High-density lipoprotein (HDL) cholesterol is a robust biomarker of atherosclerotic cardiovascular disease (CVD) events. In observational studies and many clinical trials of statin therapy, HDL cholesterol levels are inversely associated with CVD events. In the Emerging Risk Factors Collaboration that included 302,430 participants enrolled in 68 long-term prospective studies, for every 15 mg/dL increase in HDL cholesterol, the hazard ratio for coronary heart disease (CHD) events was 0.71 (95% confidence interval: 0.68–0.75) in models that were stratified by sex and trial group and further adjusted for age, systolic blood pressure, smoking status, history of diabetes mellitus, and body mass index.1 These associations remained significant in models that included triglycerides and non-HDL cholesterol.

Low levels of HDL cholesterol are inversely associated with apolipoprotein B–containing lipoproteins; thus, HDL cholesterol has been considered no more than a biomarker for atherogenic lipoproteins. Several studies have investigated the association between HDL cholesterol, atherogenic lipoproteins, and CVD.2–4 In multivariate models that include apolipoprotein B or low-density lipoprotein (LDL) particle concentration, HDL cholesterol was not associated with increased cardiovascular risk. In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) that included study participants with low levels of HDL cholesterol (men <45 mg/dL and women <47 mg/dL) and nonelevated triglycerides (<400 mg/dL), the risk of future CHD events was linearly associated with baseline and 6-month levels of apolipoprotein B but not with HDL cholesterol.4 These analyses indicate that low levels of HDL cholesterol are not associated with CVD events after adjustment for atherogenic lipoprotein concentrations.5

Clinical trials of high-intensity statin therapy have not confirmed the prognostic importance of HDL cholesterol in the prediction of CVD events. In the Treating to New Targets (TNT) trial, HDL cholesterol was not associated with future...
CVD events in patients randomized to treatment with atorvastatin 80 mg daily. In the dalcetrapib (dal)-Outcomes trial, HDL cholesterol levels were unrelated to the future risk of CVD events in subjects with prior acute coronary syndrome (ACS) who were randomized to either placebo or dalcetrapib therapy on the background of high-intensity statin therapy. Similarly, in a high-risk primary prevention trial, HDL cholesterol was not associated with future risk of CVD events in participants treated with rosuvastatin 20 mg daily. The lack of association between HDL cholesterol and CVD events in patients treated with high-intensity statins is consistent with observational studies that statistically adjust for atherogenic lipoprotein concentration.

Genetic Epidemiology: HDL Cholesterol
Genetic epidemiology does not support a causal relationship between HDL cholesterol and future risk of myocardial infarction. In a Mendelian randomization study that included 20 studies (12,482 myocardial infarction cases and 95,407 controls), single nucleotide polymorphisms in the endothelial lipase gene (LIPG Asn396Ser) had higher HDL cholesterol levels and similar nonlipid and other lipid risk factors for myocardial infarction. Although classic epidemiological studies predicted that a 1 standard deviation increase in HDL cholesterol would be associated with a reduced risk of myocardial infarction (odds ratio 0.62 [95% confidence interval: 0.58–0.62]), a 1 standard deviation in HDL cholesterol in carriers of the LIPG 396Ser allele was not associated with risk for myocardial infarction (odds ratio 0.93 [0.68–1.26; P=0.63]). This Mendelian randomization study provided important data concerning the lack of involvement of the cholesterol content in HDL particles in the causal pathway for CVD events.

Clinical Trials, HDL Cholesterol, and Concomitant Use of High-Intensity Statins
Clinical trials with pharmacological therapies that increase the cholesterol content of HDL particles have failed to establish this convenient metric as an effective strategy for the prevention of CVD events. These therapies have included niacin/niacin-lariporprant and cholesteryl ester transfer protein (CETP) inhibitors (Table 1). Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) and The Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trials investigated the effect of niacin and niacin/lariporprant, respectively, in CHD patients with LDL cholesterol levels at the recommended target <70 mg/dL. Elevations in HDL cholesterol were not associated with fewer CVD events. Instead, there was harm from niacin treatment as shown by the increased risk of infections. CETP inhibition with multiple agents (torcetrapib, dalcetrapib, and anacetrapib) has failed to reduce CVD events in high-risk patients treated with high-intensity statin therapy. The failure of torcetrapib was ascribed to off-target toxicity from renin–angiotensin–aldosterone activation and weak activity of dalcetrapib. Early termination of the evacetrapib clinical trial Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes (ACCELERATE) was unexpected, not only based on the LDL cholesterol–lowering efficacy of evacetrapib, but also in the increase in very small HDL particles (pre-β1 HDL) and macrophage cholesterol efflux capacity. Based on the event rates observed in statin-treated patients, early termination of ACCELERATE may not have allowed sufficient time to detect a treatment difference in CVD events.

