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Review

A critical appraisal of the measurement of serum 'cholesterol efflux capacity' and its use as surrogate marker of risk of cardiovascular disease

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ABSTRACT

The 'cholesterol efflux capacity (CEC)' assay is a simple *in vitro* measure of the capacities of individual sera to promote the first step of the reverse cholesterol transport pathway, the delivery of cellular cholesterol to plasma HDL.

This review describes the cell biology of this model and critically assesses its application as a marker of cardiovascular risk. We describe the pathways for cell cholesterol export, current cell models used in the CEC assay with their limitations and consider the contribution that measurement of serum CEC provides to our understanding of HDL function *in vivo*.

1. High density lipoprotein and reverse cholesterol transport

The well-established inverse correlation between serum levels of HDL-cholesterol (HDL-C) and incidence of atherosclerotic cardiovascular disease observed in many clinical and epidemiological studies has led to the view that HDL is actively protective against the development of atherosclerosis [1]. HDL particles possess a number of potentially antiatherogenic properties including antioxidative, anti-inflammatory, antiapoptotic and antithrombotic activities [2]. However the best understood activity of HDL is its central role in the mechanism of reverse cholesterol transport (RCT; Fig. 1). In this process, excess free (unesterified) cholesterol (FC) from peripheral cells is transferred to extracellular HDL through the action of cellular cholesterol transporters such as ABCA1 and ABCG1. Following its efflux to HDL, this FC may be esterified in the plasma by the enzyme lecithin:cholesterol acyltransferase (LCAT) and transferred to the core of the HDL particle. The cholesterol is subsequently transported from HDL to the liver, either directly or after transfer to apolipoprotein B-containing lipoproteins such as low density lipoprotein (LDL) by the cholesteryl ester transfer protein (CETP). The cholesterol can then be secreted from liver into in the bile, either unmodified or after conversion to bile acids. A proportion of this material is lost from the body in faeces.

In the development of atherosclerotic lesions, cholesterol accumulates as large cytoplasmic deposits of esterified cholesterol in macrophage 'foam cells' within the arterial intima. This process is believed to be important in driving the growth and development of the atherosclerotic plaque. Logically it suggests that the ability of arterial macrophages to export their excess cholesterol is inefficient. This failure could reflect an inability of the arterial macrophages to export their excess cholesterol and/or inefficiency at some point in the reverse

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; apoE, apolipoprotein E; ABCA1, ATP-cassette binding transporter A1; ABCG1, ATP-cassette binding transporter G1; ACAT, acyl CoA cholesterol acyl transferase; ATP, adenosine triphosphate; CAD, coronary artery disease; CAC, coronary artery calcium; cAMP, cyclic adenosine monophosphate; CE, cholesteryl ester; CETP, cholesterol ester transfer protein; CEC, cholesterol efflux capacity; CT, computed tomography; CVD, cardiovascular disease; CLA-1, CD36 and LIMPII analogous-1 (human homologue of SR-BI); FC, free cholesterol; FH, family history of CAD; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HMDM, human monocyte-derived macrophages; hsCRP, high sensitive C-reactive protein; LCAT, lecithin cholesterol acyltransferase; LDL, low-density lipoprotein; LDLR, LDL receptor; LXR, liver X receptor; MI, myocardial infarction; NCEH, neutral cholesteryl ester hydrolase; PEG, polyethylene glycol; PL, phospholipid; PLTP, phospholipid transfer protein; PPAR, peroxisome proliferator-activator receptor; RCT, reverse cholesterol transport; RNA, ribonucleic acid; RXR, retinoid X receptor; SR-BI, scavenger receptor class B type 1; STEMI, ST elevation myocardial infarction; VLDL, very low-density lipoprotein

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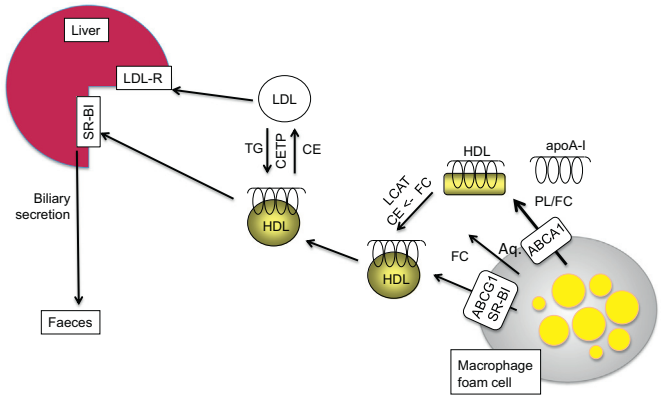

Fig. 1. The reverse cholesterol transport pathway.
Unesterified cholesterol (FC) from peripheral cells, including foam cell macrophages, is exported mainly to HDL species in the intimal fluid. ABCA1, ABCG1 and SR-BI, plus aqueous diffusion (Aq.) can all contribute to the export process. ABCA1 interacts preferentially with lipid free apoA-I and smaller HDL particles; ABCG1, SRBI and aqueous diffusion export to all lipidated HDL species. Following transfer to HDL, FC molecules in the phospholipid surface of the particle are esterified by lecithin:cholesterol acyltransferase (LCAT) and the cholesteryl esters (CE) moved to the HDL core. HDL can deliver the CE directly to the liver *via* hepatic SR-BI, or indirectly after transfer of CE to LDL, mediated by cholesteryl ester transfer protein (CETP). A proportion of cholesterol in the liver is excreted in the faeces either as FC or following its conversion to bile acids.

