Commentaries on Cutting Edge Science

Cholesterol Efflux Capacity as a Novel Biomarker for Incident Cardiovascular Events
Has High-Density Lipoprotein Been Resuscitated?

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HDL cholesterol efflux capacity and incident cardiovascular events
Rohatgi et al

Although low levels of high-density lipoprotein cholesterol (HDL-C) represent a strong independent risk marker inversely associated with cardiovascular disease (CVD), large interventional trials have failed to translate the increases in HDL-C into reductions in cardiovascular events. As HDL plasma levels represent a pool of the different HDL subfractions, which may vary in their structure and function, its levels may not capture adequately the dynamic process of reverse cholesterol transport. This has led attention to be focused on markers of HDL function, for example, cholesterol efflux capacity, further supported by a recent population-based cohort study that has related it with incident atherosclerotic cardiovascular events, suggesting HDL hypothesis may need to be revisited.

HDL particles are responsible for the reverse cholesterol transport, the physiological mechanism by which the cholesterol in peripheral tissues and cells is transferred to the liver for biliary excretion.\(^{1,2}\) Reverse cholesterol transport is considered an important atheroprotective mechanism because it facilitates the removal of excess cholesterol from lipid-laden macrophages in the arterial wall and the subsequent reduction in the proinflammatory responses; in its first steps, the reverse cholesterol transport requires HDL particles to act as extracellular acceptors of cholesterol from these cells.\(^{1,2}\) The cholesterol efflux capacity (CEC), that is, the ability of HDL particles to accept cholesterol esters from cholesterol-loaded macrophages, represents, therefore, a key process within the mechanism of reverse cholesterol transport.\(^{1,2}\) In animal models, cholesterol efflux can modulate the severity of atherosclerosis, and in humans, CEC has been related to prevalent coronary artery disease independently of HDL-C levels in cross-sectional studies.\(^{3}\)

In a recent article published by Rohatgi et al in the *New England Journal of Medicine*, CEC was for the first time reported to be inversely associated with incident atherosclerotic cardiovascular events in a multiethnic population-based cohort.\(^{4}\) These authors studied over 2900 individuals free from CVD at baseline from the Dallas Heart Study. Participants were 30 to 65 year of age, 43% males, 49% blacks, and had a median low-density lipoprotein cholesterol and HDL-C at baseline of 104 and 47 mg/dL, respectively. Interestingly, in contrast to HDL-C levels, which were associated with multiple traditional cardiovascular risk factors or metabolic variables and other lipids, the CEC was only weakly or not correlated with these parameters; in fact, traditional risk factors, together with exercise activity and alcohol intake, explained only 3% of the variance in CEC (versus 35% of the variance in HDL-C levels). In the long-term follow-up (n=2416 participants, 9.4 years median follow-up), baseline HDL-C levels were not associated with cardiovascular events (adjusted hazard ratio [HR] 1.08, 95% confidence interval [CI] 0.59–1.99). In contrast, increasing quartiles of CEC were inversely associated with the primary end point of atherosclerotic CVD (defined as a first nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or death from cardiovascular causes) or the secondary outcome of total CVD (atherosclerotic CVD plus peripheral revascularization and hospitalization for heart failure or atrial fibrillation). In a fully adjusted model (including traditional risk factors, HDL-C level, and HDL particle concentration), the highest CEC quartile, compared with the lowest quartile, was related to a 67% lower risk of atherosclerotic CVD (HR 0.33, 95% CI 0.19–0.55) and a 58% lower risk of total CVD (HR 0.42, 95% CI 0.27–0.65). These associations remained when CEC was modeled as a continuous variable (per 1-SD increase in CEC levels, atherosclerotic CVD: HR 0.68, 95% CI 0.55–0.84; total CVD: HR 0.79, 95% CI 0.67–0.94) and were consistent in subgroup analyses stratified by baseline cardiovascular risk. Finally, risk prediction for atherosclerotic CVD events (discrimination and reclassification indexes) were improved when CEC was added to traditional risk factors (C-statistic: from 0.827 to 0.841, \(P=0.02\); net reclassification index: 0.37, 95% CI 0.18–0.56).\(^{4}\)

