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The Role of LCAT in Atherosclerosis

DOMINIC S. NG

Abstract

Lecithin–cholesterol acyltransferase (LCAT) is one of the major modulators of plasma high-density lipoprotein cholesterol (HDL-C) and plays a central role in the reverse cholesterol transport (RCT) process. Clinically, partial LCAT deficiency is common in a number of chronic disorders at risk for coronary heart diseases (CHD) but the role of LCAT in atherosclerosis remains controversial. Patients with monogenic causes of complete LCAT deficiency appear not to be prone to premature CHD. On the other hand, recent studies on patients with monogenic-based partial LCAT deficiency suggest they may be at increased atherogenic risk. Animal models with transgenic overexpression of the LCAT genes showed variable degrees of antiatherogenic properties except in one transgenic mouse model. LCAT knockout mouse models from different laboratories showed conflicting findings in their predisposition to aortic atherosclerosis. On the other hand, a number of studies using the LCAT knockout mice revealed significant impact of LCAT deficiency on not only lipoprotein metabolism but also in systemic oxidative stress, intrahepatic lipid, and glucose metabolism, each of which may individually modulate atherogenesis. Much remain to be learned with respect to the impact of LCAT deficiency on various proatherogenic pathways to better delineate its pathophysiologic link to atherosclerosis. This is of particular significance in patients suffering from common atherosclerosis-prone disorders that are known to be associated with partial LCAT deficiency, including diabetes and renal insufficiencies.

Keywords: atherosclerosis; high-density lipoprotein (HDL); glomerulopathy; lecithin–cholesterol acyltransferase (LCAT); lipoprotein-X (LpX); triglycerides, glucose

Abbreviations: LCAT, lecithin–cholesterol acyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CETP, cholesteryl ester transfer protein; PLTP, phospholipids transfer protein; ABCA1, ATP-binding cassette A1; PC, phosphatidylcholine; SR-BI, scavenger receptor class B type I

Introduction

High-Density Lipoprotein and Atherosclerosis—Epidemiologic Evidence

Numerous large-scale epidemiological studies persistently demonstrated an inverse relationship between plasma high-density lipoprotein cholesterol (HDL-C) level and the risk of coronary heart disease (CHD). An aggregate analysis of four of the largest US studies, which include the Framingham Heart Study [1], the Lipid Research Clinic Prevalence Mortality Follow-up Study, Lipid Research Clinic Primary Prevention Trial, and Multiple Risk Factor Intervention Trial [2], estimated that each 1 mg/dL (0.02 mmol/L) elevation of HDL-C is associated with a 2–3% reduction in CHD risk, a magnitude comparable to that for low-density lipoprotein (LDL) lowering. While this quantitative relationship may be valid in an epidemiologic context, and may also reflect some biological function of the circulating lipoprotein fraction, the use of a single HDL-C level as a risk predictor require caution. The heterogeneity in the HDL–CHD relationship is best exemplified by studying kindreds with monogenic causes of low HDL-C, namely with mutations in the apolipoprotein apoA-I, lecithin–cholesterol acyltransferase (LCAT), and ATP-binding cassette A1 (ABCA1) genes. Individuals homozygous for mutations in these genes uniformly develop severe HDL deficiency but the affected are not equally at risk of premature CHD. This is particularly the case with patients with complete LCAT deficiency.

The Role of LCAT in HDL Metabolism and Reverse Cholesterol Transport

LCAT was first described as a plasma enzyme, which mediates the transfer of fatty acids at the *sn-2* position from phosphatidylcholine (PC) to free cholesterol (FC), forming the neutral lipid cholesterol ester (CE) and lysophosphatidylcholine (LPC) [3]. It is a 416 amino acid glycoprotein synthesized and secreted primarily by the liver. mRNA messages of the LCAT gene have also been detected in testes and the brain [4] but their physiological significance are not well understood.

Upon secretion by the liver, LCAT circulates in plasma bound primarily to HDL but it is also found in apoB-containing lipoproteins, especially the LDL. Kinetic studies in humans have provided an estimate that approximately 70% of plasma CE are formed in the HDL and 30% in the apoB-containing particles [5]. Esterification of FC on apoB-containing particles and HDL by LCAT are coined by the terms β- and α-activity, respectively, and each plays different role in lipoprotein metabolism.

