index as assessed by coronary IVUS and in coronary score as assessed by quantitative coronary angiography. However, the treatment did not result in a significant change in atheroma volume or nominal change in plaque volume compared with placebo. Evidence in this trial also suggested that doses of rHDL >40 mg/kg resulted in abnormalities of liver function, leading to a cessation of the 80-mg/kg dose arm.

These early results are promising and have provided the rationale for embarking on additional larger human trials with newer formulations of rHDLs that appear to be free of any liver toxicity.

HDL delipidation

Another novel technique related to the concept of rHDL infusion involves the collection of plasma that is subsequently subjected to a process that selectively removes lipid from HDLs. The resulting lipid-poor HDLs resemble the apoA-I/phospholipid rHDLs described in the previous section. They are then reinfused back into the patient. In one small human trial that involved 28 patients with ACS, 7 once-weekly treatments resulted in a numerical trend toward a decrease in atheroma volume compared with baseline.¹³⁷ Further larger studies that use this approach are currently being planned.

CETP inhibitors

Despite the failure of 2 previous trials, the hypothesis that CETP inhibitors will be antiatherogenic in humans is

still being tested in studies with anacetrapib and evacetrapib, 2 CETP inhibitors that are much more potent than dalcetrapib and which do not share the off-target adverse effects of torcetrapib.

Anacetrapib

As evidenced in the 18-month, Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib trial that included >1600 participants, all of whom were being treated with effective doses of statins, treatment with anacetrapib was shown to be free of the safety issues observed with torcetrapib.¹³⁸ In this trial of statin-treated patients, anacetrapib increased the level of HDL-C from 41 mg/dL at baseline to an on-treatment level of 101 mg/dL (an increase of approximately 140%) and decreased LDL-C from a baseline of 81 mg/dL to an on-treatment level of 45 mg/dL (a decrease of approximately 35%). Treatment with anacetrapib had no effect on blood pressure or on electrolyte or aldosterone levels.

Prespecified adjudicated CVD events occurred in 16 patients treated with anacetrapib (2.0%) and 21 patients receiving placebo (2.6%) ($P = .40$). Significantly fewer patients in the anacetrapib group than in the placebo group underwent revascularization (8 vs 28; $P = .001$).¹³⁸

The Results of the Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib trial provided the comfort needed to embark on a much larger clinical outcome trial with anacetrapib. The Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification trial (ClinicalTrials.gov number NCT01252953) is a phase 3 trial designed to determine whether treatment with anacetrapib given at a daily dose of 100 mg reduces the risk of a composite end point (coronary death, MI, or coronary revascularization) in patients with circulatory problems who have their LDL-C optimally treated with a statin. It is planned to randomly assign 30,000 subjects to anacetrapib 100 mg daily or matching placebo with a predicted follow-up of approximately 5 years. This study will include men and women with a history of MI, cerebrovascular atherosclerotic disease, peripheral arterial disease, or diabetes mellitus with other evidence of symptomatic CHD. This study is ongoing.

Evacetrapib

Evacetrapib is another potent and selective inhibitor of CETP. The biochemical effects, safety, and tolerability of evacetrapib were assessed in a 12-week randomized, placebo-controlled trial that included 398 patients with elevated LDL-C or low HDL-C levels.¹³⁹ Evacetrapib was given either as monotherapy or in combination with a statin. When given as monotherapy, evacetrapib produced dose-dependent increases in HDL-C of 54% to 129% and decreases in LDL-C of 14% to 36%. When given to statin-treated patients, evacetrapib at a daily dose of 100 mg increased HDL-C by 78.5% to 88.5% and decreased

LDL-C by 11% to 14%.¹³⁹ A large phase 3 clinical outcome study that uses evacetrapib has now commenced.

New PPAR- α , - δ , and - γ agonists

Fibrates have long been known to increase the concentration of HDL-C, although the magnitude of the increase is modest.¹⁴⁰ Although evidence of the cardioprotective properties of fibrates is compelling in subjects with elevated plasma TGs and low levels of HDL-C,^{140,141} the benefits cannot be explained in terms of the observed increase in concentration of HDL-C. It has been suggested that fibrates enhance the ability of HDL to promote reverse cholesterol transport (RCT), although the clinical importance of this remains to be established. Newer peroxisome proliferator-activated receptor (PPAR)- α agonists are in development that are said to promote greater increases in the concentration of HDL-C, although details of such compounds are currently not available. Published results with such agents are awaited with interest. Dual PPAR- α/γ agonists in development have also been reported to promote a greater increase in the concentration of HDL-C than observed with agents displaying PPAR- α activity alone.¹⁴² A clinical trial with one such agent, aleglitazar, is currently under way. Results are awaited with interest.

RVX-208

RVX-208 is a small molecule that increases production of apoA-I by stimulating its gene transcription.¹⁴³ It increases the concentrations of both HDL-C and apoA-I in humans,¹⁴⁴ with evidence of an increase in the pre β HDL particles that are known to be the preferred acceptors of cholesterol released from cells by the ABCA1 transporter. The effects of RVX-208 on human coronary atherosclerosis are currently being studied in an IVUS trial.¹⁴⁵

Role of HDL in RCT

Plasma levels of HDL-C are inversely associated with incident CAD and CVD events such as MI and mortality. This highly consistent observation led to the HDL hypothesis that interventions to raise HDL-C will result in reduced risk of CAD. However, recent findings have raised serious questions about the validity of this hypothesis or the emphasis on whole body cholesterol excretion vs a focus on macrophage cholesterol efflux.⁷³ A number of genetic polymorphisms associated with HDL-C have no association with CAD.⁵⁶ Furthermore, several clinical trials that involve interventions that raise HDL-C levels (niacin, CETP inhibitors) have failed to show reductions in CVD events.^{11,12,128} In light of these results, it is difficult to ascertain the validity of the HDL hypothesis.

A plausible alternative to the HDL hypothesis is one that focuses on HDL function rather than HDL-C levels per se. RCT (the ability to promote cholesterol efflux from

macrophages and transport it to the liver for biliary excretion) is one of the best-characterized HDL functions. Through the RCT pathway, cholesterol efflux from arterial foam cells onto HDL particles leads to reductions in atherosclerotic lesion size.¹⁴⁶ Data from animal models show that increasing plasma apoA-I through intravenous infusion or hepatic overexpression promotes macrophage RCT and prevents or regresses atherosclerosis.¹⁴⁷ Even in humans, the infusion of large amounts of reconstituted apoA-I particles reduces the burden of coronary atherosclerosis.^{9,10} Thus, a detailed molecular understanding of the mechanisms by which HDL and apoA-I promote cholesterol efflux and transport it back to the liver for biliary excretion is not only of biological interest but is potentially of considerable therapeutic value.

The metabolism of HDL is intimately connected to its role in RCT. The biosynthesis of HDL is complex (Fig. 14). ApoA-I is synthesized in the liver and intestine and rapidly lipidated locally via the cholesterol transporter ABCA1. Studies in mice have shown that ABCA1 expressed by the liver and small intestine is responsible for generating most of the nascent discoidal HDL. Lipid-poor apoA-I is also an acceptor of cholesterol efflux via ABCA1 from macrophages. Once bound to nascent discoidal HDL, free cholesterol is esterified by lecithin-cholesterol acyltransferase (LCAT), and the cholesteryl ester is sequestered in the hydrophobic core of the HDL particle. With progressively greater lipidation, the particle becomes larger and more spherical, resulting in the progressive formation of HDL₃ (smaller) and HDL₂ (larger) particles. Large HDL₂ particles are acceptors of cholesterol efflux via ABCG1 from macrophages.¹⁴⁸

Mature HDL can transfer its cholesterol to the liver indirectly via CETP-mediated transfer to apoB-containing lipoproteins, with subsequent uptake by the liver, or directly via selective uptake by the SR-B1 (Fig. 15). Once delivered to the liver, cholesterol can be excreted directly into the bile as cholesterol or after conversion via 7- α hydroxylase to bile acids and, unless reabsorbed by the intestine, is ultimately excreted in feces. In addition, evidence also suggests that HDL-derived cholesterol can be transported to the intestinal enterocyte and excreted without first going through the hepatobiliary route,¹⁴⁹ although the quantitative contribution of this pathway in humans is uncertain.

