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Lecithin: Cholesterol Acyltransferase and Atherosclerosis:

Another High-Density Lipoprotein Story That Doesn't Quite Follow the Script

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Abstract

Plasma concentrations of high-density lipoprotein cholesterol (HDL-C) are strongly, consistently, and independently inversely associated with risk of atherosclerotic cardiovascular disease (CVD).[1] A series of animal studies in the 1990s, primarily involving overexpression of the major HDL protein apolipoprotein A-I (apoA-I) with subsequent increases in HDL-C, showed reduced progression or even regression of atherosclerosis, fitting nicely with the "HDL hypothesis" that raising HDL-C is causally associated with benefit. However, the last decade has seen several observations that do not follow this simple script. Some examples include the following: (1) the demonstration that scavenger receptor class BI knockout mice have increased HDL-C but increased atherosclerosis[2]; (2) the suggestion that some persons with high HDL-C levels have "dysfunctional" HDL that may not be protective[3]; and (3) the observation that the cholesteryl ester transfer protein inhibitor torcetrapib raised HDL-C levels considerably but did not decrease, and indeed increased, cardiovascular risk.[4,5] These developments have brought into major question the simple hypothesis that higher HDL-C directly and causally results in reduced atherosclerosis and challenge the approach of developing therapies that raise HDL-C levels.

Keywords

Editorials; atherosclerosis; cholesterol; cholesterol; HDL; lipoproteins

One of the paradoxes in the field of HDL for nearly 3 decades has been the fact that monogenic disorders of extreme low HDL-C are not generally associated with obviously accelerated atherosclerosis. The first report of this phenomenon was the apoA-I Milano mutation, heterozygous carriers of which have HDL-C levels <5th percentile but no increased CVD.[6] A number of additional apoA-I point mutations causing very low HDL-C but no increased risk of CVD have since been described, confirming the fundamental observation. Most of these mutant apoA-I proteins are rapidly catabolized rather than secreted in a defective manner, but this is an inadequate explanation for the lack of CVD given that most garden-variety low HDL-C is also due to increased catabolism and not reduced production.

A second cause of extremely low HDL-C is Tangier disease, caused by loss-of-function mutations in both alleles of the ABCA1 gene responsible for transporting cholesterol and

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phospholipids out of cells. The lack of obviously increased CVD in Tangier disease despite the fact that HDL-C and apoA-I levels are usually <5 mg/dL and often virtually undetectable has been a bit of a mystery and is usually ascribed to the relatively low levels of low-density lipoprotein cholesterol that are also seen in Tangier disease.[7] However, Tangier disease is very rare, and therefore this issue remains in the realm of the anecdotal. Individuals heterozygous for mutations in ABCA1 have low levels of HDL-C, generally <10th percentile, and are much more common. Although some reports based on selected patients have suggested some modestly increased CVD risk,[8] a recent population-based study indicated that individuals carrying ABCA1 mutations with low HDL-C levels are at no increased risk of CHD compared with the general population.[9] Mouse studies suggest that hepatic and (secondarily) intestinal ABCA1 are the most important tissues expressing ABCA1 for maintaining normal plasma HDL-C and apoA-I levels,[10,11] but this does not explain the striking lack of premature CVD in persons with 1 or 2 mutated ABCA1 alleles. Given the apparent importance of the ABCA1 pathway in transporting cholesterol out of macrophages,[12] the lack of cardiovascular risk is even that much more confounding.

The third monogenic cause of extremely low HDL-C is lecithin:cholesterol acyltransferase (LCAT) deficiency, a form of which is also known as fish-eye disease. LCAT is responsible for the synthesis of cholesteryl esters in human plasma by catalyzing a reaction in which a fatty acyl residue from the sn-2 position of phosphatidylcholine is transferred to the 3-beta-hydroxy group of cholesterol, resulting in formation of cholesteryl esters.[13] LCAT is clearly a critical enzyme in the metabolism of HDL as individuals with loss-of-function mutations in both alleles (LCAT deficiency) have HDL-C levels that are generally <10 mg/dL.[14,15] Deficiency of LCAT-mediated cholesterol esterification in plasma leads to inability to form mature HDL particles with a cholesteryl ester core and rapid catabolism of circulating apoA-I and apoA-II.[16] The classic form of LCAT deficiency is characterized by progressive corneal opacification, hemolytic anemia, and progressive renal insufficiency that eventually leads to end-stage renal disease. A variant in which some LCAT activity can be detected on low-density lipoprotein (but not HDL) is known as fish-eye disease, which, as its name suggests, also involves the corneas, but fish-eye disease generally spares the kidneys. Remarkably, premature CVD is not a consistent feature of LCAT deficiency or fish-eye disease despite the extremely low plasma levels of HDL-C and apoA-I.[14,15] However, the number of LCAT-deficient patients is limited, and some fish-eye disease patients with premature coronary heart disease have been described, making a broader investigation of this issue critically important to the field.