By way of its primary enzymatic action, LCAT plays a major role in HDL metabolism, especially in the reverse cholesterol transport (RCT) pathway, a multistep process by which cholesterol in the peripheral tissues is transferred

back to the liver for eventual elimination from the body (Fig. 2.1). To date, the RCT pathway continues to be considered as one of the major mechanisms by which HDL confers its cardioprotective effects [6]. The first step of RCT entails the efflux of cellular cholesterol, along with phospholipids, onto the cholesterol acceptors, with the lipid-free apoA-I and the disc-shaped pre-β-HDL being most avid [7]. Esterification of tissue-derived FC by LCAT transfers the neutral CE into the lipoprotein core, hence sustaining a chemical gradient to accept more FC from tissues. The accumulation of core CE converts the disc-shaped HDL to spherical particles. The mature HDL can further acquire other apoproteins from triglyceride-rich lipoprotein particles. The mature HDL particles can deliver its CE content directly into the liver through the selective uptake process mediated by scavenger receptor class B type I (SR-BI). On the other hand, the tissue-derived CE in HDL can also be transferred to the apoB-containing lipoproteins, in exchange for TG, through the action of

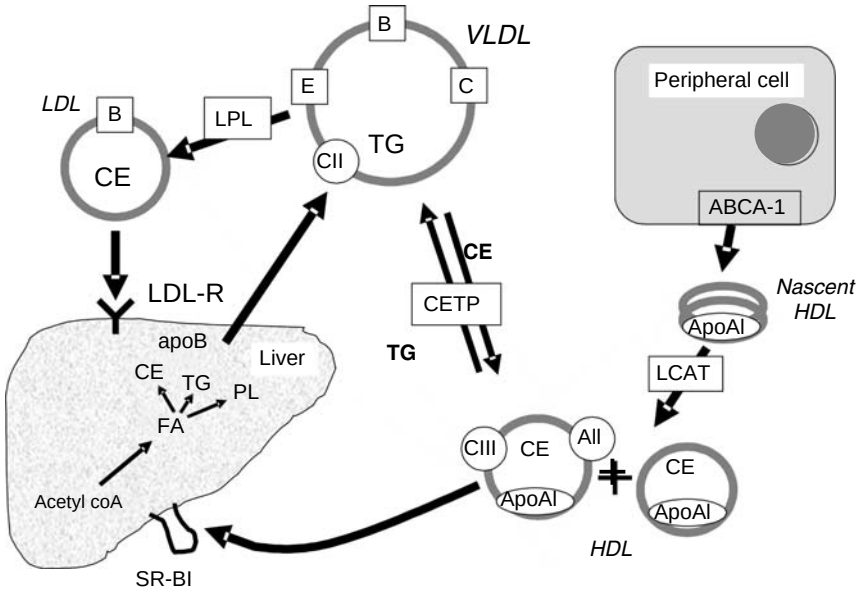

FIGURE 2.1. Schematics of the reverse cholesterol transport (RCT) pathway and the role of LCAT in HDL metabolism. Key steps in the RCT pathway include (1) cholesterol efflux: the transport of free cholesterol from peripheral cells (e.g., arterial wall macrophages) onto the circulating nascent HDL or lipid-free apoA-I (not shown)—mediated by the ATP-binding cassette AI (ABCA1) transporter; (2) cholesterol esterification: cell-derived cholesterol are esterified by lecithin–cholesterol acyltransferase (LCAT), generating neutral esterified cholesterol (CE) which enters the core; (3) lipid transfer: exchange of CE from HDL with triglyceride-rich lipoproteins for TG—mediated by cholesteryl ester transfer protein (CETP). The CE of the mature HDL can also be taken up directly into the liver—mediated by scavenger receptor class B type I (SR-BI). LPL, lipoprotein lipase; LDL-R, low-density lipoprotein receptor.

cholesteryl ester transfer protein (CETP). When the apoB-containing lipoproteins are taken up by the liver, the tissue-derived cholesterol can be channeled for elimination from the body through the biliary cholesterol pathway [8].

More recently, Nakamura et al. [9] examined the differential efficiency of LCAT activity in the generation of CE in various HDL fractions. These authors reported LCAT is associated with various size fractions of HDL and the smallest HDL identified in this experimental regime is consistent with a pre-β-HDL/LCAT complex. Among the various size fractions, the small pre-β-HDL-associated LCAT was responsible for the majority of the LCAT-mediated esterification of CE in HDL. Furthermore, these authors also examined the role of HDL size on the activity of CETP and reported a preferential transfer of CE from the small HDL fractions, including those derived from the pre-β-HDL. Continued activity of LCAT leads to the "maturation" and formation of the α-migrating HDL. Meanwhile, the larger HDL are subject to the modulatory action of phospholipids transfer protein (PLTP), replenishing the pool of pre-β-HDL. In this paradigm, one could envision a pool of HDL that would recycle between the smaller "metabolically active" form and the more mature form, and the cycle will continue to fuel the net movement of cell-derived cholesterol towards the liver for removal.

LCAT is one of the several major modulators of the plasma HDL-C levels, the other being apoA-I, ABCA1, and CETP. In humans subjects with genetic deficiency of LCAT, those who are homozygous a functional LCAT gene mutation develop severe HDL deficiency and the ones heterozygous for a defective LCAT gene develop intermediate levels of HDL deficiency [10, 11]. The marked reduction in HDL-C in the LCAT deficient subjects is attributed primarily to an accelerated catabolism of the HDL particles, with those containing apoA-I and apoA-II being more rapidly cleared than those containing apoA-I alone [12]. The residual circulating lipoproteins in the HDL density range include (a) the discoidal particles containing apoA-I, apoA-II, and occasionally apoE which often form rouleaux and (b) the small apoA-I containing particles that are rich in FC and PL. Interestingly, plasma from LCAT deficient subjects have been demonstrated to be equally effective in mediated cholesterol efflux in fibroblasts [13] but this finding was not shared by other [14, 15]. It is tempting to postulate that the residual HDL in LCAT deficient subjects are functionally sufficient for the majority of the esterification of the cell-derived cholesterol [9] although confirmatory evidence is lacking.