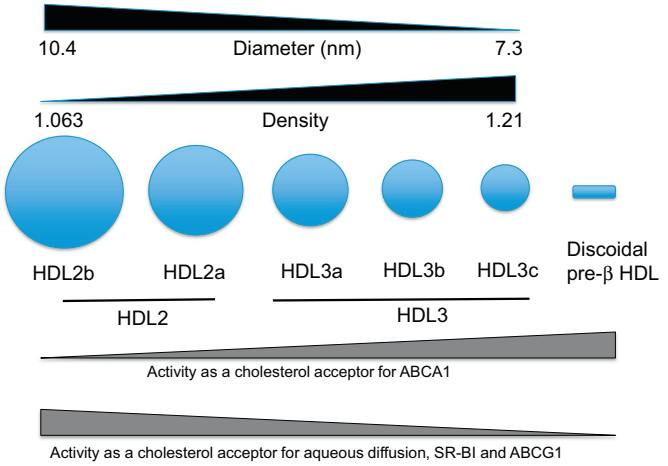

Fig. 2. HDL heterogeneity.
Properties are shown of HDL particles classified according to density. Two HDL (HDL2 and HDL3) subclasses were initially identified based on their separation by ultracentrifugation and subsequently categorised into further subclasses [5]. The larger, less dense particles contain relatively more lipid and less protein than the smaller particles. Discoidal pre-beta HDL is not usually separated by centrifugation but is smaller and more dense than the smallest of the spherical particles. Relative activities as acceptors for cholesterol efflux are provided on a per particle basis (see text for more details).

cholesterol transport pathway.

The inverse relationship between plasma HDL-C levels and risk of atherosclerosis suggest that the availability of HDL as a mediator of RCT may be limiting in some subjects and that the elevation of HDL-C levels is a potential therapeutic target. However the association between HDL-C and cardiovascular risk is not always direct at the individual level. For example, several drug trials aimed at elevating plasma HDL-C have failed to reduce cardiovascular events [3] and several specific genetic mutations leading to increased levels of HDL-C do not seem to reduce the risk of CVD [4]. This has led to the idea that HDL quality is important as well as HDL quantity. Changes in quality could arise through differences in relative amounts of different types of HDL particles present in an individual, or to changes in the structure of the particles through metabolic and/or covalent processes such as oxidation and glycation, leading to formation of 'dysfunctional' HDL (reviewed in [2]). Neither of these processes would necessarily be reflected by measurement of HDL-C levels.

Plasma HDL is a very heterogenous mixture of particles, differing in size, density, shape and protein and lipid composition (reviewed in [5,6]). They are mostly spherical particles, comprising a surface coat of phospholipid and free cholesterol and a core mainly of esterified cholesterol. A minor proportion of HDL has very little cholesteryl ester core and is discoidal in shape. The major protein component of HDL is apolipoprotein A-I (apoA-I), which provides both structure and activity to the particles, but HDL also contains numerous other apolipoproteins and proteins. The HDL particle population can be classified according to size, density, protein content or mobility in non-denaturing electrophoresis. Fig. 2 provides a simple overview of the spectrum of HDL particles separated on the basis of their density. These particles also differ in their capacity to stimulate the various pathways for cell cholesterol export (described in detail below), so that variations in the relative amounts of specific HDL particles would be expected to alter the overall efficiency of the HDL population to stimulate cholesterol export. In addition to differences in size and density, variations in relative amounts of different phospholipid species and protein cargoes can also profoundly affect HDL function and activity, including their cholesterol efflux activity [7,8].

Separate analysis of the individual HDL subpopulations is technically complex and time-consuming and at the present time is not suitable for large-scale analysis and screening. With this background, there has recently been an intense interest in the development of methods to directly assess various aspects of HDL functionality as predictors of cardiovascular risk. These aim to directly assess the biological activity of the whole HDL population in individual subjects. One of these uses an *in vitro* assay to assess the capacity of human plasma or serum to participate in the first step in the reverse cholesterol transport pathway, the removal of cholesterol from peripheral cells. This serum cholesterol efflux capacity (CEC) assay appears from early studies to correlate better with cardiovascular outcomes than HDL-C levels [9].

The aim of this review is to explain in detail the cell biology of this model and to critically assess its application in the assessment of cardiovascular risk. We will first provide a detailed description of the pathways available for cholesterol export from cells and their requirements for specific types of HDL particles. This will be followed by a description of the development of the serum CEC assay and details of a range of clinical studies in which variants of this assay have been correlated with cardiovascular outcomes. Finally we will provide a critical analysis of the currently used methods and suggest additional considerations that may improve or extend assessment of this component of the RCT pathway and its role in the development of atherosclerosis.

2. Cellular cholesterol homeostasis

All mammalian cells need FC as it has an important role in the maintenance of membrane structure, fluidity and permeability. Cells can synthesise cholesterol or acquire it exogenously from circulating lipoproteins, predominantly low-density lipoprotein (LDL), *via* a receptor-mediated uptake pathway (Fig. 3). Normally LDL is internalised *via* the LDL-receptor (LDL-R), which is expressed on all cells. Other receptors, such as CD36 and Scavenger Receptor A (SRA) may mediate uptake of modified forms of LDL by macrophages in the intima (reviewed in [10]). Additionally, some LDL may be internalised by pinocytosis [11]. However, because most cells cannot degrade cholesterol, they have a number of mechanisms to avoid the accumulation of excess free cholesterol, which would have a deleterious impact on membrane structure. These controls include feedback mechanisms to regulate both