In contrast to ABCA1 heterozygotes, LCAT heterozygotes have not been as extensively or systematically studied. Reduced LCAT activity had been reported to be associated with increased carotid intima-media thickness (IMT) in a previous report,[17] but this was not definitive. In this issue of *Circulation*, the most definitive study to date on the relationship of LCAT to atherosclerosis is reported. Calebresi et al[18] systematically studied 12 individuals with 2 loss-of-function LCAT mutations and 28 heterozygous carriers with 1 mutation and compared them with 80 matched healthy controls. They confirmed the extremely low HDL-C levels in the subjects with 2 mutations and that heterozygosity for LCAT mutations also resulted in significantly lower levels of HDL-C compared with controls. However, they found that not only was carotid IMT not increased in the LCAT mutation carriers, but there was a strong suggestion of a gene dose-dependent reduction in carotid IMT. Although the conclusion that LCAT-deficient subjects are protected from atherosclerotic CVD cannot be confidently drawn from this study, it suggests that LCAT deficiency does not increase risk. Nevertheless, the total numbers studied are relatively small, and IMT remains a surrogate for hard cardiovascular end points. An important complementary study would be a population-based approach of rare LCAT mutations and their association with coronary heart disease end points similar to that reported recently for ABCA1 mutations.[9] In addition, common

variants in LCAT have been reported to be genome-wide and significantly associated with variation in HDL-C levels,[19] and a "mendelian randomization" study of common LCAT variants associated with variation in HDL-C and their association with coronary heart disease events would be of substantial interest.

The fact that reduced LCAT leading to reduced HDL-C does not result in increased atherosclerosis is all the more surprising because of the critical role that LCAT has been traditionally believed to play in the process of reverse cholesterol transport (RCT), the primary mechanism by which HDL is believed to protect against atherosclerosis.[20] LCAT has long been believed to be critical for promoting RCT by maintaining a free cholesterol gradient between cells in the periphery and plasma HDL.[21] However, there are reasons to question an essential role for LCAT in RCT. First, although passive diffusion was previously considered the predominant mechanism of cellular cholesterol efflux, it is now recognized that facilitated efflux of macrophage cholesterol via transporters such as ABCA1 and ABCG1 is quantitatively important in RCT[22] and atherogenesis,[12] and thus efflux is less likely to be sensitive to a free cholesterol gradient. Indeed, LCAT is not required for the normal ability of human serum to promote cholesterol efflux ex vivo.[23] Second, in humans, HDL is capable of directly delivering large amounts of unesterified cholesterol directly to the liver with subsequent targeting to the bile.[24,25] Indeed, we recently showed, using a validated method for assessing macrophage to feces RCT in mice, that neither LCAT overexpression nor loss of function had much effect on macrophage RCT.[26] Thus, although LCAT is clearly important for normal HDL metabolism, it may not be nearly as critical a player in maintaining a normal rate of macrophage RCT as has been traditionally believed, consistent with the results of Calibresi et al relative to its effects on carotid IMT.

A review of the literature on the relationship between LCAT expression and atherosclerosis in mice reveals conflicting reports but is nevertheless largely consistent with the interpretation that LCAT expression is not atheroprotective. LCAT-deficient mice were reported to have reduced atherosclerosis compared with controls despite much lower HDL-C levels[27,28] (although another report suggested increased atherosclerosis[29]). LCAT transgenic mice were reported to have no reduction or even increased atherosclerosis compared with controls despite much higher HDL-C levels[30–33] (although LCAT transgenic rabbits had both higher HDL-C levels[34] and reduced atherosclerotic lesions[35]). Clearly, more work is needed, possibly in more humanized animal models, to resolve the role of LCAT in atherogenesis.

Why does LCAT expression not appear to be associated with atherosclerosis in mice or humans despite its clear effect on plasma HDL-C levels? One hypothesis is that only a subfraction of HDL is actually responsible for atheroprotection and that LCAT expression influences this subfraction in a direction opposite that of its effects on overall HDL-C levels. Specifically, it has been suggested that "pre-β HDL" or lipid-poor apoA-I, which serves as the acceptor for ABCA1-mediated cholesterol efflux, is a critical HDL subfraction that protects against atherosclerosis. Reduced cholesterol esterification might be expected to produce increased lipid-poor pre-β HDL, and vice versa. Indeed, humans with LCAT deficiency have been reported to have increased plasma levels of pre-β HDL.[36] Our studies using plasma from LCAT-overexpressing and knockout mice revealed that LCAT expression was inversely associated with ABCA1-dependent cholesterol efflux from macrophages (ie, overexpression with reduced and knockout with increased ABCA1 efflux).[26] On the other hand, careful studies of factors influencing ABCG1-dependent efflux from macrophages suggested that the LCAT content of HDL under certain circumstances, such as cholesteryl ester transfer protein deficiency or inhibition, was a major predictor of ABCG1 efflux.[37,38] Further studies are required to probe the effects of differences in LCAT

expression on measures of HDL function, including but not limited to cholesterol efflux, in both animal models and humans and in different metabolic milieu.

These studies challenge the long-held belief that LCAT is atheroprotective and that increasing its activity would likely be beneficial in reducing CVD. At least 1 approach to pharmacological LCAT activation, an apoA-I peptide known at the “RLT peptide” or ETC-642, entered clinical development,[39] although very little information is publicly available. LCAT may be another example of a gene product that modulates HDL-C levels but does not otherwise follow the script, in that changes in HDL-C as a result of LCAT activity have little relationship to atherosclerotic CVD. Therapeutic targeting of HDL and RCT with the goal of inhibiting or regressing atherosclerosis may be better achieved through targets other than activating or upregulating LCAT.

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