The metabolic fate of HDL-C and apoA-I, however, is not so simple and unidirectional (Fig. 16). HDL can be remodeled by lipases such as hepatic lipase and endothelial lipase, which hydrolyze HDL TGs and phospholipid, respectively.⁷³ This results in a reduction in the volume of HDL particles and promotes the dissociation of apoA-I from the particles, de facto regenerating lipid-poor apoA-I that can then once again function as a cholesterol acceptor via ABCA1, but is also at risk of catabolism via the kidneys. In addition, the HDLs are continuously interacting with apoB-containing lipoproteins via the action of CETP and phospholipid transfer protein that contribute to the

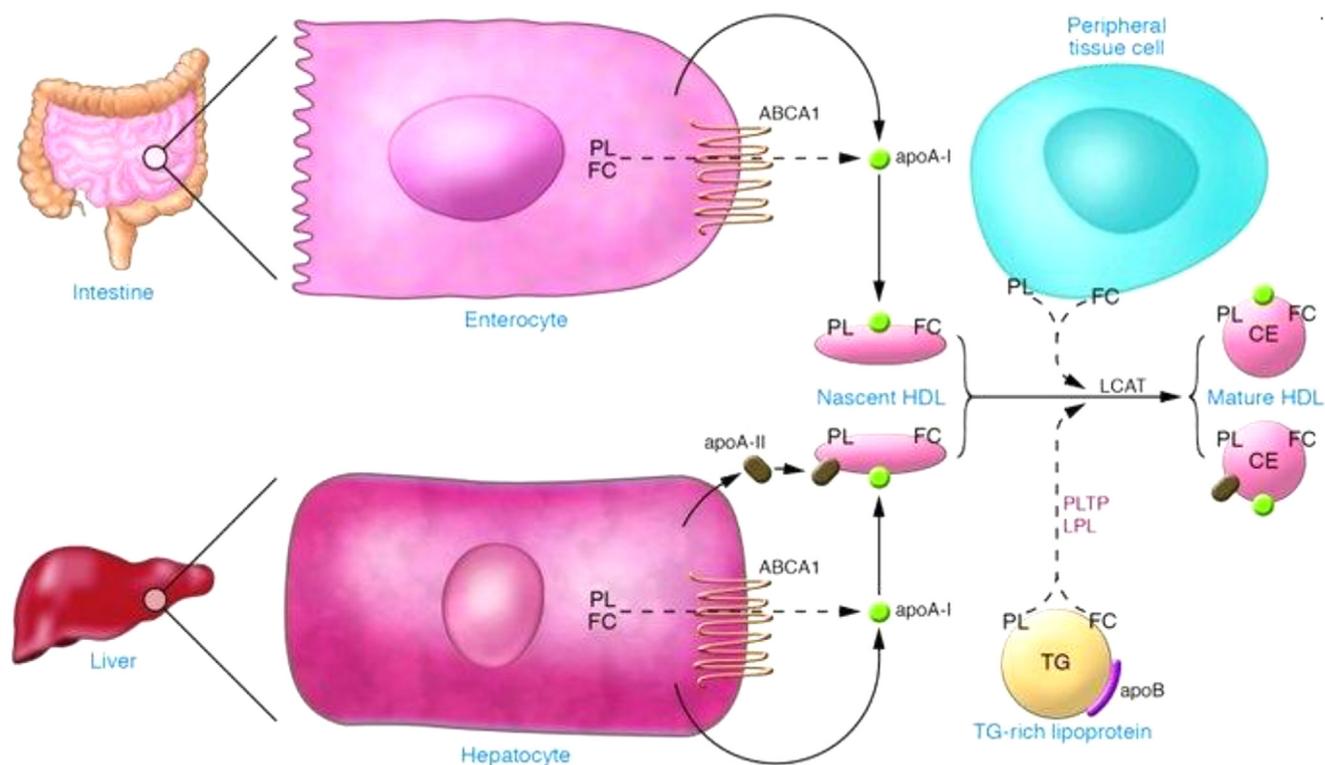


Figure 14 Biosynthesis of HDL. Reprinted with permission from Rader.⁹⁹ ABCA1, adenosine triphosphate-binding cassette transporter-1; apo, apolipoprotein; CE, cholesteryl ester; FC, free cholesterol; HDL, high-density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LPL, lipoprotein lipase; PL, phospholipid; PLTP, phospholipid transfer protein; TG, triglyceride.

dynamic exchange of cholesteryl esters for TGs and phospholipids between HDL and apoB-containing lipoproteins. Thus, circulating levels of HDL particles and of the cholesterol they carry are determined by the equilibrium between factors that affect production and factors that affect catabolism.

The concept of RCT was first introduced in 1968 by Glomset.¹⁵⁰ The physiological need for this process is clear, as nonhepatic cells acquire cholesterol through the uptake of lipoproteins and de novo synthesis, yet (with the exception of steroidogenic tissues that convert cholesterol to steroid hormones) they are unable to catabolize it. Because an excess of intracellular free cholesterol is toxic, one of the pathways that cells have developed to balance cholesterol intake and de novo synthesis and maintain viability is the efflux of excess cholesterol from peripheral cells to extracellular HDL acceptors, followed by its transport to the liver and its enteric excretion. The pathways by which peripheral cholesterol that is effluxed from tissues to apoA-I or HDL gets back to the liver for excretion are covered above. Essentially, after esterification of free cholesterol by LCAT, the cholesteryl ester in the HDL core has 2 primary routes to the liver: direct uptake by SR-B1 or transfer to apoB-containing lipoproteins by CETP with subsequent uptake by the liver. HDL-derived cholesterol arriving at the liver, after hydrolysis by neutral or lysosomal lipases, can be effluxed by the hepatocyte back into the plasma by the ABCA1 transporter to apoA-I as an acceptor.

Alternatively, it can be routed to the bile as free cholesterol or after conversion to bile acids. Ultimately, RCT is complete when the cholesterol and bile acids are excreted from the body in the feces.

Relationship of macrophage cholesterol efflux to atherosclerosis

Although every peripheral cell can efflux cholesterol and contribute to RCT, it is the RCT from macrophages that is directly relevant to the prevention and management of atherosclerosis. Multiple pathways are known by which excess cholesterol in foam cells can be removed by HDL^{73,151} (Fig. 17). The 2 most important pathways quantitatively are the ABCA1 and ABCG1 transporters, which efflux cholesterol to lipid-poor apoA-I and mature HDL, respectively. Macrophage SR-B1 can promote cholesterol efflux to mature HDL but is quantitatively less important in cholesterol-loaded cells. Lipid-loaded macrophages express apoE, which facilitates the efflux of cholesterol.

The concept that macrophage-specific RCT is relevant to atherosclerosis is supported by numerous studies in animal models. With the use of a method that specifically assesses macrophage RCT, it has been found, for example, that (1) mice overexpressing apoA-I have increased, and mice deficient in apoA-I have reduced, macrophage RCT^{147,152}; (2) hepatic SR-B1 expression is a positive regulator of macrophage RCT inverse to its effects on plasma HDL-C

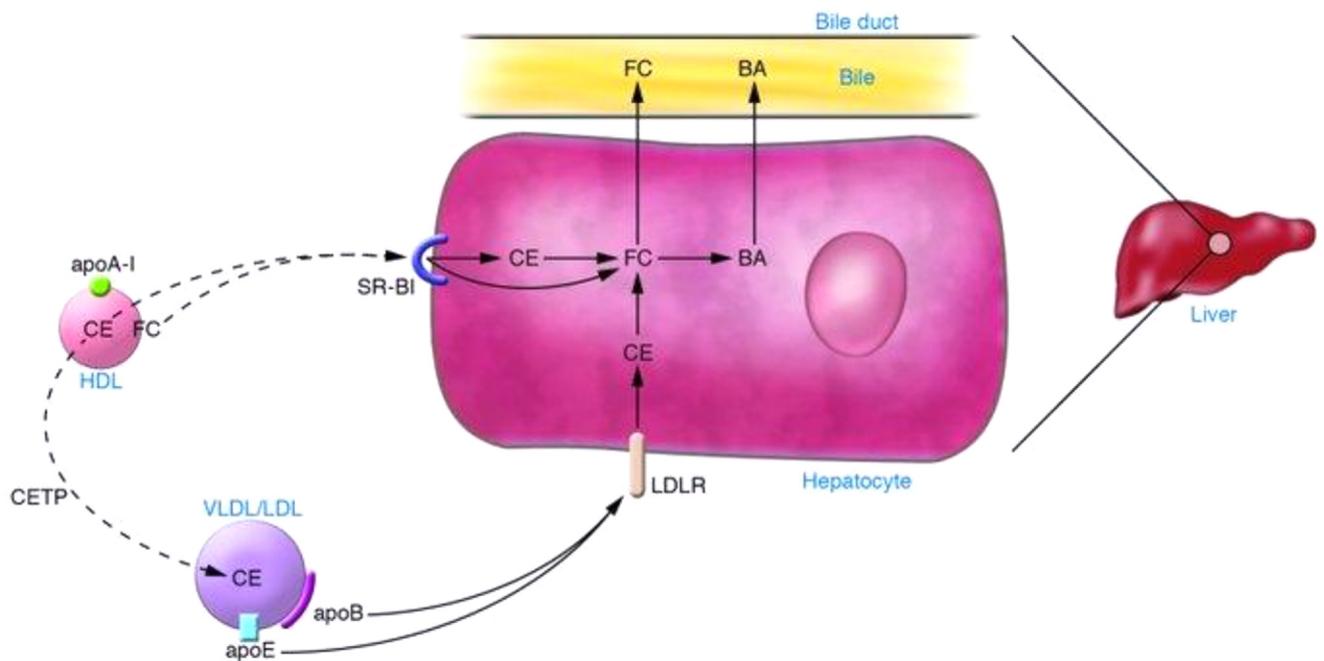


Figure 15 RCT from mature HDL to liver. Reprinted with permission from Rader.⁹⁹ Apo, apolipoprotein; BA, bile acids; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; RCT, reverse cholesterol transport; SR-B1, scavenger receptor class B type 1; VLDL, very low-density lipoprotein.

