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EDITORIAL

Re-examining the high-density lipoprotein hypothesis

A low level of high-density lipoprotein cholesterol (HDL-C) is an important cardiovascular risk factor, and is commonly seen in patients with type 2 diabetes mellitus. An inverse relationship between HDL-C concentration and cardiovascular risk has been consistently shown in clinical and epidemiological studies. In the well-known Framingham Heart Study, HDL-C was found to be the best predictor of cardiovascular mortality. The recent Emerging Risk Factors Collaboration Study has also shown that the strong inverse association between the risk of coronary artery disease and HDL-C levels remained even after adjusting for other lipid and non-lipid risk factors. As a key independent cardiovascular risk factor, measurement of HDL-C has been incorporated into the majority of the risk stratification models for prediction of cardiovascular risk. It has been estimated that cardiovascular risk decreases by approximately 2–3% per 1-mg/dL increase in HDL-C. In light of the strong experimental and epidemiological data supporting a protective role for HDL in atherosclerosis, substantial research efforts have been directed at developing strategies to harness the 'atheroprotective' function of HDL, and raising HDL-C has become a potential therapeutic target over the past decade.

However, the 'HDL hypothesis' suggesting that HDL plays a causal role in the pathogenesis of atherosclerotic cardiovascular disease has recently been challenged by large-scale human genetic studies. The first genetic study to cast doubt on the causal role of HDL used a Mendelian randomization approach to determine whether single-nucleotide polymorphisms (SNPs) associated with increased levels of HDL-C were also linked to changes in the risk of coronary heart disease. Voight *et al.*[1] reported that SNPs associated with high levels of low-density lipoprotein cholesterol increased the risk of myocardial infarction, but surprisingly, no relationship could be found between SNPs that raised HDL-C and coronary heart disease risk. Furthermore, subsequent genome-wide association studies also failed to show correlations between the magnitude of the effect size of additional HDL-associated SNPs identified and the magnitude of the effect size on coronary heart disease. These analyses have therefore created skepticism about the causal role of HDL in atherosclerotic cardiovascular disease.

Another severe blow to the HDL hypothesis comes from the disappointing results of a number of clinical trials on agents raising HDL-C. So far, pharmacological interventions that increase HDL-C concentration have not shown improvement in cardiovascular outcomes when added to standard statin therapy[2]. Two large trials with the currently available major HDL-C raising agent, niacin – the Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: impact on Global Health outcomes (AIM-HIGH) trial and the Heart Protection Study 2 – Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) – failed to show significant cardiovascular benefit. This would suggest that raising HDL-C levels, at least with niacin, did not confer any additional clinical benefit on top of that achieved by statin therapy. Inhibitors of cholesteryl ester transfer protein (CETP), which are being developed specifically as HDL-C raising drugs, increase HDL-C to a much greater extent than niacin. Short-term clinical trials have demonstrated up to 60–70% increase in HDL-C with some of the more potent CETP inhibitors. However, torcetrapib, the first CETP inhibitor to enter clinical development, not only failed to reduce cardiovascular risk but actually increased cardiovascular and total mortality due to its off-target effects. The Dal-OUTCOMES study, a phase 3 placebo-controlled randomized controlled trial of dalcetrapib in patients with a recent acute coronary syndrome, was stopped early after no significant improvement in overall or cardiovascular related mortality and related events was found. It has just been announced that the development of another CETP inhibitor, evacetrapib, would be discontinued after independent data monitoring committee advised a halt to the phase 3 randomized trial in patients with high risk coronary artery disease [Assessment of clinical effects of cholesteryl ester transfer protein inhibition with evacetrapib in patients at a high-risk for vascular outcomes (ACCELERATE)] due to insufficient efficacy. This leaves the Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification (REVEAL) trial as the only ongoing phase 3 cardiovascular outcome trial of CETP inhibitor in patients with established vascular disease. The trial is expected to be completed in 2017 and the results will be critical in determining whether CETP inhibitor has a place in clinical care.

So is the 'HDL hypothesis' truly defunct? It should be pointed out that the HDL hypothesis has always been related to HDL function rather than to HDL-C content. Simply raising levels of HDL-C level cannot be directly inferred to as improving HDL function. Likewise, in human genetic studies, the extent to which the SNPs that are associated with increases in HDL-C are also linked to relevant changes in HDL function is uncertain. It is clear that the complex nature of HDL metabolism and function cannot be determined by just quantifying HDL-C alone. HDL particles are highly heterogeneous and

