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Time to ditch HDL-C as a measure of HDL function?

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Abstract

Purpose of review—Epidemiological and clinical studies link low levels of HDL cholesterol (HDL-C) with increased risk of atherosclerotic cardiovascular disease (CVD). However, genetic polymorphisms linked to HDL-C do not associate consistently with CVD risk, and randomized clinical studies of drugs that elevate HDL-C via different mechanisms failed to reduce CVD risk in statin-treated patients with established CVD. New metrics that capture HDL's proposed cardioprotective effects are therefore urgently needed.

Recent findings—Recent studies demonstrate cholesterol efflux capacity (CEC) of serum HDL (serum depleted of cholesterol-rich atherogenic lipoproteins) is an independent and better predictor of incident and prevalent CVD risk than HDL-C. However, it remains unclear whether therapies that increase CEC are cardioprotective. Other key issues are the impact of HDL-targeted therapies on HDL particle size and concentration and the relationship of those changes to CEC and cardioprotection.

Summary—It is time to end the clinical focus on HDL-C and to understand how HDL's function, protein composition and size contribute to CVD risk. It will also be important to link variations in function and size to HDL-targeted therapies. Developing new metrics for quantifying HDL function, based on better understanding HDL metabolism and macrophage CEC, is critical for achieving these goals.

Keywords

HDL; cholesterol efflux capacity; cardiovascular risk; HDL proteomics; HDL particle concentration

Introduction

Clinical and epidemiological evidence has established a causal relationship between high levels of LDL cholesterol (LDL-C) and cardiovascular disease (CVD). Moreover, the success of therapeutic interventions that lower LDL-C has further confirmed that LDL-C is

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Conflicts of interest

J.W. Heinecke is named as a co-inventor on patents from the US Patent Office on the use of HDL markers to predict the risk of cardiovascular disease. Dr. Heinecke has served as a consultant for Kowa, Merck, Amgen, Bristol Meyer Squibb, GSK, and Pacific Biomarkers.

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in the causal pathway of CVD. Multiple clinical trials have proven that statin therapy reduces all-cause mortality for both women and men [1]. Recently, therapies with antibodies against proprotein convertase subtilisin–kexin type 9 (PCSK9), an enzyme that attaches and internalizes LDL receptors into lysosomes for degradation, were able to reduce LDL-C levels beyond those promoted by statins or any other lipid-lowering treatment [2, 3]. Initial data on the PCSK9 inhibitors suggest a reduction in cardiovascular events [2–4].

The success with LDL-C lowering drugs has not been shared with drugs designed to elevate HDL cholesterol (HDL-C), despite the strong inverse relationship between HDL-C levels and CVD, which has long been known [5]. Modulating HDL therapeutically has proved to be more challenging than first expected, possibly because of the complex dynamic of HDL particles.

Niacin, the first drug approved to treat dyslipidemia, decreases triglycerides and LDL-C and raises HDL-C levels. However, two independent clinical trials with niacin added to statin therapy failed to show clinical benefits [6, 7]. So far, clinical trials with inhibitors of cholesteryl ester transfer protein (CETP), which promotes the equilibration of cholesteryl esters and triglycerides in plasma lipoproteins, also have been disappointing. Three clinical trials with CETP inhibitors—torcetrapib, dalcetrapib, and evacetrapib, designed to specifically raise HDL cholesterol—were stopped due to increased mortality (torcetrapib) [8] or lack of CVD risk reduction (dalcetrapib [9] and evacetrapib [10]). It is noteworthy that evacetrapid also decreased LDL-C levels significantly [10]. The lack of benefit of lowering LDL-C raises the possibility that the CETP inhibitor was adversely affecting the cardioprotectve functions of HDL. Finally, the results of an ongoing clinical trial with anacetrapib (ClinicalTrials.gov Identifier: NCT01252953) may determine whether modulating plasma lipids with CETP inhibitors is beneficial.

Here, we review the challenges in therapeutically modulating HDL, and discuss recent outcomes in light of our current understanding of HDL metabolism and function.

Maturation of HDL

Several studies have reviewed HDL metabolism [11–13]. In short, when apoA-I, HDL's major protein, binds to adenosine-triphosphate-binding cassette transporter A1 (ABCA1), it acquires phospholipids and free cholesterol, giving rise to discoidal particles called pre-β HDL. This first step in HDL maturation is thought to be key to removing excess cholesterol from macrophages. The formation of spherical, mature HDL particles occurs in the bloodstream when lecithin cholesterol acyltransferase (LCAT) esterifies free cholesterol in pre-β HDL generating cholesteryl esters that then migrate to the core, and forming mature spherical HDL particles. Mature HDL can promote cholesterol efflux from cells via adenosine-triphosphate-binding cassette transporter G1 (ABCG1).

CETP replaces the cholesteryl esters in HDL with triglycerides taken from lipoproteins that contain apolipoprotein B (apoB) [14]. Therefore, pharmacological inhibition of CETP increases levels of large, cholesteryl ester-enriched HDL particles in the circulation.