concentrations¹⁵³; (3) liver X-receptor- α ¹⁵⁴ and PPAR- α ¹⁵⁵ agonists significantly increase macrophage RCT in mice; and (4) probucol increases macrophage RCT despite reducing plasma levels of HDL-C.¹⁵⁶ Multiple other studies have assessed macrophage-specific RCT by genetically and

pharmacologically manipulating mice, and the effects on RCT (in contrast to the effects on HDL-C concentrations) are largely consistent with the effects of the same interventions on atherosclerosis.¹⁴⁶ Overall, the dynamic rate of macrophage RCT correlates much better than steady-state

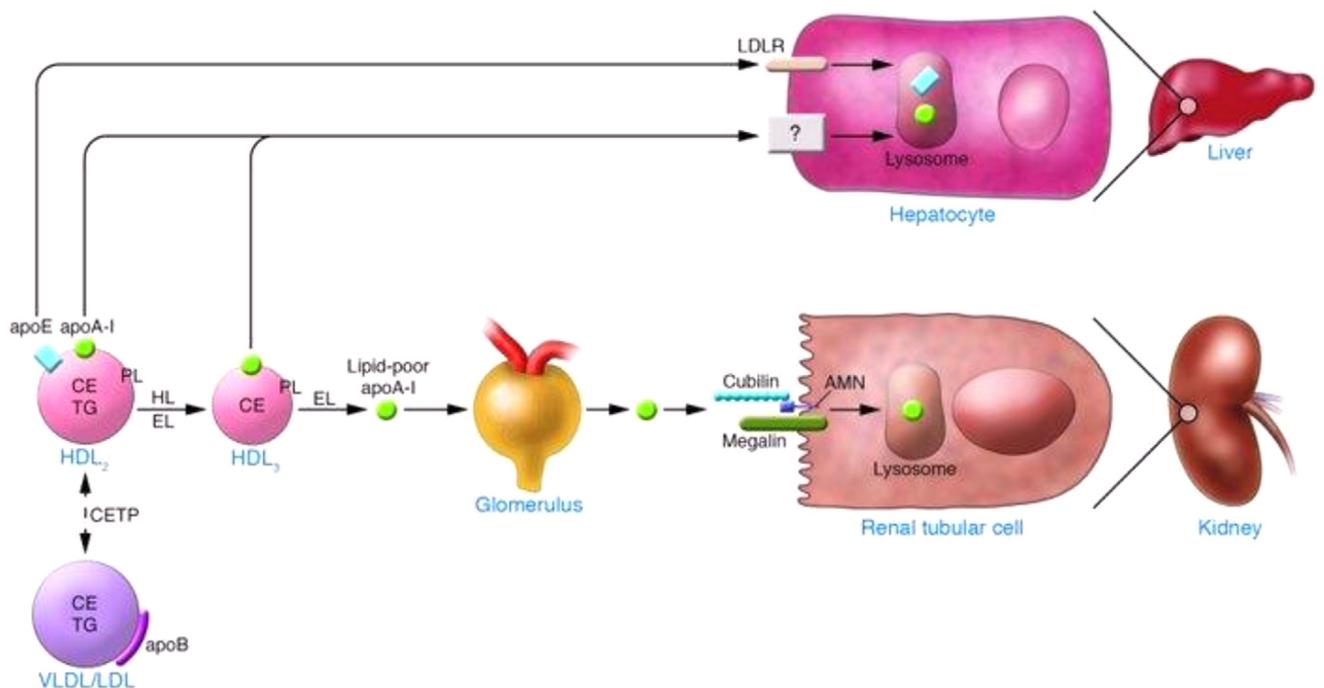


Figure 16 Metabolic fates of HDL and apoA-I. Reprinted with permission from Rader.⁹⁹ AMN, amnionless; apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; PL, phospholipid; SR-B1, scavenger receptor class B type 1; TG, triglyceride; VLDL, very low-density lipoprotein.

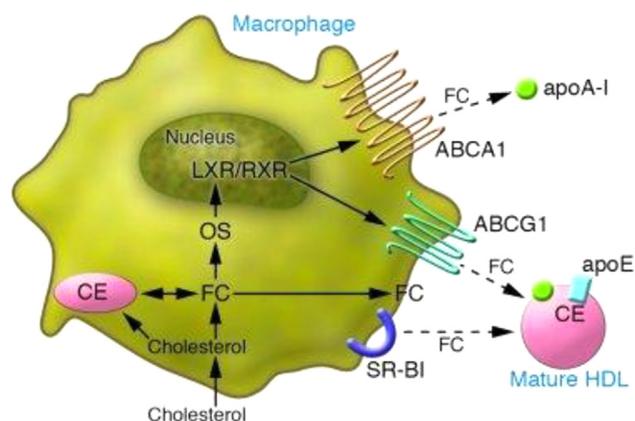


Figure 17 Pathways for removal of excess cholesterol in foam cells by HDL. Reprinted with permission from Rader.⁹⁹ ABCA1, adenosine triphosphate-binding cassette transporter A1; ABCG1, adenosine triphosphate-binding cassette transporter G1; apo, apolipoprotein; CE, cholesteryl ester; FC, free cholesterol; HDL, high-density lipoprotein; LXR/RXR, liver X-receptor β /retinoid X-receptor α ; OS, oxysterol; SR-B1, scavenger receptor class B type 1.

plasma HDL-C level with atherosclerosis, suggesting that methods to assess macrophage-specific RCT may be more useful in dissecting the molecular regulation of RCT because it is relevant to atherogenesis and that the promotion of cholesterol efflux from macrophages, more than just the increase in HDL-C levels, is a potential therapeutic approach to preventing or regressing atherosclerotic disease.⁷³

Robust and sensitive methods for assessing RCT in humans are needed to assess novel therapies targeted toward raising HDL and RCT. In a study that tested the cholesterol efflux capacity of HDL, measured with the use of an established *ex vivo* assay,¹⁵⁷ efflux capacity was found to be a significant predictor of atherosclerotic CVD, independent of HDL-C and apoA-I levels.¹⁵⁸ In *in vitro* studies in cholesterol-loaded human macrophages the ABCA1 pathway seems to be the predominant pathway for cholesterol efflux.¹⁵⁹ The observation that the efflux capacity of HDL may be a more important parameter in determining risk of atherosclerosis than plasma levels of HDL-C¹⁵⁷ has spurred interest in the concept of HDL function and its relationship to CVD.

Although this *ex vivo* approach supports the concept that the capacity of HDL in promoting efflux is more important than the serum concentration of HDL-C, a more effective approach would be to develop a clinical method to determine cholesterol flux in humans and in particular the efflux from cholesterol-loaded macrophages in coronary plaques. Some investigators have shown that the quantification of fecal sterol mass and bile acid excretion is a viable surrogate for RCT. For example, an acute intravenous bolus infusion of apoA-I in humans was found to result in a significant increase in fecal sterol excretion, suggesting a promotion of RCT,¹⁶⁰ whereas no increase in fecal sterol excretion in the steady state was reported in patients who were receiving CETP inhibitors.¹⁶¹ However, this approach

is not macrophage specific, is unlikely to be very sensitive, and may have limited utility in the chronic steady-state setting because of counter-regulatory pathways involved in biliary cholesterol excretion and fecal sterol absorption.

An isotope kinetic modeling technique has been developed that involves the intravenous infusion of stable isotopically labeled cholesterol for approximately 24 hours, with frequent blood sampling for analysis of plasma free cholesterol and cholesteryl ester and for isotope enrichments of fecal sterols by mass spectrometry.¹⁶² With the use of a multicompartmental model, this method allowed the calculation of the key steps in RCT, namely the rates of whole body efflux of free cholesterol from tissues into the plasma compartment, after correcting for red blood cell exchange with plasma-free cholesterol, esterification of free cholesterol to cholesteryl ester, clearance of cholesteryl ester from the blood, and flux from plasma cholesterol into fecal bile acids and neutral sterols. With the use of this method, an approximate 40% reduction in efflux despite normal esterification and fecal excretion rates was observed in subjects with hypoalphalipoproteinemia due to *ABCA1* or *APOA1* genetic alterations.¹⁶³

Similar to the fecal sterol excretion method, this method measures whole body cholesterol efflux and is not macrophage specific. Thus, it remains to be established whether it will have utility in determining cholesterol efflux specifically from cholesterol-loaded coronary macrophages in humans after therapeutic intervention. Nevertheless, other metrics of the RCT pathway generated by this approach, including plasma cholesterol ester production and clearance rates, plasma cholesterol flux into specific neutral sterols and bile acids, and the quantitative role of the red cell membrane as a lipoprotein-independent pathway for cholesterol transport are potentially useful in helping to understand the mechanisms underlying RCT.

Recently, a macrophage-specific method was developed.¹⁶⁴ This method is based on early studies conducted to investigate the mechanisms that regulate cholesterol metabolism by Nilsson and Zilversmit¹⁶⁵ that showed when a saturated solution of radiolabeled unesterified cholesterol mixed with albumin to stabilize the solution was administered to rats as an intravenous bolus, the tracer rapidly disappeared from the blood compartment, followed by its reappearance in the circulation. It was further reported that the disappearance of the tracer from the blood compartment was due to the rapid uptake by reticuloendothelial cells.¹⁶⁵ Schwartz et al^{166–169} used a similar preparation (“particulate cholesterol”) to study cholesterol metabolism in humans. Radiolabeled cholesterol or its precursor, mevalonic acid, was administered in subjects with or without bile fistula, and tracer data obtained from plasma and bile were then analyzed with multicompartmental analysis. Their results were consistent with rapid clearance of the cholesterol-albumin complexes from the blood compartment and subsequent reappearance of the tracer on circulating HDL as free cholesterol, suggesting that this approach may specifically measure the efflux of cholesterol

from macrophage cells to HDL particles as sole acceptor. On the basis of these data, feasibility studies were conducted in humans to show that the administration of a bolus of nanoparticulate ^3H -cholesterol was rapidly removed by the reticuloendothelial system and then labeled cholesterol gradually reappeared in the blood, potentially reflecting physiological cholesterol efflux in vivo.¹⁶⁴ With additional validation, this may be a method for assessing macrophage-specific RCT in humans in vivo.