Low levels of HDL-C represent a strong independent risk marker inversely associated with CVD,\(^{3}\) and its levels remain a predictor of cardiovascular events even in patients treated with statins and among those with low levels of low-density lipoprotein cholesterol (ie, below 70 mg/dL). Despite these observations, it remains unclear whether these associations are causal. In fact, Mendelian randomization analyses of genetic
mechanisms that result in higher levels suggest that HDL-C raising per se may not uniformly result in reductions of CVD risk. Furthermore, trials involving pharmacological interventions which increase plasma HDL-C levels (i.e., niacin or cholesteryl ester transfer protein [CETP] inhibitors [CETPi]) have failed to translate this into CVD reduction (e.g., AIM-HIGH [niacin], HPS2-THRIVE [niacin], ILLUMINATE [torcetrapib], or DAL-OUTCOMES [dalcetrapib]). Added now to the controversy is the lack of association between HDL-C levels and cardiovascular events observed by Rohatgi et al, what might suggest that plasma HDL-C levels could vary in its accuracy as a CVD risk marker depending on the population studied. HDL plasma levels represent a pool of the different HDL subfractions, which vary in structure and size, protein and lipid composition and, more importantly, may vary in their function. HDL-C levels do not capture adequately the dynamic process of reverse cholesterol transport, and furthermore, only a small percentage of the cholesterol transported by the HDL particles are derived from peripheral tissues, for example, lipid-laden macrophages in the arterial wall. Notwithstanding the evidence of the inverse association of plasma HDL-C levels with CVD events, all the aforementioned issues has led to an increasing attention paid, not only to HDL-C levels, but also to different biomarkers which may reflect HDL function or metabolism as well, as an attempt to better assess the role of HDL in cardiovascular risk. In this respect, the association between CEC and incident cardiovascular events found by Rohatgi et al supports an important role for HDL function and reverse cholesterol transport mechanisms in cardiovascular risk, beyond HDL-C levels, HDL particle concentration, or traditional risk factors.

Cholesterol efflux from peripheral tissues to HDL particles is a complex process that depends on the cholesterol status of the cells and the concentration and composition of the extracellular acceptors (apolipoprotein-A1, HDL subfractions, or apolipoprotein-E-related HDL), as well as the expression of membrane cholesterol transporters. Cholesterol efflux from macrophages can take place by passive diffusion or be facilitated by the adenosine triphosphate–binding cassette transporters A1 (ABCA1) and G1 (ABCG1), the scavenger receptor class B type I (SR-BI), or the endogenous production of lipid-poor apolipoprotein-E. These pathways use different HDL subfractions as acceptors. ABCA1 constitutes an important pathway in cholesterol-loaded macrophages that promotes cholesterol efflux to lipid-poor apoA1 (pre-beta or very small HDL particles), initiating the formation of larger HDL particles; subsequently, ABCG1 interacts with nascent and mature HDL particles (small, medium, large, and very large HDL particles). Both ABC transporters act in a coordinated fashion and have additive contributions to cholesterol efflux; however, in animal studies, while ABCA1 deficiency leads to an increase in atherosclerosis, ABCG1 deficiency results are more conflicting, though the combined deficiency produces accelerated atherosclerosis, and the role of macrophage SR-BI in CEC and atherosclerosis remains uncertain.