Finally, cholesteryl esters and free cholesterol in HDL are taken up by the liver and excreted as bile acids. The uptake mechanisms for cholesterol are poorly understood, but one important pathway for cholesteryl esters involves the scavenger receptor B-1 (SR-B1) [15]**.

Cholesterol level is a poor biomarker for HDL complexity

Maturation produces diverse HDL particles that vary in size, density, and lipid and protein composition. ApoA-I and apolipoprotein II account for 90 % of HDL's protein content, but close to 100 other HDL proteins have been described as HDL associated [16]. Measuring HDL-C levels takes only the cholesterol mass into account, but cholesterol accounts for no more than 20% of a particular HDL particle, and its level may vary up to 10-fold, depending on a particle's size [17]. Thus, a confounder in interpreting HDL-C as a measure of circulating HDL concentration is the relative balance between large and small HDL particles [18]. HDL particle concentration, which provides information regarding the actual number of HDL particles in the circulation, can be measured by nuclear magnetic resonance (NMR) [19] or ion mobility analysis [20, 21]. Calibrated ion mobility analyses further demonstrate that precise measurements of small, medium, and large HDL particles can be obtained from plasma [21]. Therefore, evaluating changes in HDL subpopulations in response to therapy might be a better metric than HDL-C levels for predicting risk.

As well as varying in size, HDL particles vary in protein content, as HDL's proteome is very rich. Alterations in protein cargo have been associated with pathological states, such as inflammation [22], poor response to therapy [23], autoimmune disease [24], and diabetes [25]. Further improvements in quantitative techniques [26, 27] and their application to clinical studies should identify panels of protein biomarkers that may be pharmacologically modifiable to reduce risk.

Another important—though not unrelated—variable is HDL's ability to promote cholesterol efflux from cultured macrophages with samples derived from serum, termed cholesterol efflux capacity (CEC). CEC has been a stronger predictor of prevalent and incident coronary artery disease than HDL-C in multiple clinical trials [28–30]. *In vitro* assays of CEC of HDL may include four possible pathways: aqueous diffusion, SR-B1 receptor, and ABCA1 and ABCG1 transporters.

The physiological importance of ABCA1-driven efflux is evident in humans with Tangier disease, which is characterized by defective ABCA1 transport and, consequently, low HDL-C and early atherosclerosis onset [31]. Also, the ABCA1 transporter is induced by excess cellular cholesterol in atherosclerotic plaques [32]. Therefore, it is logical to assume that assays measuring CEC emphasize the ABCA1 pathway. However, other pathways are starting to emerge as important players in atherosclerotic disease. For example, the SR-B1 receptor is responsible for the selective uptake of HDL cholesteryl esters into cells [33], but it can also promote free cholesterol efflux to HDL [34]. A rare loss-of-function variant in the gene encoding the SR-B1 receptor raises HDL-C levels and the concentration of large HDL particles, but it also increases the risk of CVD [15]**. This observation stresses once more that HDL-C is a poor surrogate for functionality. It also highlights the importance of

considering all the steps in reverse cholesterol transport—not only cholesterol removal from peripheral tissues but also its delivery to the liver for elimination. The emerging role of the SR-B1 receptor is reviewed elsewhere [35].

A limitation of measuring cholesterol efflux capacity is that it does not address HDL's ability to deliver its cholesterol to the liver for excretion. Recently, a preclinical test used ^{3}H-cholesterol nanoparticles to selectively trace macrophage-specific reverse cholesterol transport in vivo in humans [36]*. More studies are needed to address the feasibility of using this approach in translational and clinical studies.

HDL-C–raising therapies, particle number, protein cargo and function

Therapies that increase the concentration of large HDL particles stimulate overall HDL efflux in a process that is mainly independent of the ABCA1 pathway. Thus, studies showed that niacin improved [37, 38] or did not change [39] HDL's overall efflux capacity without improving ABCA1-specific efflux [37, 38]. Importantly, the improvement in total efflux correlated with the increase in HDL-C [38]. Treatment of patients with the CETP inhibitors torcetrapib and dalcetrapib also elevated total efflux capacity but not ABCA1-specific efflux [40, 41]. A small study showed that anacetrapib increased cholesterol efflux capacity in a process mainly dependent on ABCG1 but also on the ABCA1 receptor [42]. In a different fashion, evacetrapib increased total and ABCA1-mediated cholesterol efflux capacity of serum HDL in dyslipidemic patients [43]. That increase was mirrored by increased plasma levels of pre-β_1 HDL, but the evacetrapib trial was stopped early due to lack of benefit [10]. Interestingly, infusion of the disk-shaped HDL mimetic CSL112 causes a dramatic rise in small HDL particles and ABCA1-efflux capacity [44]. Extensive remodeling of discoidal and spherical HDL particles was proposed as a mechanism [45]. The results of a clinical trial with CSL112 are awaited.