HDL-mediated cholesterol efflux from macrophages,¹⁵⁸ or measurements of the flux of cholesterol from macrophages to the liver and feces,¹⁴⁶ seem to correlate better with atherosclerotic burden than with HDL-C levels. Thus, it may be time to modify the HDL cholesterol hypothesis to the HDL flux hypothesis in which interventions to promote cholesterol efflux and RCT may reduce CHD risk, regardless of whether it raises plasma HDL-C levels.⁷³ This change will guide a way forward in the development of HDL-targeted therapeutics. To establish this paradigmatic shift, a number of issues need to be clarified. First, as outcome studies with drugs that increase HDL-C levels, such as CETP inhibitors, are currently in progress, there is a major need to address how these drugs influence the rate of RCT in humans and to relate this to the outcomes seen with these drugs. Second, clinical outcomes studies of interventions that promote cholesterol efflux and RCT, including infusions of reconstituted apoA-I/phospholipid particles and strategies to upregulate pathways of macrophage cholesterol efflux, such as liver X-receptor agonists and miRNA (miR-33) inhibitors, are ultimately required to test the HDL flux hypothesis. Third, a deeper understanding of the complex biology of HDL metabolism and its relationship to RCT and atherothrombotic events is urgently needed. This might lead to biomarkers of HDL flux and functionality that are more informative than simple measurements of HDL-C levels. Finally, new loci found in humans, which associate not only with plasma HDL-C but also with CHD risk,⁵⁶ may harbor genes that influence HDL flux through a mechanism that directly affects atherosclerosis and thus may be of particular interest as therapeutic targets.

Recent clinical trial and genetic studies suggest the need to evaluate therapies that affect HDL function rather than simply HDL-C elevation. Perhaps, moving from a focus on the HDL-C hypothesis to a focus on the HDL flux hypothesis will permit a biologically based reassessment of the optimal therapeutic approach to targeting HDL for reducing CVD risk.⁷³

Proteome and lipidome: HDL compositional heterogeneity and function

HDL particles are macromolecular complexes of lipids and proteins that are largely assembled in the extracellular space and then remodeled in the circulation with the participation of lipid transfer proteins, enzymes, and cell surface proteins.¹⁷⁰ Once formed, HDL performs multiple

biological functions, many of which *may* contribute to atheroprotection. The HDLs are primarily responsible for the reverse transport of cholesterol from cells in the periphery, including lipid-laden macrophages in the atherosclerotic plaque, to the liver for catabolism. Within this pathway, HDL and many of its apolipoproteins can promote lipid removal from cells through multiple mechanisms¹⁷¹ and can deliver cholesteryl esters to the liver via the process of selective uptake.¹⁷² In vivo animal models clearly show that genetic lowering of plasma HDL decreases the appearance of macrophage-derived cholesterol in the feces.¹⁵² Aside from their lipid transport functions, HDLs can prevent oxidative modification of LDL, thus inhibiting generation of macrophage foam cells in the vessel wall.¹⁷³ Importantly, HDLs also have clear anti-inflammatory traits (reviewed in Rye et al²). HDL can inhibit the expression of proinflammatory cell adhesion molecules on endothelial cells¹⁷⁴ and can modulate the activity of macrophage chemotactic factors that signal the infiltration of surface-adhered monocytes into the vessel wall.¹⁷³ Readers interested in more detail on lipid transport, antioxidative, and anti-inflammatory functions of HDL are directed to recent reviews.^{4,170} Interestingly, there is also a host of lesser known, but potentially highly important, HDL functions. For innate immunity, HDLs contain bacteriocidal factors in several species of fish¹⁷⁵ as well as humans^{176,177} and can neutralize toxins released during infection, including enterohemolysin,¹⁷⁸ lipopolysaccharide, and lipoteichoic acid.^{179–183} Indeed, HDL is also the source of trypanolytic factor that protects humans from *Trypanosoma brucei*. In addition, HDL has documented roles in hemostasis,¹⁸⁴ undergoes dramatic compositional rearrangements in the acute phase response,¹⁸⁵ and plays roles in apoptosis, stem cell differentiation, and even glucose homeostasis.¹⁸⁶ These reports beg many questions as to how HDL can mediate such a diverse array of functions. This has prompted tremendous interest in understanding the composition, structure, and subparticle constitution of HDL. Determination of the main physical, chemical, and hydrodynamic features of HDL was achieved during the period up to the early 1980s, and, concomitantly, the earliest experimental evidence that HDL constitutes a heterogeneous continuum of pseudomicellar, quasi-spherical particle subpopulations was provided.¹⁷⁰

With the advent of highly resolutive technologies at the molecular level, as exemplified by mass spectrometry, it has become possible to probe not only the complexity of the protein components of HDL particles, the “proteome,” but also that of the lipid components, the “lipidome.”⁸⁷ Thus, the possibility of understanding the nature of protein–protein, lipid–protein, and lipid–lipid interactions within HDL particles, and in turn their relationships to HDL functionality, is on the horizon.³ Figure 18 illustrates this new, integrated concept, which emphasizes the fact that the particle heterogeneity of HDL is determined both by the complement of lipids and of proteins in an individual particle. Given that the plasma levels of some minor HDL proteins,

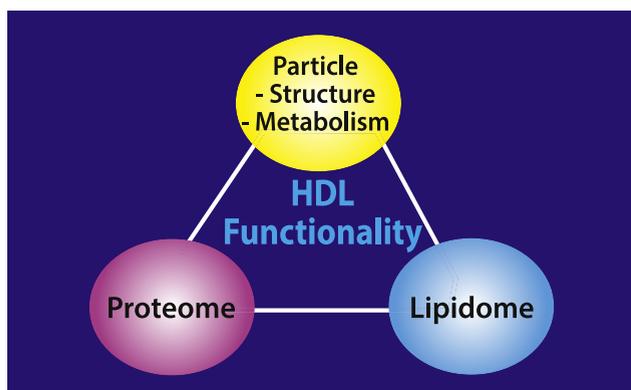


Figure 18 Schematic of HDL functionality as the integration of its proteome and lipidome. HDL, high-density lipoprotein.

such as transthyretin, are only sufficient on a molar basis to be present in 1 in 500 (or less) HDL particles, then one obtains a first glimpse of the enormous potential level of HDL particle subpopulation heterogeneity in man.

Importantly, experimental data indicate that HDL-C measurement provides not only an inadequate evaluation of HDL functionality but also that the increase in the cholesterol content of HDL may occur without clinical benefit when assessed as reduction in CV risk.^{56,158} Thus, the capacity of HDL to efflux cellular cholesterol was more informative than HDL-C level as a metric to estimate CVD risk in the studies of Khera et al,¹⁵⁸ whereas the small increment in HDL-C due to a functional variant in the endothelial lipase gene failed to manifest as a cardioprotective effect in the analyses of Voigt et al.⁵⁶ The cholesterol content of HDL is, therefore, clearly not atheroprotective per se. This finding in part reflects the fact that different HDL particle subpopulations contain differing absolute numbers of free cholesterol and of cholesteryl ester molecules per particle; large particles such as HDL₂ contain several fold greater numbers of cholesterol molecules than small HDL₃ particles.¹⁷⁰ Indeed, cholesterol-poor, ABCA1-active acceptor HDL, such as pre β particles, are significantly underestimated by HDL-C determination.⁸⁷

The next main paradigm in the study of HDL is to relate new understanding of the structure and composition of individual HDL particle subpopulations to their function, and in turn, to the risk of CVD. For example, lipid-poor forms of apoA-I, sometimes referred to as pre β HDL, are the most efficient and specific acceptors of cellular cholesterol through the ABCA1 transporter, whereas large, cholesteryl ester-rich HDL₂ are efficient cholesterol acceptors through both the SR-B1 receptor and ABCG1 pathways.⁷³ The relationship of such particles to CVD remains unclear, however. Indeed, a single HDL particle species may possess multiple biological activities relevant to atherosclerosis, such as cellular cholesterol efflux activity, antioxidative, and anti-inflammatory activity; we have yet to determine which of these is most relevant to atheroprotection and ultimately to reduction in CVD risk.

In light of this, it is the goal of this section to summarize current knowledge of the different features of HDL

compositional heterogeneity and to relate them, as far as possible, to specific functions. In addition, therapeutic approaches to normalizing the altered structure, metabolism, and function of HDL typical of certain cardiometabolic disease states are addressed.