The data from the Rohatgi et al study suggest that promoting cholesterol efflux from cholesterol-loaded macrophages may be a therapeutic strategy that could effectively reduce the risk of atherosclerotic CVD in the long-term. However, whether the observational association between lower CEC and incident CVD can be translated into a reduction in cardiovascular events by pharmacologically promoting cholesterol efflux is unclear. The CETPi drugs substantially increase the HDL-C levels by reducing the transfer of cholesterol esters from HDL particles to apoB-containing lipoproteins in exchange for triglycerides. Thus, higher HDL levels are mainly caused by the increase in the larger type-2 HDL fractions which, in turn, have been described as promoting ABCG1- and SR-BI-dependent cholesterol efflux. In fact, the CETPi torcetrapib and dalcetrapib have been found to increase CEC to varying degrees, principally via non-ABCA1 pathways. Yvan-Charvet et al, in hypercholesterolemic individuals free from CVD, observed that torcetrapib at doses of 60 mg daily (the dose used in the ILLUMINATE trial) only modestly increased CEC after the rise in HDL-C levels; however, torcetrapib 120 mg/d increased the cholesterol efflux more significantly and beyond that expected by the rise in HDL-C levels (therefore, suggesting an increase in the function of HDL particles), principally via ABCG1 pathways. In the dal-ACUTE trial, in postacute coronary syndrome participants, dalcetrapib 600 mg/d increased total cholesterol efflux by 9.5%, via non-ABCA1 pathways; pre-beta-1 HDL particles (the rate-limiting acceptor of cholesterol efflux by ABCA1 pathway) did not significantly increase; and accordingly, changes in ABCA1-specific efflux were not significant. Despite the fact that CETPi seems to generate functional HDL particles and the described increase in CEC, the ILLUMINATE trial (with >80% of participants having CVD) demonstrated an increased morbidity and mortality with torcetrapib, and the dal-OUTCOMES trial with dalcetrapib in patients with a recent acute coronary syndrome was stopped prematurely for clinical futility (though it was shown to be safe). The apparent disconnect between the observational data reported by Rohatgi et al and those of therapeutic interventions, which despite increasing CEC have failed to demonstrate reductions in CVD, may be explained by distinct factors, such as (1) study design; (2) background therapy: for example, statins may decrease CEC or prebeta-1 HDL particles; (3) the doses used in trials: for example, the improvement in CEC achieved with torcetrapib or dalcetrapib in trials could be insufficient to restore impaired CEC in subjects with established CVD; (4) the effects of CETPi on other lipid parameters, such as the increase in apolipoprotein-A or HDL-related ApoE (which can alter the function of the different HDL subfractions); (5) the differing cholesterol efflux pathways: for example, torcetrapib and dalcetrapib mainly increase CEC via non-ABCA1 pathways, whereas the association between CEC and CVD reported by Rohatgi et al primarily evaluate ABCA1-mediated CEC; (6) the differences in the populations: for example, whilst in ILLUMINATE and dal-OUTCOMES all or almost all patients had CVD at baseline, participants in the Rohatgi et al study were free from CVD, relatively young (median age 42 years) and the number of subjects with reduced HDL-C was relatively low (46% of women and 35% of men [HDL-C <50 or <40 mg/dL, respectively]); therefore, deleterious mechanisms that may impair CEC in patients with CVD are likely not present in the healthier Rohatgi et al study population, and this may influence the ability of CEC to predict risk in the different populations. For instance, in patients with established CVD, oxidative damage of apoA1 (induced by
myeloperoxidase) has been described, resulting in the impairment of apoA1 to promote ABCA1-mediated cholesterol efflux, as well as inhibiting activation of lecinthin cholesterol acyltransferase enzyme and thus reducing HDL maturation, and (7) off-target effects (torcetrapib).

The CETPs anacetrapib and evacetrapib, currently being evaluated, as well as the new compound TA-8995, have been reported to produce larger increments in HDL-C levels and may promote cholesterol efflux to a greater degree than earlier drugs tested, as a result of more potent CETP inhibition. In this respect, anacetrapib is reported to increase CEC up to 2.4-fold at the dose of 300 mg daily, and evacetrapib to increase CEC by 21% (combined with statins) or 28% (monotherapy), as well as a 20% to 32% increase in pre-beta-1 HDL levels.

Both anacetrapib and evacetrapib have been found to also increase ABCA1-mediated efflux in addition to the non-ABCA1 pathways, which may be a consequence of the potency of CETP inhibition and may have clinical importance. Large trials to evaluate CVD outcomes with anacetrapib (REVEAL trial, with 30,000 participants and results expected in 2017) and evacetrapib (ACCELERATE trial, with 12,000 individuals and expected in 2016) are currently ongoing; these studies, which have not been stopped for futility or safety, could shed light on questions still unresolved about CEC.

The interpretation of the Rohatgi et al findings must take into account the population from which the data are derived. On the one hand, the overall low cardiovascular risk of participants and relatively normal HDL-C levels in a high percentage of subjects do not allow us to extrapolate the data to subjects with higher cardiovascular risk, established CVD, or with abnormal (higher or lower) HDL-C levels. Furthermore, the ethnic distribution of this cohort does not reflect the general population distribution. Finally, the lack of assessment of non-ABCA1-mediated cholesterol efflux pathways does not allow us to evaluate the importance of alternative mechanisms of cholesterol efflux or additional regulatory factors. Furthermore, we currently lack Mendelian randomization data supporting CEC as a causal factor in atherosclerosis rather than as a risk marker.

Despite these potential limitations, the data reported by Rohatgi et al provide an important step forward in our understanding of the role of HDL, or more precisely, the function of HDL, with respect to atherosclerotic risk. They suggest understanding of the role of HDL, or more precisely, the function of HDL, with respect to atherosclerotic risk. They suggest that at least with respect to HDL, the function of HDL rather than HDL-C levels per se may improve cardiovascular risk assessment. Validation of the results of Rohatgi et al in other cohorts and assessment of whether CEC is a causal factor in atherosclerosis may provide the much-needed resuscitation of the HDL hypothesis.

Disclosures K.K.R. reports to having received honoraria from Roche, Lilly and Cerenis for serving on the executive steering committee of trials assessing CETP inhibition and for attending advisory boards and giving lectures. A.J.V.V. has no conflicts to declare.

References