Unresolved issues in HDL metabolism

A key issue is the identities of the *in vivo* ligands for ABCA1, which promotes the first step in cholesterol excretion from macrophages. Poorly lipidated or lipid-free apoA-I is widely considered to be the major ligand, but this species is only a minor component of total apoA-I in blood. Indeed, poorly lipidated apoA-I levels are elevated in subjects with established CVD, suggesting that they are unlikely to be cardioprotective [46, 47].

A recent study found that small HDLs also promote cholesterol efflux from macrophages by the ABCA1 pathway [48]. Importantly, small HDLs carry much less cholesterol per particle, indicating that HDL-C levels do not necessarily provide useful information on the size and concentration of this HDL subspecies. It is noteworthy that niacin and CETP inhibitors decrease HDL catabolism, thereby increasing the size of the HDL particle and raising HDL-C levels. Little is known about the concentrations of the different sizes of HDL particles in patients treated with these agents.

These observations raise the possibility that the concentrations of the various HDL subspecies might provide another key metric of HDL's cardioprotective effects. However, there is no agreement on how to accurately quantify the size and concentration of HDL, and

two widely used methods—NMR and non-calibrated ion mobility—give substantially different concentration values [20, 49]. Moreover, neither method yields a value for the stoichiometry of apoA-I per HDL particle that is physiologically plausible [21].

We recently showed that the ion mobility method of Kraus and colleagues could be a quantitative metric [21] with external calibration, an approach we call “calibrated ion mobility analysis”. Multiple lines of evidence, including analysis of reconstituted HDL particles and gold nanoparticles, showed that calibrated ion mobility analysis accurately quantifies the size and concentration of nanoparticles. In a small study, the concentration of medium-sized HDL particles was selectively lower in subjects with advanced carotid atherosclerotic disease, independent of HDL-C levels [21]. Also, altered HDL particle concentration—but not HDL-C levels—predicts endothelial dysfunction as assessed by coronary angiography, supporting the notion that the size and concentration of HDL particles is an independent predictor of CVD risk [50].

Another promising approach focuses on quantifying the protein cargo of HDL. Pioneering proteomics studies demonstrate that HDL carries a rich cargo of proteins linked to inflammation, protease inhibition and complement regulation [51]. Moreover, aggressive lipid-lowering therapy favorably alters the protein composition and function of HDL of people with CVD [38, 52, 53] raising the possibility that quantifying the HDL proteome provides insights into the therapeutic efficacy of antiatherosclerotic interventions. In future studies, it will be important to use high-throughput methods [23, 26] to quantify the HDL proteome and to link specific proteins to CVD risk, HDL particle number and HDL function.

A complicating factor in understanding HDL function is the inverse relationship between HDL-C and triglycerides. It is currently not known if efflux metrics represent cardiovascular risk in dyslipidemic or diabetic patients. Thus, patients with high triglyceride levels had significantly higher concentrations and functionality of preβ-1 particles (as measured by ABCA1 efflux) than the controls [54].

Moreover, patients with low HDL-C generally have exacerbated postprandial hypertriglyceridemia. These patients may have more cholesterol efflux from macrophages, but also have a global reduction in reverse cholesterol transport efficacy because the ultimate return of cholesterol to the liver is reduced [55]. Niacin reduced ABCA1-dependent efflux after dyslipidemic patients ingested a typical western meal. However, HDL particles isolated from patients receiving niacin displayed an enhanced capacity to deliver cholesteryl esters to hepatic cells, improving overall reverse cholesterol transport [37]. Of note, a secondary analysis of a subset of dyslipidemic patients in the AIM-HIGH trial uncovered a significant reduction in cardiovascular risk [56].

Conclusion

Multiple clinical studies demonstrate that the cholesterol content of HDL particles is a poor surrogate for proposed atheroprotective functions. As we gain insights into the complex metabolism of HDL, however, new putative players for atheroprotection will emerge. Evidence points to the need to address all the steps involved in reverse cholesterol transport,

from HDL's capacity to remove cholesterol from peripheral tissues to its ability to deliver excess cholesterol for elimination by the liver. In this process, it will be critical to link variations in HDL's size, protein composition and function to HDL-targeted therapies and populations at high risk for CVD, such as diabetics.

It is interesting to point out that changing to a Mediterranean diet, which reduced cardiovascular events in one large randomized clinical trial [57], increased cholesterol efflux capacity without changing HDL-C levels [58]**. These observations support the proposal that an anti-atherogenic diet can alter HDL function without changing its cholesterol levels, perhaps by altering the size and distribution of its particles.

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Key points

- Cholesterol level is a poor surrogate for HDL atheroprotective effects.
- Understanding HDL metabolism and macrophage cholesterol efflux is key to address HDL atheroprotection.
- Alternative metrics of HDL functionality and composition, such as cholesterol efflux capacity, HDL protein cargo and HDL particle concentration should be used to evaluate HDL's therapeutic interventions, replacing HDL-C levels.