HDL protein composition

HDL is an assembly of amphipathic proteins that stabilize lipid emulsions composed of phospholipids, cholesterol, TGs, and cholesteryl esters. In addition to structural stability, these apolipoproteins impart biological targeting of the lipid cargo to various tissues via receptors, modifying its chemical form (ie, lipolysis or esterification) or transferring it to other lipoproteins. In humans, roughly 65% of HDL protein mass is composed of apoA-I with another 15% by apoA-II. Although the presence of limited additional protein constituents has been known for many years, recent applications of techniques for soft ionization mass spectrometry have revealed the staggering complexity of the HDL proteome. By the end of 2012, at least 14 studies had applied various unbiased “shotgun” mass spectrometry techniques to characterizing the human HDL proteome. These included HDL isolated by traditional density ultracentrifugation,^{187–197} but also immunoaffinity capture¹⁹⁸ and size exclusion chromatography.¹⁹⁹ According to the HDL Proteome Watch Initiative (<http://homepages.uc.edu/~davidswm/HDLproteome.html>), 188 individual proteins have been proposed to be associated with HDL. Of these, 85 proteins have appeared in at least 3 different studies (from independent laboratories) and thus represent the best current estimate of the HDL proteome. Although HDL is well known to contain structural proteins (ie, the “apos”), enzymes (LCAT), and transfer proteins (CETP, etc) that are key to lipid metabolism, these studies found additional proteins involved in surprisingly disparate functions. For example, many proteins involved in inflammation and the immune response were identified, including numerous members of the complement cascade (C3, C4A, vitronectin, etc), protease inhibitors (antithrombin III, α -1-antitrypsin inhibitor, serine protease inhibitors, etc), and acute-phase response proteins (serum amyloid A, transthyretin, etc). Also present were proteins involved in heme and iron metabolism such as hemoglobin, transferrin, and hemopexin, as well as proteins with a host of additional enigmatic functions, ranging from platelet regulation to vitamin binding and transport. Not coincidentally, the predicted functions of many of these protein constituents line up nicely with the diverse functions attributed to HDL described earlier (Fig. 19).²⁰⁰

Given the known exchangeability of certain apolipoproteins, HDL has been viewed by some as a transient ensemble of proteins that randomly exchange. However, evidence is mounting that HDL proteins in fact segregate into compositionally stable particles. For example, Santos et al²⁰¹ have shown highly distinct HDL protein patterns with the use of a 2-dimensional gel electrophoresis system.

Lipid Metabolism and Transport ApoA-I, ApoA-II ApoA-V, ApoD ApoE, ApoF ApoC-I, ApoC-II ApoC-III, ApoC-IV ApoO, Apo(a) LCAT, PLTP, CETP PAFAH, SAA4 Pon1, Pon3 Albumin, Transthyretin ApoM, ApoB ApoL-I	Hemostasis Fibrinogen α Fibrinogen β Fibrinogen γ ApoH α -2 macroglobulin	Immune Response Platelet basic prot. Vitronectin Haptoglobin rel. prot. Ac-muramoyl amidase Ig α -1 chain C Ig γ -1 chain C Ig κ -1 chain C Ig λ -1 chain C AMBP, AZGP1 Serpin G1 Platelet factor 4 ApoL-I	Metal Binding HB α , HB β Ceruloplasmin Hemopexin Serotransferrin His-rich glycoprot. AMBP	Vitamin Transport Afamin Vit. D bind. prot. Retinol bind. prot.
ApoA-IV α -1-acid glycoprot. 1 α -1-acid glycoprot. 2 SAA1/2	Prothrombin Kinninogen Kallistatin	ApoA-IV LPS-binding protein α -2 antiplasmin	α -1 anti-trypsin Serum amyloid P ITIH4 α -1 anti-chymo. Fibronectin α -2 HS glycoprot.	Acute Phase Response/ Inflammation
	Anti-thrombin III Plasminogen Serpin G1 Serpin D1 Kallikrein	α -2 antiplasmin	Prenyl-Cys-oxid. α -1 anti-trypsin Hep. cofactor 2 ITIH4, ITIH1, ITIH2 α -1 antichymo. Serpin F1, AMBP	Proteolysis/ Inhibition
ApoJ	Comp. C9	Comp. 1S, Comp. C2 Comp. 4B, Comp. B Comp. H, Comp. C3 ApoJ		Complement

Figure 19 Functional relationships of the HDL-associated proteins detected in proteomic studies. Reproduced from Shah et al.²⁰⁰ AMBP, α -1-microglobulin/bikunin precursor; Apo, apolipoprotein; AZGP1, zinc- α -2-glycoprotein; bind, binding; CETP, cholesteryl ester transfer protein; chymo, chymotrypsin; Comp, complement; Cys-oxid, cysteine oxidation; glycoprot, glycoprotein; HB, hemoglobin; Hep, heparin; Ig, immunoglobulin; ITIH, inter- α -trypsin inhibitor heavy chain; LCAT, lecithin cholesterol acyltransferase; LPS, lipopolysaccharide; PAFAH, platelet-activating factor acetyl hydrolase; PLTP, phospholipid transfer protein; Pon, paraoxonase; prot, protein; rel, related; SAA, serum amyloid A; Vit, vitamin.

In addition, proteomic profiling of HDL density¹⁹² and gel filtration¹⁹⁹ subfractions clearly indicate that HDL proteins distribute in distinct patterns across the HDL spectrum. Strong evidence is available that major HDL activities rely on cooperative interactions between associated proteins. The most striking example of on-particle cooperation is the discovery that a specific HDL subparticle (containing apoA-I, apoL-I, and haptoglobin-related protein) can mediate the lysis of *T. brucei*, a trypanosome responsible for African sleeping sickness.²⁰² Given the extraordinary functional diversity of HDL proteins, it is easy to imagine the existence of additional unknown subspecies that may mediate HDL cardioprotection. Indeed, Jensen et al.²⁰³ used immunoaffinity chromatography on samples from 2 large prospective case-control studies to separate HDL into 2 subspecies, HDL that contains apoC-III and HDL

that does not. They found that cholesterol level in HDL containing apoC-III was significantly associated with CVD risk, whereas the cholesterol levels of the particles lacking apoC-III were associated with protection. That study supports the concept that distinct populations of HDL particles may be better biomarkers of disease risk in CVD and possibly in other inflammatory disease states or even innate immune function.

Similar studies of the other lipoprotein classes have also found protein diversity, although not to the extent of HDL. LDL, commonly associated with a single molecule of apoB, has been shown by a handful of mass spectrometry studies to contain approximately 15 to 20 distinct proteins.^{204–206} Most of these can also be found in HDL, although there are a few examples of exclusive LDL proteins such as calgranulin A.²⁰⁴ VLDL appears to have

a similar complement of proteins,²⁰⁷ although the isoform profile of these proteins (ie, posttranslational modifications) likely varies between the lipoprotein classes, perhaps with functional implications.

With the complexity of the HDL proteome largely established, investigations have turned to monitoring changes in various disease states. In a comparison of the HDL₃ protein profiles between normolipidemic subjects and patients with documented CAD,^{191,208,209} several proteins that were enriched in the patients with CAD, including apoE, apoC-IV, paraoxonase 1, complement C3, and apoA-IV, were involved in vascular inflammation. Importantly, pattern recognition analyses were able to clearly differentiate mass signatures from healthy subjects and subjects with CAD, particularly from mass markers found in apoA-I, apoC-III, and apoC-I.²⁰⁸ In a similar comparison in control subjects with stable CAD, and subjects with ACS,¹⁹³ significant differences in serum amyloid A, apoA-IV, and complement C3 levels were noted in the patients with ACS, indicating a shift to an inflammatory profile. No differences were noted in the ability of HDL from the different groups to promote various modes of cholesterol efflux; however, other HDL functionalities with regard to vascular inflammation and antioxidation were not tested. Overall, these studies indicate that HDL can change its protein composition dramatically in CAD.

The state of the HDL proteome has been evaluated in other conditions as well. For example, sex steroid withdrawal in men increased HDL-associated levels of clusterin (apoJ) while increasing the capacity of HDL to promote cholesterol efflux from macrophages.²¹⁰ A follow-up study showed that testosterone replacement in hypogonadal men promoted significant increases in paraoxonase 1 and fibrinogen α chain, while lowering apoA-IV, but had no effects on HDL-C levels or cholesterol efflux functionality.²¹¹ Because renal disease is associated with low HDL-C and increased renal disease, 2 laboratories have monitored the HDL proteome in response to chronic dialysis. Holzer et al¹⁹⁵ found that patients undergoing dialysis have increased levels of the acute-phase inflammatory proteins serum amyloid A, lipoprotein-associated phospholipase A2, and apoC-III in HDL along with decreases in phospholipid and increases in TG content. These changes corresponded with impaired cholesterol efflux function. Weichhart et al.¹⁹⁶ showed that HDL from patients with advanced renal disease lacked normal anti-inflammatory properties and correlated this with HDL enrichment of several proteins, including serum amyloid A. These studies are suggestive of a link between HDL dysfunction and increased risk of CAD in renal disease.

The effect of non-CAD chronic inflammatory disease states on the HDL proteome has also been explored. In a cohort of patients with psoriasis, a chronic inflammatory skin disease, a reduction in apoA-I levels was observed relative to controls, but these patients had increased levels of apoA-II and proteins involved in the acute-phase response. Strikingly, the ability of HDL to promote

cholesterol efflux from macrophages was negatively correlated with psoriasis severity. Overall, these studies indicate that the HDL proteome can change in a variety of inflammatory disease states and that these changes are often related to in vitro measures of HDL function. However, it remains to be seen whether these changes are secondary to other processes occurring during disease progression or if the HDL particles themselves contribute to the disease etiology.

