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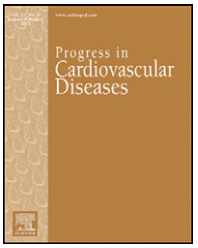

High-Density Lipoprotein Function Measurement in Human Studies: Focus on Cholesterol Efflux Capacity

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ABSTRACT

A low plasma level of high-density lipoprotein (HDL) cholesterol (HDL-C) is a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD). However, several observations have highlighted the shortcomings of using cholesterol content as the sole reflection of HDL metabolism. In particular, several large randomized controlled trials of extended release niacin and cholesteryl-ester transfer protein (CETP) inhibitors on background statin therapy have failed to show improvement in ASCVD outcomes despite significant increases in HDL-C. Reverse cholesterol transport (RCT) is the principal HDL function that impacts macrophage foam cell formation and other functions such as endothelial activation of endothelial nitric oxide synthase, monocyte adhesion, and platelet aggregation. Cholesterol efflux from macrophages to plasma/serum reflects the first critical step of RCT and is considered a key anti-atherosclerotic function of HDL. Whether this function is operative in humans remains to be seen, but recent studies assessing cholesterol efflux in humans suggest that the cholesterol efflux capacity (CEC) of human plasma or serum is a potent marker of ASCVD risk. This review describes the methodology of measuring CEC *ex vivo* from human samples and the findings to date linking CEC to human disease. Studies to date confirm that CEC can be reliably measured using stored human blood samples as cholesterol acceptors and suggest that CEC may be a promising new biomarker for atherosclerotic and metabolic diseases. Further studies are needed to standardize measurements and clarify the role CEC may play in predicting risk of developing disease and response to therapies.

A low plasma level of high-density lipoprotein (HDL) cholesterol (HDL-C) is a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD).[1] However, several observations have highlighted the shortcomings of using cholesterol content as the sole reflection of HDL metabolism. HDL-C is lower in the insulin resistance state, which also confers increased ASCVD risk. This is evidenced by the fact that the association between low HDL-C and ASCVD is attenuated by adjustment for total low-density lipoprotein (LDL) particle concentration.[2] In addition, genetic studies of low or high high-density lipoprotein (HDL) cholesterol (HDL-C) have not shown association with increased or decreased risk, respectively.[3,4] Lastly, several large randomized controlled trials of extended release niacin and cholesteryl-ester transfer

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Abbreviations and Acronyms

Apo = apolipoprotein

ASCVD = atherosclerotic cardiovascular disease

ATP = adenosine triphosphate

CAD = coronary artery disease

CEC = cholesterol efflux capacity

CETP = cholesterol ester transport protein

CKD = chronic kidney disease

CV = cardiovascular

CVD = cardiovascular disease

DM = diabetes mellitus

eNOS = endothelial nitric oxide synthase

ESRD = end-stage renal disease

HDL = high-density lipoprotein

HDL-C = high-density lipoprotein cholesterol

LCAT = Lecithin:cholesterol acyltransferase

LDL = low-density lipoprotein

LDL-C = low-density lipoprotein cholesterol

MetS = metabolic syndrome

MI = myocardial infarction

RCT = reverse cholesterol transport

TGs = triglycerides

VLDL = very low-density lipoprotein

protein (CETP) inhibitors on background statin therapy have failed to show improvement in ASCVD outcomes despite significant increases in HDL-C.[5–8] These observations on the shortcomings of using cholesterol content of HDL as a marker of risk have focused attention on other parameters of HDL metabolism to improve prediction of ASCVD risk and response to therapy.

HDL exerts several key anti-atherosclerotic functions related to cholesterol transport, endothelial and vascular function, and inflammation. Reverse cholesterol transport (RCT), the ability of HDL to accept cholesterol from the periphery and deliver it to the liver for excretion, is the principal method for HDL biogenesis from nascent lipid-poor particles to mature cholesteryl-ester laden spherical particles;[9] RCT is also considered the principal HDL function that impacts macrophage foam cell formation and other functions such as endothelial activation of endothelial nitric oxide synthase (eNOS), monocyte adhesion, and platelet aggregation.[10] Therefore, RCT is the overriding action of HDL on multiple cell types.

RCT is a complex process that has been well worked out in animal models.[11] As summarized in Fig 1, lipid-poor apolipoprotein (apo) A-I (apoA-I) interacts with the ABCA1 receptor on hepatocytes and macrophages in the periphery to accept cellular cholesterol. This cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT), leading to the formation of spherical HDL particles with a hydrophobic cholesteryl ester core. These enlarging HDL particles can continue to accept cholesterol from the periphery via other pathways and deliver it back to the liver for excretion into bile and feces. In addition, cholesterol within HDL particles can be exchanged with LDL and very low-density lipoprotein (VLDL) particles for triglycerides (TGs), leading to catabolism of HDL particles by lipases and either excretion via the kidney or re-participation in accepting cholesterol from the periphery.

Macrophage-specific cholesterol efflux is the key initial step in RCT and has been shown in genetic and pharmacologic animal studies to be more closely associated with atherosclerosis than circulating levels of HDL-C.[9] Whether this function is operative in humans remains to be seen, but recent studies assessing cholesterol efflux in humans suggest that the cholesterol efflux capacity (CEC) of human plasma or serum is a potent marker of ASCVD risk. This review serves to describe the methodology of measuring CEC *ex vivo* from human samples and the findings to date linking CEC to human disease.

Measuring Cholesterol Efflux Capacity (CEC) in Humans

There is no standardized method for measuring CEC in humans and protocols vary considerably; however, they all measure the movement of labeled cholesterol from cells to an extracellular acceptor (Fig 2).[12] In general, most studies in humans have only tested the cholesterol acceptor aspect of efflux, specifically, the differential capacity of human serum/plasma to accept cholesterol from cells in a unidirectional manner. This approach does not take into account the ability of a patient's own macrophages to efflux cholesterol and does not assess cholesterol influx, or net efflux.

Macrophages are the most relevant cell type for studies of atherosclerosis given the central role of macrophage "foam" cells in disorders of lipid accumulation. Macrophages efflux cholesterol is via several transporters, including adenosine tri-phosphate (ATP)-binding cassette transporters ABCA1 and ABCG1, scavenger receptor SRB1, as well as via aqueous diffusion. CEC assays can reflect all of these pathways in aggregate or can be modified to interrogate a specific transporter. Choice of cholesterol acceptor can have significant impact on assessment of CEC and is the largest source of variation across studies. Cholesterol acceptor mediums can range in specificity for HDL from isolated pure HDL to apo B-depleted plasma/serum to whole plasma/serum. The use of ApoB-depleted plasma eliminates the role of LDL and VLDL in assessing cholesterol efflux, making it more specific for HDL-mediated CEC. When whole or apoB-depleted plasma/serum is used, other cholesterol acceptors and shuttles such as albumin can also play a role in CEC; however, studies have shown that apoA-I, the main protein constituent of HDL particles, is responsible for ~75–80% of the CEC from macrophage cell lines with amplified ABCA1 transporter pathways.[13,14] In one small study, CEC to apoB-depleted plasma moderately correlated with CEC to isolated HDL ($r = 0.46$, $p < 0.02$) but was not correlated at all with CEC to whole plasma ($p > 0.2$).[15] Ascertaining the specific methodology used to assess CEC is critical when evaluating the reported findings in human studies. Correlations between CEC and other lipid markers can vary widely whether using whole vs. apoB-depleted plasma/serum as the cholesterol acceptor.[15]

CEC and ASCVD

Studies assessing the association between CEC and ASCVD are summarized in Table 1. Perhaps the first reported study of