A common theme in these failed HDL cholesterol trials is the background evidence–based use of high-intensity statin therapy. Statins increase expression of miR-33 and result in inhibited ATP-binding cassette transporter (ABCA1) expression. In J774 cells, incubation with statins, particularly simvastatin, impairs total cholesterol efflux capacity and ABCA1-specific efflux in a dose-dependent manner. However, differences in total cholesterol efflux capacity and ABCA1-specific efflux capacity between near equipotent doses of other statins have been inconsistent in 2 published reports. These data suggest that high-intensity statin therapy may counteract the effect of HDL therapies through miR33-induced downregulation of ABCA1-mediated cholesterol efflux and provide one potential mechanism for the lack of efficacy of HDL-raising therapies in clinical trials in which all participants received statin therapy.

Solving the HDL Puzzle: A Look Beyond HDL Cholesterol
HDLs comprise a multitude of discrete subpopulations that differ in composition, metabolism, cellular interactions, and functional properties. Expansion of the core cholesterol in HDL particles interferes with apoA-I-mediated cholesterol efflux via ABCA1 and alters the proteome and lipidome, resulting in species that are less effective in antioxidant and anti-inflammatory properties. Of the HDL functional assays, only macrophage cholesterol efflux has been validated in prospective studies.

Classic Epidemiology and HDL Particles
Small HDL particle concentration is associated with lower risk of recurrent cardiovascular events in men with a prior myocardial infarction, whereas large HDL particle concentration was associated with higher cardiovascular risk in statistical models that adjusted for LDL particle concentration. Several prospective studies and clinical trials of lipid-modifying therapies...
show that total HDL particle concentration is a more robust biomarker of cardiovascular risk than HDL cholesterol.25–27

Under some circumstances, HDL particles or free apoA-I in the arterial wall becomes dysfunctional.24 Among acute myocardial infarction patients with ST-segment elevations and serological evidence of an acute-phase response (elevations in C-reactive protein, serum amyloid A, and interleukin-6), small HDL (HDL3b) and very small HDL particles (HDL3c) exhibited reduced antioxidative activity (maximum −68%; P < 0.05, on a unit HDL mass basis) and diminished cholesterol efflux capacity from THP-1 cells (maximum −32%, P < 0.01, on a unit phospholipid mass basis).28 HDL subpopulations in ST-segment-elevation myocardial infarction patients were enriched in lysophosphatidylcholine (≤3.0-fold; P < 0.05) and phosphatidic acid (≤8.4-fold; P < 0.05). Enrichment of proinflammatory bioactive lipids was greater in HDL3c than in HDL3b fractions. In addition to these diminished salutary HDL functions in ACS, myeloperoxidase mediates site-specific modifications in both circulating and arterial apoA-I that diminish ABCA1-mediated efflux and increase vascular inflammation.29 In ACS patients, selective myeloperoxidase modifications in apoA-I (oxidized-tryptophan32, 3-chlorotyrosine, 3-nitrotyrosine32, oxidized methionine38) were associated with higher CHD event rates.29,30

Genetic Epidemiology: HDL Particles

Evidence supports a role for 2 HDL-associated proteins (phospholipid transfer protein [PLTP] and paraoxonase [PON]) and cardiovascular risk. HDL-associated PON1 and PON3 are calcium-dependent HDL-associated enzymes that impede the peroxidation of LDL particles; thus, loss of these enzymes decreases the anti-oxidative/anti-inflammatory capacity of HDL.

Genetic association between small HDL particles, total HDL particles, and CVD events has been limited to a gene score based on PLTP polymorphisms. In an analysis of 5 case–control studies of cardiovascular events, 2 variants (rs378114 and rs6065904) showed reproducible associations with lower hepatic PLTP transcription and plasma activity, increased number of small HDL particles, and lower CVD risk.33 The odds ratio for the highest versus lowest gene score was 0.69 (95% confidence interval: 0.55–0.86; P = 1.0 × 10−3). In a case–control study of 1115 European ancestry partici-

Clinical Trials and HDL Particles

Therapeutic interventions that increase small HDL particles while having less effect on cholesterol loading of HDL particles have yielded more favorable results than therapies that overload HDL particles with cholesterol. The Veterans Administration High-Density Lipoprotein Intervention Trial (VA-HIT) investigated the effect of gemfibrozil therapy in CHD patients with low levels of HDL cholesterol. This trial was conducted before high-intensity statin therapy was the established mantra for secondary prevention of CVD events; thus, important lessons from this trial have been ignored. As compared with placebo treatment, the gemfibrozil group had 6% higher HDL cholesterol levels and 27% fewer CVD events.35 Changes in HDL cholesterol accounted for 6% of the risk reduction in the main trial. In a nested case–control study that included 364 men with a new CHD event (non-fatal myocardial infarction or cardiac death) and 697 age-matched controls, gemfibrozil-treated participants had higher concentrations of small, medium, and total HDL particle numbers.4 On-trial increases in small HDL particle numbers were predictive of 33% lower multivariate-adjusted risk (odds ratio 0.67 [0.57–0.79] per 1 standard deviation increase in small HDL particle number) of CHD events. In a model that included total HDL and LDL particle number, the risk of CHD events was 2.4-fold higher (upper versus lower quartile). From this analysis, increased flux of very small HDL particles may represent a more effective strategy for HDL therapies. Imaging studies with other therapies that increase very small HDL particles support the concept that expansion of the HDL pool through generation of very small HDL particles has a net benefit on atherosclerosis. Specific examples of therapies that increase flux through the reverse cholesterol transport pathway include infusions of apoA-1 and apoA-1 Milano and delipidation of HDL.36,37

