Clinical and epidemiological studies show a robust, inverse association of high-density lipoprotein (HDL) levels with cardiovascular disease (CVD) risk. Moreover, genetically engineered mice provide compelling evidence that HDL is atheroprotective in hypercholesterolemic animal models. These observations have triggered intense interest in targeting HDL for therapeutic intervention.

Most clinical studies have used HDL-cholesterol (HDL-C) as the metric for quantifying HDL’s cardioprotective effects. However, recent evidence has raised doubts about elevating HDL-C being therapeutic. For example, genetic variations that associate with altered HDL-C levels do not strongly associate with altered CVD risk. Also, prospective clinical trials of niacin and cholesteryl ester transfer protein inhibitors (2 drugs that elevate HDL-C levels by different mechanisms) failed to reduce cardiac events in statin-treated subjects with established CVD. Moreover, when mice lack certain proteins involved in HDL metabolism—such as SR-B1, the liver receptor for HDL—both HDL-C levels and atherosclerosis increase dramatically. Thus, quantifying HDL-C does not necessarily assess HDL’s proposed ability to lower CVD risk.

Many lines of evidence indicate that one of HDL’s cardioprotection tasks is to mobilize excess cholesterol from artery wall macrophages. For example, mouse studies demonstrate that increased hepatic expression of apolipoprotein A-I, the major HDL protein, increases cholesterol export from macrophages and retards atherosclerosis.

Two pathways for sterol export involve the membrane-associated ATP-binding cassette transporters (ABC) ABCA1 and ABCG1, which are highly induced when macrophages accumulate excess cholesterol. Thus, atherosclerosis increases markedly in hypercholesterolemic mice when myeloid cells are deficient in ABCA1. Also, humans with ABCA1 deficiency had Tangier’s disease and accumulate macrophages laden with cholesterol in many tissues. These observations support the proposal that HDL, ABCA1, and sterol efflux from cells are important regulators of sterol balance in human macrophages.

The relevance of sterol efflux from macrophages in humans has been difficult to assess because it accounts for only a small fraction of overall reverse cholesterol transport from peripheral tissues to the liver. To measure efflux capacity, de la Llera-Moya et al pioneered the use of serum HDL (serum depleted of the atherogenic lipoproteins low-density lipoprotein and very low-density lipoprotein, which deliver cholesterol to macrophages) with cultured J774 macrophages radiolabeled with cholesterol. They demonstrated that the cholesterol efflux capacity of human serum HDL varies markedly, despite similar levels of HDL-C. Thus, HDL-C level is not the major determinant of macrophage sterol efflux in this system.

Using the same model system, investigators found strong, inverse associations between the cholesterol efflux capacity of serum HDL and prevalent coronary artery disease. Differences in efflux capacity of serum HDL correlated with altered efflux by the ABCA1 pathway in macrophages. Moreover, efflux capacity remained a strong predictor of prevalent coronary artery disease after adjustment for HDL-C levels. This study provided the first strong clinical evidence that a proposed functional property of HDL might be more relevant to human atherosclerosis than HDL-C levels.

The efflux capacity of serum HDL with J774 macrophages can also be assessed with fluorescently labeled cholesterol, which primarily measures cholesterol export by ABCA1. A recent study of a large, population-based cohort initially free of CVD demonstrated that sterol efflux assessed by this method associates strongly and negatively with the risk of future cardiac events. This association persisted after multivariate adjustment, suggesting that impaired HDL function affects incident cardiovascular risk by processes distinct from those involving HDL-C and other traditional lipid risk factors. Taken together, these observations provide strong evidence that HDL’s capacity to accept cholesterol via ABCA1 is a functional metric, relevant to atheroprotection, that is independent of HDL-C.

HDL is not a homogeneous population. It is a collection of particles that range in size from <7 nm to >14 nm and contains >80 different proteins. Most HDL particles are spherical with a core of hydrophobic lipids (cholesteryl ester and triglycerides). However, the major initial acceptor for cholesterol excreted by cells seems to be prebeta HDL, a quantitatively minor species of plasma HDL made of poorly lipidated apolipoprotein A-I.