HDL lipid composition

The number of individual molecular lipid species present in the HDL lipidome is high (Table 5); indeed, >200 of them were recently identified,^{212,213} and it is safe to predict that this number will only grow with the development of available technologies.²¹⁴ Cholesterol is by far the most well-known component of the HDL lipidome because, in the form of HDL-C, it represents the main risk factor for CVD. Cholesterol is present in HDL in esterified and free forms, which, together with phospholipids and TGs, constitute 4 major lipid classes of HDL. Phospholipids and free cholesterol form the surface lipid monolayer of HDL, whereas cholesteryl esters and TGs build the hydrophobic lipid core. Cholesterol is, however, far from being a quantitatively major lipid carried by HDL. Indeed, phospholipids quantitatively predominate in the HDL lipidome,

Table 5 Broad range of lipid species isolable from HDL

Dihydroceramide
Ceramide
Monohexosylceramide
Dihexosylceramide
Trihexosylceramide
GM3 ganglioside
Sphingomyelin
Sphingosine-1-phosphate
Phosphatidylcholine
Alkylphosphatidylcholine
Alkenylphosphatidylcholine (plasmalogen)
Lysophosphatidylcholine
Lysoalkylphosphatidylcholine (lysoplatelet activating factor)
Phosphatidylethanolamine
Alkylphosphatidylethanolamine
Alkenylphosphatidylethanolamine (plasmalogen)
Lysophosphatidylethanolamine
Phosphatidylinositol
Lysophosphatidylinositol
Phosphatidylserine
Phosphatidylglycerol
Bis(monoacylglycero)phosphate
Cholesterol ester
Cholesterol
Diacylglycerol
Triacylglycerol

Information was kindly provided by Prof. Peter Meikle, Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

accounting for 20% to 30% of total HDL mass; they are followed by cholesteryl esters (14–18 wt%), TGs (3–6 wt%), and free cholesterol (3–5 wt%).

HDL contains diverse molecular classes of phospholipids among which phosphatidylcholine and sphingomyelin predominate; indeed, phosphatidylcholine accounts for approximately 70% of HDL phospholipid. Most of phosphatidylcholine in HDL is accounted for by the 18:2/16:0, 18:2/18:0, and 20:4/16:0 species.²¹⁵ In addition, HDL carries significant amounts of phosphatidylinositol, lysophosphatidylcholine, phosphatidylethanolamine, and plasmalogens.^{212,215,216} Minor HDL phospholipids include phosphatidylglycerol, phosphatidylserine, phosphatidic acid, and cardiolipin.^{213,217,218} Phosphatidylinositol, phosphatidylserine, and phosphatidic acid are negatively charged phospholipids that determine the net surface charge of HDL,^{217,219,220} thereby modulating interactions with extracellular matrix, enzymes, and other protein components. The family of HDL cholesteryl esters is less diversified; the major molecular species of cholesteryl ester includes cholesteryl linoleate that accounts for >50% of total cholesteryl ester in HDL.²¹⁵ HDL also carries numerous molecular species of TGs that are dominated by those containing oleic, palmitic, and linoleic acid moieties.²¹² Finally, HDL contains multiple minor bioactive lipids, including ceramides, lysosphingolipids, glycosphingolipids, gangliosides, sulfatides, diacylglycerides, monoacylglycerides, free fatty acids, lipophilic vitamins, and antioxidants.^{212,221–227} Biologically active lysosphingolipids carried by HDL are represented by sphingosine-1-phosphate, sphingosylphosphorylcholine, and lysosulfatide.²²⁸ HDL is the major carrier of sphingosine-1-phosphate in the circulation, accounting for, together with albumin, >90% of plasma sphingoid base phosphates.²¹⁶

Importantly, HDL particle subpopulations differ in their content of lipids. Although no differences are found between HDL subspecies in particle contents of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lysophosphatidylcholine, cholesteryl ester, and total fatty acids, HDL content of sphingomyelin (expressed as percentage of total lipids) decreases 2-fold with HDL density from HDL_{2b} to HDL_{3c}.²¹⁵ This result suggests that in contrast to other major lipid classes, the sphingomyelin pool is not in equilibrium across HDL subpopulations, reflecting the slow rate of transfer of sphingomyelin through the aqueous phase.²²⁹ Similarly, the HDL content of free cholesterol decreases 2-fold from HDL_{2b} to HDL_{3c}.²¹⁵ As a result, the cholesteryl ester-to-free cholesterol ratio increases with HDL density, in parallel with LCAT activity,²³⁰ consistent with the observation that small HDL constitutes a major site of cholesterol esterification within the HDL particle pool.²³¹ Finally, sphingosine-1-phosphate is enriched in small, dense HDL₃ (40–50 mmol/mol HDL) compared with HDL₂ particles (15–20 mmol/mol),^{215,232,233} potentially reflecting enrichment of HDL₃ in apoM,¹⁹² a specific carrier for sphingosine-1-phosphate.²³⁴

HDL lipids exert major effects on HDL function. Thus, cholesterol efflux capacity of HDL via SR-B1 is proportional to the HDL content of phospholipids.²³⁵ The ability of HDL to efflux cellular cholesterol, but also its capacity to protect LDL from oxidation, depends on the physical state of phospholipids, with a more fluid liquid-crystal phospholipid surface monolayer resulting in more efficient cholesterol acceptor particles^{236,237} that display higher anti-oxidative activity.²³⁸ HDL-associated lysosphingolipids possess anti-inflammatory, cytoprotective, antiapoptotic, and vasodilatory functions.^{239,240} In particular, sphingosine-1-phosphate acts via increased nitric oxide production and improved survival of endothelial cells, interacting with sphingosine-1-phosphate receptors and activating intracellular signaling cascades that include the small G-protein Rac, Src kinase, phosphatidylinositide 3-kinase, protein kinase B, extracellular signal-related kinase, and mitogen-activated protein kinase.^{241–244}

The HDL lipidome can be altered under conditions of dyslipidemia, insulin resistance, and increased systemic inflammatory tone. Such alterations may involve core enrichment in TGs with cholesteryl ester depletion as a consequence of elevated CETP activity, depletion of surface phospholipid as a consequence of phospholipase activation, and increased abundance of free cholesterol as a consequence of decreased LCAT activity.¹⁷⁰ As a result, HDL function, primarily cholesterol efflux capacity, can be attenuated; direct functional relevance of such compositional alterations, however, remains to be established.

miRNAs in HDL

HDL is known to mediate specific cell signaling events in cells of the vasculature. For example, it can interact with SR-B1 to modulate nitric oxide production in the endothelium, and apoA-I can interact with ABCA1 to promote the availability of intracellular cholesterol pools and to promote anti-inflammatory cellular responses.²⁴⁵ However, recent work has found that a new class of signaling molecules, microRNAs (miRNAs), may be a key mode of HDL-based cellular communication. These molecules are capable of regulating cellular processes via mRNA targeting and repressing translation of key cellular mediators. Although miRNAs have been long associated with extracellular lipid vesicles and microparticles, Vickers et al²⁴⁶ have recently provided evidence that HDL may be a key miRNA conduit. They demonstrated that HDL isolated from human plasma contained a complement of miRNAs (with miR-135a, miR-118-5p, and miR-877 among the most commonly found) that was unique from the complement typically found in circulating exosomes. HDL from subjects with familial hypercholesterolemia exhibited a different miRNA profile than that from healthy persons. Strikingly, HDL was capable of delivering these mediators to recipient cells via an SR-B1-dependent pathway. miRNA from familial hypercholesterolemia-HDL was found to alter gene expression profiles in human hepatocytes differently than HDL

from healthy subjects. Although this field of research is still emerging, the implications of different miRNA cargos on HDL particles and their potential for altering cell function in disease states may open exciting avenues for understanding, and exploiting, HDL's role in modulating atherogenesis and CAD-related end points.

The promise of therapeutic approaches to alter HDL composition and function

The antiatherogenic, cardioprotective effects associated with elevated HDL-C levels appear to derive from the capacity of HDL particles to exert a spectrum of anti-atherogenic and vasculoprotective effects.²⁴⁷ Common metabolic diseases associated with accelerated atherosclerosis and premature CVD, and notably type 2 diabetes mellitus and metabolic syndrome, are frequently characterized by subnormal levels of HDL particles of defective antiatherogenic function. Clearly, innovative therapeutic strategies targeted to normalize HDL metabolism, structure, and function are critically required, and particularly with a view to enhancing cholesterol efflux, with consequent reduction in plaque cholesterol content and inflammation. In this way, plaque stabilization, regression, or both may be achieved, with the potential for reduction in CV events.

As yet, little knowledge is available of the effects of lipid-modulating agents on HDL composition and function, and this is primarily limited to niacin and to CETP inhibitors. Niacin, which may induce elevation of HDL-C levels by up to 30% at the 2-g/d dose, has been shown to favor normalization of the altered HDL proteome in subjects displaying CHD.²⁰⁹ Moreover, HDL from niacin-treated subjects displays a minor increment in cholesterol efflux activity, primarily reflecting a small increase in HDL particle numbers (<10%).

