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.

Small HDL Promotes Cholesterol Efflux by the ABCA1 Pathway in Macrophages
Implications for Therapies Targeted to HDL

Jay W. Heinecke

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...
apoprotein 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 apoprotein 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 cholesteryl 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 HDL particles. In this issue, Du et al10 challenge this notion by proposing a new model for HDL particle size and function. By examining the affect of size on sterol efflux; quantifying sterol efflux via ABCA1 and ABCG1 from multiple types of macrophages (bone marrow–derived mouse macrophages, a human macrophage cell line, and primary human monocyte–derived macrophages); and quantifying cholesterol efflux with both radiolabeled cholesterol and by sterol mass. The latter point is significant because, under certain circumstances, efflux of radiolabeled cellular cholesterol can be dissociated from changes in overall sterol balance in macrophages. In future studies, it will be critical to determine the mechanistic basis for the variations in efflux capacity seen with different sizes of HDL.

The work of Du et al10 suggests a new model for how HDL promotes sterol efflux from macrophages (Figure B): both lipid-free/poorly lipidated apolipoprotein A-I and small HDL particles promote sterol efflux by the ABCA1 pathway. As in the current model, cholesterol derived from both LCAT and ABCG1 increases the size of HDL. Because LCAT converts discoidal HDL and small HDL to larger particles, the enzyme might interfere with macrophage sterol export by the ABCA1 pathway. It is noteworthy that overexpression of LCAT in hypercholesterolemic mice increases both HDL-C and atherosclerosis. Moreover, humans deficient in LCAT have very low HDL-C levels but no major increase in CVD risk.

These observations may have important implications for therapies targeted to increase the cardioprotective subspecies(s) of HDL. Importantly, both the drugs that failed to reduce cardiac risk in the clinical trials increase the concentration of HDL_C, the large form of HDL.1 If smaller forms of HDL (eg, HDL_3) are indeed the main promoters of sterol efflux from macrophages by ABCA1, the drugs may have been ineffective because they failed to increase the concentration of the small HDL particles that promote efflux by this pathway.

Two key unresolved issues are the concentrations of the different sizes of HDL subspecies in blood, and how the different subspecies in blood contribute to the sterol efflux capacity of serum HDL with macrophages. The report by Du et al strongly implies that different-sized HDLs have distinct functional capacities and furthermore that small, cholesterol-poor particles play important roles in mobilizing cholesterol from macrophages. HDL levels are generally measured indirectly as the cholesterol content of an HDL particle can vary >10-fold. At a given level of HDL-C, it is theoretically possible to have many small cholesterol-poor particles or far fewer large, cholesterol-rich particles. Thus, a key issue confounding the interpretation of HDL-C as a measure of HDL concentration and function is the relative balance between large and small HDL particles. An additional complexity is the difference in the stoichiometry of apolipoprotein A-I in large and small HDL particles, which makes interpretation of apolipoprotein A-I levels (or HDL protein content, the method used by Du et al10) problematic for assessing HDL particle size and concentration. These issues highlight the need for new HDL metrics, which are not biased toward certain subpopulations, such as large cholesterol-rich HDL.

We recently developed a method to measure the concentration and size of HDL particles in plasma. Termed calibrated ion mobility analysis, the method uses protein standards to convert ion mobility analysis signal intensity, a relative

**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 the HDL cholesterol hypothesis.

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Disclosures
None.

References

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