Niacin increases levels of HDL cholesterol, but it has a marginal effect on HDL particles.38 Ex vivo macrophage cholesterol efflux from J774 was not improved with niacin in AIM-HIGH participants,39 and in another study, cholesterol efflux from THP-1 cells was marginally improved.40 In addition, niacin-treated participants enrolled in AIM-HIGH neither enhanced or diminished HDL antioxidant/anti-inflammatory capacity.

CETP inhibitors have variable efficacy on cholesterol efflux. Both torcetrapib and anacetrapib facilitate cholesterol efflux from cholesterol-loaded human THP-1 macrophages via ABCG1-mediated pathway,40,41 and torcetrapib increases hepatic uptake of cholesterol esters via SR-B1.42 Although dalcetrapib was initially reported to mediate macrophage cholesterol efflux from cells via ABCA1-mediated pathways,43 these results were not corroborated in a human
ACS study. The dal-ACUTE trial investigated the effects of dalcetrapib on markers of HDL function within 1 week after an ACS. After 4 weeks of treatment with dalcetrapib, total cholesterol efflux capacity increased by 9.5% compared with placebo primarily through an increase in non–ABCA1-mediated transport. In contrast, evacetrapib and TA-8995 increased very small HDL particles/pre-β1 HDL, and ABCA1-mediated efflux in dose-ranging studies of non-ACS patients. In A Multi-Centre, Randomised, Double-Blind, Placebo-Controlled, Parallel-Group Study of TA-8995 in Patients With Mild Dyslipidaemia, Alone and In Combination With Statin Therapy (TULIP), the very small HDL particles was highly correlated with ABCA1-mediated efflux.

Despite the improvement in cholesterol efflux, detailed analysis of the proteome and lipidome with CETP inhibitors has not been reported. Thus, it is possible that CETP inhibitors create particles with less antioxidant/anti-inflammatory properties and perhaps dysfunctional in certain atheroprotective properties.

Solving the Puzzle
Efforts to solve the HDL puzzle require high throughput assays that accurately quantify discrete HDL species based on their protein and lipid components (Figure). HDL speciation may allow for the identification of specific HDL populations with functional or dysfunctional properties. The macrophage cholesterol efflux assay is an established method that has been used to characterize both functional and dysfunctional HDL. Subsequent steps require evaluation of discrete HDL populations in the prediction of atherosclerosis and cardiovascular events. The independent contribution of discrete HDL populations with functional and dysfunctional properties should be incorporated in Mendelian randomization studies. After these steps have been satisfied, therapies can be developed with more confidence than the prior era, which has emphasized cholesterol loading of HDL particles.

Conclusions
HDLs comprise a multitude of subpopulations that differ in composition, metabolism, cellular interactions, and functional properties. Despite established heterogeneity of HDL particles, the cholesterol transported in the HDL fraction is the established metric used to describe these highly complex lipoproteins. However, the emphasis on HDL cholesterol as a therapeutic target has resulted in multiple failures. Unraveling the HDL puzzle requires more sophisticated approaches in characterization of HDL subclasses and associated functional properties. Evaluation of the association between specific HDL subpopulations and CVD events in Mendelian randomization studies represents an important step before evaluation in clinical trials of HDL-modifying therapies.

Disclosures
Dr Rosenson was in the Advisory Boards/Consulting of Akcea, Amgen, Astra Zeneca, Eli Lilly, GSK, Regeneron, Sanofi. The author had honoraria from Kowa and research grants to institution Amgen, Astra Zeneca, Catabasis, Sanofi.

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**Significance**

High density lipoproteins (HDLs) are conventionally described by their cholesterol carrying capacity. Although HDL cholesterol concentration is convenient analytic measure used to estimate the low density lipoprotein (LDL) cholesterol concentration, it is not in the causal pathway for coronary heart disease and it is unrelated to the multifarious atheroprotective functions. Advances in HDL therapeutics require strategies that emphasize improving or restoring HDL function rather than simply increasing its cholesterol cargo.
The High-Density Lipoprotein Puzzle: Why Classic Epidemiology, Genetic Epidemiology, and Clinical Trials Conflict?
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Arterioscler Thromb Vasc Biol. 2016;36:777-782; originally published online March 10, 2016; doi: 10.1161/ATVBAHA.116.307024

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