Recent findings with anacetrapib, a potent CETP inhibitor that raises levels of HDL-C >100%, have clearly indicated that its action improves both HDL efflux capacity from macrophages and the anti-inflammatory activity of HDL as determined by its ability to suppress toll-like receptor-4-mediated inflammatory responses in this cell system; moreover, a marked increase in the cholesteryl ester-to-TG ratio in HDL from anacetrapib-treated subjects has been reported.^{148,248} Subsequently, HDL particle analysis revealed that anacetrapib increased large HDL₂ with increases in levels of HDL apoA-I, apoA-II, apoE, and apoC-III. The relationship between the potential changes in the HDL proteome and lipidome to specific changes in function remains indeterminate in these pioneering studies. Nonetheless, it is relevant that this agent has been shown to promote RCT and bulk cholesterol excretion in a hamster model.²⁴⁹

Pharmacologic modulation of the miRNA content of HDL particles with a view to potentiating mechanisms of vascular repair or plaque stabilization is highly attractive but remains to be exploited.

Proteome and lipidome conclusions and perspectives

Plasma HDL particles display a high level of compositional heterogeneity that is directly linked to their heterogeneity in biological activities. Available data suggest that at least some biological functions of HDL can be confined to individual subpopulations of distinct proteome or lipidome or both. Linking composition of distinct HDL subpopulations to specific atheroprotective functions calls for further studies. When successful, identification of such clinically relevant HDL subpopulations may be followed by assessment of their circulating levels and biological activities under conditions associated with accelerated atherosclerosis. As a corollary, further development of HDL-based therapies can be envisaged to specifically target beneficial subspecies among those constituting the plasma HDL pool.

Dysfunctional HDL: What is dysfunctional HDL, and is it clinically relevant?

Clinical and epidemiologic studies show a robust, inverse association of HDL-C level with CVD risk.^{250,251} Moreover, hypercholesterolemic mice with genetically engineered deficiencies in proteins implicated in HDL metabolism have strongly atherosclerotic phenotypes,²⁵² providing compelling evidence that HDL is a key modulator of atherosclerosis in animal models. These observations have triggered intense interest in targeting HDL for therapeutic intervention.

However, several lines of evidence weaken the hypotheses that HDL-C levels relate to CVD status and that elevating HDL-C is necessarily therapeutic. For example, genetic variations that associate with altered HDL-C do not strongly affect CVD risk.^{56,253} Certain drugs that elevate HDL levels, such as fibric acid derivatives, show no clear clinical benefit.¹¹ Moreover, certain genetically engineered deficiencies in proteins involved in murine HDL metabolism greatly increase both HDL-C levels and atherosclerosis.^{252,254} Such observations have led some investigators to conclude that higher HDL levels do not prevent CVD. We believe the correct interpretation is that HDL-C levels do not necessarily reflect its antiatherosclerotic effects in either humans or animal models and that HDL-C is not the correct target for intervention. Therefore, we need to understand which forms of HDL are cardioprotective and how they function in the artery wall.

HDL has potent anti-inflammatory effects in vivo

Recent studies indicate that HDL is anti-inflammatory in vivo,²⁵⁵⁻²⁵⁸ and this property may contribute significantly to its ability to inhibit atherosclerosis. Indeed, many lines of evidence support the proposal that atherosclerosis is a chronic inflammatory disorder.²⁵⁹ For

example, macrophage accumulation in the arterial intima and increased markers of systemic inflammation are hallmarks of CVD.

Proteins proposed to have anti-inflammatory properties, such as paraoxonase 1 and clusterin, are cotransported with HDL in plasma.^{255,258} Importantly, levels of those proteins are markedly altered in humans with CVD and in mice that are susceptible to atherosclerosis.^{258,260–262} Loss of anti-inflammatory proteins, perhaps in concert with gain of proinflammatory proteins, may thus be other key steps to HDL dysfunction.

Serum HDL mobilizes cholesterol from macrophages

Cell-based assays have provided key insights into many aspects of lipoprotein metabolism. With the use of that approach, de la Llera-Moya et al¹⁵⁷ have shown that the ability of human serum HDL to promote sterol efflux from cultured macrophages varies markedly, despite similar levels of HDL-C and apoA-I. Thus, HDL-C level is not the main determinant of macrophage sterol efflux in that system. Those investigators also demonstrated that the efflux capacity of serum HDL associated strongly and negatively with CVD status in 2 independent human populations.¹⁵⁸ That association was also independent of HDL-C and apoA-I levels. Differences in efflux capacity of serum HDL correlated with efflux by the ABCA1 pathway in macrophages.

Taken together, these observations suggest that the capacity of the serum HDL particle to promote sterol efflux from macrophages reflects its functionality, raising the possibility that efflux assays could provide insights into HDL biology and CVD risk. If robustly linked to CVD status, quantifying HDL-mediated sterol efflux from macrophages could prove useful in mechanistic studies, assessment of CVD risk, and evaluation of therapeutic interventions. However, cell-based assays are technically demanding and therefore unlikely to be widely applicable to clinical studies. It is therefore critical to develop quantitative, high-throughput assays that assess HDL function and can show independence from HDL-C levels.

Dysfunctional HDL conclusions

How should dysfunctional HDL be assessed, given that investigators have used many different definitions?^{262–264} We believe those differences arise because multiple pathways impair HDL's functions in vivo. For example, systemic inflammation may alter HDL's sterol efflux capacity by modulating its content of serum amyloid A, an acute-phase protein.²⁶⁵ Moreover, many lines of evidence indicate that oxidized lipids in HDL promote monocyte adhesion to endothelium, a key early step in atherogenesis.²⁶² In addition, several studies have reported a strong association between damage by myeloperoxidase

to apoA-I, HDL's major protein, and impaired sterol efflux by the ABCA1 pathway.²⁶⁴ Another important factor may be the absolute concentration of HDL particles, which might be a better metric for HDL concentration than HDL-C.^{266,267} Thus, many observations suggest that both local (artery wall) and systemic (inflammation, altered TG metabolism) pathways can generate dysfunctional HDL.

Despite intense interest, however, it is not yet clear whether the concept of dysfunctional HDL could improve clinical practice by better predicting risk or assessing new interventions that target HDL. It will therefore be critical to determine whether any metrics proposed for dysfunctional HDL provide clinically useful information in large and diverse populations.

HDL consensus summary of conclusions and recommendations

A. Epidemiology

1. Low serum levels of HDL-C have been found repeatedly to be the best predictor of CHD in observational studies, especially in men older than 50 years. After adjustment for established covariates, high levels of HDL-C in general correlate with low risk, whereas low levels correlate with higher risk of CHD. This is established from cohorts around the world and independent of race, ethnicity, and sex. However, most studies did not adjust for LDL particle concentration or apoB levels that may confound this association.
2. Data from observational cohorts are somewhat inconsistent. Extremely low HDL-C is not consistently associated with premature CHD development, and extremely high HDL-C is not consistently associated with atheroprotection in a reliable manner.
3. Epidemiologic inconsistency has also arisen among levels of HDL-C, CHD events, and monogenic abnormalities that result in extremely low (apoA-I^{Milano} and Paris, Tangier disease) or high HDL-C (CETP and endothelial lipase loss-of-function polymorphisms show little correlation, whereas loss-of-function variants in phospholipid transfer protein associate with increased HDL-C and reduced risk of CHD) and have not reliably demonstrated premature CHD or longevity, respectively.³
4. HDL-C levels are inversely related to weight, waist circumference, TGs, insulin resistance, systemic inflammatory tone, and cigarette smoking, all of which can confound the true relationship between HDL-C and risk of CHD. Isolated low HDL-C occurs in <1% of the population.
5. Given that the amount of cholesterol in an HDL particle is not likely to confer atheroprotection, serum

HDL-C levels (or the change in them) may not be the proper parameter to assess adequately the contribution of HDL to CHD risk.

B. Clinical trials

1. Evidence is robust from animal studies that use infusible apoA-I/HDL and viral hepatic transfection with apoA-I to suggest that HDL particles are antiatherogenic and can regress established atherosclerotic plaque.
2. Evidence is growing that HDLs can modulate inflammation, oxidation, endothelial function, insulin secretory capacity, and other processes that affect atherogenesis.
3. The hypothesis that HDL-C-targeted therapies in humans will reduce clinical CVD events has still not been substantiated. In fact, 4 recent human trials with HDL-C-targeted agents did not show a reduction in CV events.
4. The results of ILLUMINATE, dal-OUTCOMES, AIM-HIGH, and HPS2-THRIVE have placed the HDL cholesterol hypothesis in question. However, plausible reasons for why these trials failed suggest that the HDL hypothesis has still not been rigorously and conclusively tested.
5. Some of the agents used to raise HDL-C in clinical trials had adverse off-target effects that may have offset the potential benefits of the HDL raising.
6. A reduction in clinical CVD events may require a much greater increase in HDL-C/HDL particles than has been achieved in the trials with niacin and CETP inhibitors.
7. Increasing the level of HDL-C may be of little value when the concentration of LDL-C is very low, as was the case in each of the failed trials.
8. We need much more research to understand the reason for the unexpected results in these failed trials.
9. The potential for changing other components or functions of HDL with beneficial effects remains an important concept. Interventions that increase the concentration of HDL-C may not be accompanied by an increase in other protective properties of HDL particles.
10. The inverse relationship between the concentration of HDL-C and CV risk observed in population studies may represent an epiphenomenon rather than reflecting an ability of HDL to protect. This proposition is not, however, supported by the animal studies in which increasing HDL is demonstrably antiatherogenic.
11. Effects (regression, stabilization) on the development of atherosclerosis in animals (or humans with CSL 111, apoA-I_{Milano}, delipidated HDL) do not necessarily translate into effects on clinical events in humans. The relationship between plaque regression and risk of CV events is yet to be more fully defined.
12. In addition to ongoing trials with anacetrapib and evacetrapib, investigations are under way with other novel agents that will further probe the effect of HDL raising with the following:
 - a. HDL infusions
 - b. HDL mimetics
 - c. Newer CETP inhibitors
 - d. Liver X-receptor agonists
 - e. Farnesoid X-receptor agonists
 - f. RVX-208
 - g. Novel PPAR- α , - γ , - δ agonists
 - h. miRNA inhibitors