Pioneering studies by Francis et al demonstrated that lipid-free apolipoprotein A-I promotes cholesterol efflux from cells and that this pathway is defective in Tangier’s disease fibroblasts. Other lipid-free or lipid-poor apolipoproteins can also promote cholesterol efflux by ABCA1. In contrast, lipid-free
apolipoprotein A-I fails to promote sterol efflux from cells by the ABCG1 pathway. Instead, the major acceptor for free cholesterol export by ABCG1 is spherical HDL. Efflux by ABCG1 increases as the phospholipid surface layer of spherical HDLs enlarges.

In this model, lipid-free or poorly lipidated apolipoprotein A-I accepts sterol (and phospholipid) from cells by a pathway involving ABCA1 (Figure A) to become discoidal HDL particles that lack a cholesteryl ester core. Lecithin-cholesterol acyltransferase (LCAT) then converts the newly accepted cholesterol to cholesteryl ester, which—being more hydrophobic—migrates into the core of the nascent HDL particle to generate a more mature spherical HDL. This form of HDL becomes an acceptor for cholesterol exported from cells by ABCG1, and this second wave of cholesterol enlarges the HDL particles.

A key feature of the current model is that discoidal and more mature forms of HDL do not promote sterol efflux by the ABCA1 pathway (Figure A). The model also implies that increased LCAT activity should promote sterol efflux from macrophages. In this issue, Du et al10 challenge this notion by providing strong evidence that small, dense HDL particles are potent acceptors of cholesterol exported from macrophages by the ABCA1 pathway. Moreover, they show that the major mediator of cholesterol export from macrophages to HDL is ABCA1.

Strengths of their studies include using carefully defined sizes of both reconstituted HDL particles and human HDL to examine the affect of size on sterol efflux; quantifying sterol

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**Figure. Current (A) and proposed (B) models for how different high-density lipoprotein (HDL) species promote cholesterol efflux from macrophages.** Both ABCA1 and ABCG1 are transmembrane proteins but they promote sterol efflux by different mechanisms. ABCA1 exports cholesterol from the plasma membrane to cholesterol acceptors, whereas ABCG1 is an intracellular sterol transporter that enriches the plasma membrane with cholesterol. See the text for additional details. Apo indicates, apolipoprotein; CE, cholesteryl ester; FC, cholesterol; and LCAT, lecithin-cholesterol acyltransferase.
measurement, to a metric that quantifies the absolute concentration of HDL particles. Using this method, we found that the total concentration of HDL particles in plasma to be 13.4 μmol/L, with a mean plasma apolipoprotein A-I value of 48.8 μmol/L, indicating that each HDL particle contains 3.6 molecules of apolipoprotein A-I (assuming that all HDL particles contain apolipoprotein A-I). This value is in excellent agreement with the stoichiometry of human HDL determined by other methods. Moreover, calibrated ion mobility analysis also yielded values for the size of the HDL subspecies that agree well with those obtained by orthogonal methods. As expected, both HDL-C and apolipoprotein A-I levels were poor predictors of HDL particle number; both the metrics under-represented small HDLs. In future studies, quantification of HDL size and concentration should be a valuable tool for investigating the roles of specific HDL subspecies and pathways in regulating the efflux capacity of serum HDL.

Recent clinical and genetic studies clearly demonstrate that elevating HDL-C does not necessarily reduce CVD risk. Therefore, it is time to end the clinical focus on HDL-C and to understand—at the mechanistic level—how HDL’s functional properties contribute to that risk. It will also be important to link changes in HDL’s size and function to genetics and HDL-targeted therapies. The development of new metrics for quantifying HDL function, based on a better understanding of macropage reverse cholesterol transport, is essential for achieving these goals.

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Disclosures
None.

References

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