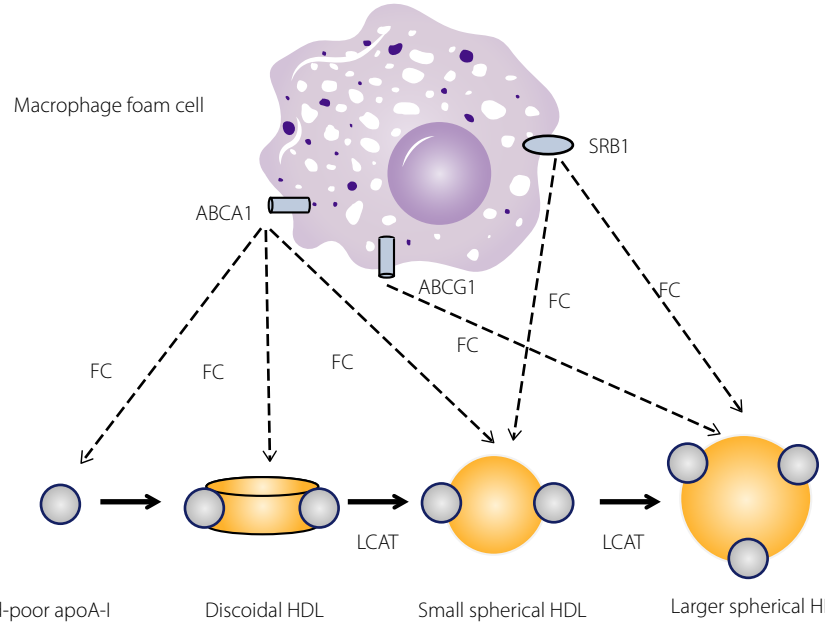

Figure 1 | Cellular cholesterol efflux to high-density lipoprotein (HDL). Efflux of free cholesterol (FC) from cells to extracellular HDL-based acceptors is mediated by the action of active transporters and passive diffusion. This first step of reverse cholesterol transport is influenced by the physicochemical properties of HDL and the interaction of these HDL subclasses with cellular transporters. The adenosine triphosphate-binding cassette transporter A1 (ABCA1) interacts with cholesterol-deficient and phospholipid-depleted apolipoprotein A-I complexes, whereas ABCG1 and scavenger receptor B1 (SRB1) interact with spherical HDL particles of various sizes. After efflux to HDL, FC is esterified in the plasma by lecithin-cholesterol acyltransferase (LCAT), and is ultimately transported from HDL to the liver, either directly through SRBI or after transfer to apolipoprotein B-containing lipoproteins by the cholesteryl ester transfer protein for ultimate disposal in the feces.

consist of multiple forms and components, and this heterogeneity in HDL structure is intrinsically related to their functional diversity. Measurement of HDL-C content does not necessarily reflect the distribution or overall abundance of HDL subspecies. Structure-function analyses have shown that HDL-C is a rather poor indicator of the functionality of HDL. Experimental data have suggested that HDL has multiple protective functions against atherosclerosis. One of the best characterized functions of HDL is its role in reverse cholesterol transport. By acting as extracellular cholesterol acceptors in the circulation, HDL can promote cholesterol efflux from cells like macrophages in the artery wall (Figure 1). In addition, HDL has anti-oxidative, anti-inflammatory and anti-apoptotic properties. HDL has been reported to block oxidative modification of LDL and decrease adhesion molecules expression. It also promotes endothelial repair and ameliorates endothelial dysfunction. Which subclass (es) of HDL species are responsible for which aspects of biological activities of HDL is poorly understood. To make matters even more complicated, changes in the proteome and/or lipidome of HDL particle may adversely affect the functionality of HDL. A number of pathological conditions including diabetes mellitus can trigger structural and functional alterations in HDL and many proteins other than apolipoproteins may bind to HDL. One such example is serum amyloid A, and binding of serum amyloid A impairs the ability of HDL to efflux cholesterol from cells[3]. As a result, HDL can lose its atheroprotective properties, and become dysfunctional and pro-inflammatory under certain circumstances. The degree of loss of normal HDL function and gain of dysfunction might differ with respect to the different biological activities of HDL. How changes in the proteome and/or lipidome of HDL affect the functionality of HDL is currently under intense investigations.

Clinical measures to determine the different aspects of HDL function are being developed and evaluated in a number of research laboratories. The most widely-studied and well-characterized HDL function is probably its cholesterol efflux capacity. *Ex vivo* assays have been used to assess the capacity of individual patient HDL specimens or serum to remove cholesterol from cultured cholesterol-loaded macrophages. The relationship between HDL cholesterol efflux capacity and cardiovascular disease has been examined in cross-sectional and longitudinal studies. It has been shown that HDL-C levels only explain a fraction (approximately one-third) of the variance in the cholesterol efflux capacity of HDL. An independent inverse association between HDL cholesterol efflux capacity and incident cardiovascular events has been shown both in the Dallas Heart Study and in the European Prospective Investigation of Cancer-Norfolk study[4,5]. Measurement of this classic function of HDL has an even stronger relationship with coronary heart disease than measures of HDL-C, and the relevance of HDL function-

ality to cardiovascular disease has finally been confirmed in these clinical studies. Taken together, available data show that HDL cholesterol efflux capacity is predictive of prevalent and incident coronary heart disease independent of HDL-C, and evaluation of HDL function rather than HDL mass such as HDL-C might be much more informative.

In conclusion, HDL-C is not a good surrogate marker of HDL function. Measurement(s) of HDL function rather than HDL-C might be a better tool to evaluate the therapeutic response of interventions targeting HDL, and could also further improve the assessment of cardiovascular risk. However, standardized assays of HDL function are not yet available for routine clinical use, and a number of issues need to be addressed. As HDL has multiple functions, there is currently no consensus on which function(s) of HDL are key to its antiatherogenic effect, and what the optimal test(s) to characterize HDL function are. Because of the heterogeneity of HDL, measurement of HDL particles and distribution of subspecies needs to be validated in order to standardize assays of functionality with HDL quantification. The assays of HDL function also need to be reproducible and cost-effective. HDL is one of the most biologically variable molecules, and the 'HDL function hypothesis' still holds potential as a means to reduce cardiovascular disease.

DISCLOSURE

The author declares no conflict of interest.

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