C. HDL particles and subclasses

1. A need exists to move beyond HDL-C as a surrogate for HDL particles, particle concentrations, and subfractions. HDL particles and subfractions should be more specifically defined in terms of size, density, charge, and composition. Such measures should be correlated with prospective risk of CVD, whether protective or detrimental.
2. HDL-C refers only to the cholesterol content of HDL particles. Targeting the cholesterol content of an HDL particle for elevation with drugs does not make much sense unless it reliably reflects RCT capacity, which is unlikely.
3. The cholesterol content of HDL does not represent many important HDL functions that are related to CVD risk. Specific examples include the relative cholesterol efflux from the macrophage mediated by ABCA1, as well as antioxidant, anti-inflammatory, antiapoptotic, anti-infective properties, and capacity to modulate insulin secretion.
4. A need exists to establish the most informative clinical measures of HDL particles/subfractions so as to
 - a. Improve CVD risk assessment,
 - b. Develop therapies that could influence the content of specific components of HDL that have atheroprotective properties,
 - c. More clearly establish the effect of specific therapies on HDL raising or functionality or both, and
 - d. Correlate effect of specific types of functional HDL particles or subfractions with risk reduction.
5. Standardize methods that measure specific features of HDL functionality. Validation will require prospective, observational studies and interventional studies with agents that change the functional features in question.
6. Conduct research to identify certain HDL subclasses with specific properties with the use of other analytical methods such as proteomics, lipidomics, and functional measures such as capacity for RCT.
7. HDL-C is a biomarker of CVD risk but not a target of therapy. Measures of HDL particles/subfractions may be more useful than HDL-C in
 - a. Assessing the effectiveness of CVD risk management and

- b. Being a more appropriate target of therapy.
 8. Levels of various HDL particles and subfractions are strongly correlated with serum TGs and concentrations of subclasses of atherogenic lipoproteins (chylomicron remnants, VLDL-C, IDL-C, LDL-C, and the number of LDL particles). Thus, the relationships between HDL-C and CVD risk must consider concentrations of the full spectrum of circulating lipoproteins.
 9. Future studies for management of dyslipidemia are needed to determine the predictive utility of HDL subclass concentrations, and the functional properties of individual HDL particle sizes and subfractions.
 10. Prospective, randomized studies should be designed to more precisely and quantitatively ascertain the effect of specific interventions on HDL functionality and role in risk modulation.
- D. HDL proteome and lipidome
1. The composition of HDL is much more complex than previously appreciated. The molecular cargo of HDL particles regulates functionality and can vary as a function of genetic and metabolic milieu.
 2. It is urgent to more fully identify and characterize the large number of proteins, enzymes, apoproteins, bioactive lipids, phospholipids, fatty acids, and miRNAs carried by HDL particles and how these affect functionality. Without this information, it will be difficult to fully understand how or why HDL is protective or injurious, depending on clinical circumstances.
 3. Predicted functions from known protein constituents imply that HDL plays roles in lipid transport and exchange, inflammation, innate immunity, hemostasis, extracellular matrix remodeling, complement, insulin secretion, metal ion transport, and modulation of endothelial function, among many others.
 4. The HDL lipidome is potentially even more complicated, and the presence of low abundance and potentially bioactive (ie, signaling, antioxidant or pro-oxidant, anti-inflammatory or proinflammatory) lipids may have important functional or pathologic significance.
 5. HDL also carries miRNA cargos that are distinct from typical microparticle cargos, and these *may* be important mediators of HDL signaling and vascular cell function.
 6. HDL is a phospholipid-based platform for the extracellular assembly of proteins and lipids to form particles that perform distinct and diverse functions. Many of these functions may be important for CAD but for other disease states as well.
 7. It is important to quantitatively characterize differences in HDL's proteome and lipidome in clinically specific and discrete human populations (CAD, diabetes, other chronic inflammatory states) and how these affect function and risk of CHD.
 8. It is critical to identify and characterize specific HDL particles (both from a protein and lipid standpoint) that may be cardioprotective. This may involve methods such as physical isolation, tandem affinity purification, immuno-coprecipitation, chemical cross-linking, coseparation, and bioinformatic analyses.
 9. Identifying which proteins and lipids colocalize in discrete populations of HDLs and establishing whether they can be therapeutically manipulated are issues of high priority for further investigation.
 10. HDL is not just a singular molecular entity. Its functions are not entirely reflected by HDL-C or apoA-I levels, 2 of the most commonly used HDL surrogates to characterize risk.
 11. With more understanding, it may become possible to do the following:
 - a. Identify and pharmacologically raise subpopulations of HDL that have desirable atheroprotective effects; such treatments may or may not raise HDL-C.
 - b. Produce clinical assays that measure HDL or its components that better identify persons who are at heightened risk of atherosclerotic vascular disease.
- E. Dysfunctional HDL
1. Animal models have provided strong evidence that HDL can become dysfunctional and lose its cardioprotective actions. However, it remains unclear under what circumstances dysfunctional HDL is relevant to humans.
 2. The molecular basis for generating dysfunctional HDL is poorly understood in both animal models and humans.
 3. It is critical to develop standardized, high-throughput assays that can assess HDL function and be applied in human studies. Such assays—when applied in large and diverse populations—should determine whether new HDL metrics related to loss of function or dysfunction provide clinically useful information.
- F. Extant questions
1. Why have recent clinical outcomes trials shown no benefit?
 - a. Baseline HDL-C levels not low enough
 - b. Use of concomitant therapies
 - c. Some agents not potent enough
 - d. Unanticipated off-target toxicities
 - e. Choice of combination therapies (eg, niacin or fibrates in combination with a statin)
 2. How can we reconcile the positive outcomes with surrogate measures and the absence of benefit in clinical outcomes trials (eg, niacin)?
 3. Are surrogate end points (eg, quantitative coronary angiography, cIMT, and IVUS) informative measures of atherosclerosis stabilization or regression?
 4. What is the effect of studying different patient populations on results?
 5. What are the effects of HDL-raising therapies in patients with low HDL-C or high TGs?

6. Why have numerous post hoc subgroup analyses shown significant clinical outcomes benefit in patients with low HDL-C or high TGs?
 7. To what extent does the fact that all CETP inhibitor trials to date have taken all comers, without specific HDL-C entry criteria, contribute to their lack of observed benefit?
 8. Do we need to address the role of LDL-C, VLDL-C, IDL-C, remnant particles, apoB, and Lp(a) reduction and other lipoprotein effects of CETP inhibitors and niacin?
 9. Despite the use of aggressive secondary prevention interventions and guideline-directed medical therapy, Why does residual CHD risk remain appreciable?
 10. Should HDL particles or specific measures of HDL function be used as inclusion criteria in future studies?
 11. What is the most appropriate assay for HDL functionality? Rates of RCT, inhibition of oxidation or inflammation, etc?
 12. What will the outcomes of ongoing or planned clinical outcomes studies of novel HDL-targeted therapies suggest?
 13. Should more extensive study of novel agents be performed with the use of intermediate end points before launching large-scale outcomes studies?
 14. To what extent does the occurrence of a recent ACS alter the relationship between HDL/HDL-C levels/functionality and outcomes?
- G. Clinical recommendations
1. Serum HDL-C is an extremely important biomarker of risk for CV events and is appropriately incorporated into quantitative CV risk models.
 2. Currently, evidence from clinical trials is insufficient to recommend HDL-targeted therapy. No evidence supports raising HDL-C levels to some arbitrarily defined HDL-C threshold (ie, >40 mg/dL in men or >50 mg/dL in women).
 3. No new guideline for the management of dyslipidemia will be recommending pharmacologic intervention for low HDL-C, given the absence of positive data from randomized, prospective studies.
 4. In patients with established CHD who are able to achieve and maintain optimal levels of LDL-C and non-HDL-C on statins, current data do not support additional clinical benefit with additional lipid-altering agents.
 5. Patients who are unable to achieve their LDL-C and non-HDL-C goals on a statin should continue to be considered for combination therapy. The adjuvant therapy should be used with the goal of lowering LDL-C and non-HDL-C to risk-stratified target levels.
 6. For patients with metabolic syndrome or insulin resistance, the likely best approach to raising HDL-C is lifestyle modification (dietary modification, weight

loss, exercise, and smoking cessation), as set forth by the National Cholesterol Education Program ATP III.

7. The results of clinical trials should not be extended to patient populations not represented by the study population.

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