LCAT and Atherosclerosis—Clinical Studies

Monogenic Disorders—Humans

The human LCAT gene is located in the q12–22 region of chromosome 16. Up until recently, 40 mutations have been reported (HGMD; http://uwcmml1s.uwcm.ac.uk/mg/search/119359.html). Calabresi et al. [11] reported an additional

15 novel mutations more recently. In addition to a marked reduction in HDL-C, other lipoprotein phenotypes in homozygotes and compound heterozygotes further distinguish the LCAT mutations into two distinct syndromes, the complete LCAT deficiency (CLD) and the fish-eye disease (FED). The CLD is characterized by a complete or near-complete deficiency of LCAT activity in the plasma with the absence of cholesterol esterification in all lipoprotein classes. Furthermore, marked increase in total plasma FC/CE ratio and an accumulation of the phospholipid precursor are also hallmarks of the CLD syndrome. Clinically, despite the disruption of the RCT pathway and the severe low level of HDL-C, CLD subjects are paradoxically not particularly predisposed to premature CHD [10, 16–18]. Instead, there is a high prevalence of glomerulopathy in these subjects [19]. Other phenotypes include modest hypertriglyceridemia (HTG), presence of LpX vesicles and mild anemia [20]. In FED, LCAT activity is absent selectively in the HDL fractions. In these subjects, HDL-C is markedly reduced but cholesterol esterification in the apoB-containing lipoprotein particles is relatively preserved. FED patients are not associated with renal complications. However, a number of FED mutations had been found to be associated with premature coronary artery disease (CAD) [21, 22] but the underlying pathogenesis remains obscure.

Although subjects with complete LCAT deficiency appear to be associated with a paradoxical absence of premature CAD in spite of severe HDL deficiency, little is known about the predisposition of partial LCAT deficient subjects based on earlier studies. More recently, Ayyobi et al. [23] reported a 25-year longitudinal follow-up of a large Canadian LCAT deficient kindred concerning their vascular risk. Carotid ultrasound for intima-medial thickness (IMT) and brachial artery flow-mediated dilatation (FMD) were used as surrogate markers for cardiovascular endpoints. These authors reported that, based on two homozygotes, nine heterozygotes, and four unaffected family members, the heterozygotes are associated with pronounced IMT abnormalities including detection of atherosclerotic plaques whereas for the homozygotes, the IMT are only minimally increased. A similar vascular evaluation by IMT in 68 carriers of various known FED mutations showed significant increase in IMT progression in both heterozygotes and homozygotes [24].

Putting it all together, data from families with genetic causes of LCAT deficiency suggest that FED mutations may confer higher risk of CAD than those with CLD. On the other hand, subjects homozygous for CLD mutations are generally not at risk of accelerated CAD but the heterozygotes with partial LCAT deficiency, appear to be more prone. In light of the clinical observations that partial LCAT deficiency being detected in a variety of common diseases known to be at high risk of premature CAD, e.g., diabetes [25, 26], uremia [27], cigarette smoking [28] etc., the proatherogenic potential of partial LCAT deficiency require confirmation as this would form the basis for LCAT being a potential therapeutic target.

The LCAT–Atherosclerosis Controversy

Transgenic Mouse Models

To investigate the role of LCAT in atherosclerosis, a number of animal models have been generated. LCAT transgenic mice have been created by a number of laboratories. As expected, a modest overexpression of the human LCAT gene in mice resulted in a moderate elevation of total plasma HDL-C levels and an increase in the HDL particle size. In case of the human LCAT gene being coexpressed with the human apoA-I transgene, a mouse strain showed a humanlike polymodal size distribution of HDL, modest particle size increase was observed in each individual HDL subfractions attributable to increased accumulation of CE [29]. Meanwhile, there was a modest reduction in fasting plasma TG. These data demonstrated that overexpression of LCAT *in vivo* resu *in vivo* a "favorable" lipid profile. However, transgenic mice with a modest overproduction of LCAT failed to impact on aortic atherosclerotic lesions [30, 31]. On the other hand, high-level overexpression (>100-fold) of human LCAT resulted in not only a marked increase in plasma HDL-C, but there was also appearance of a large-sized, HDL1-like subfraction [32]. Surprisingly, this strain of high-expressor mice showed a paradoxical increase in diet-induced aortic atherosclerosis [33]. On the other hand, the same level of overexpression of human LCAT in rabbits was found associated with a significant reduction in aortic atherosclerosis. More detailed analyses of the lipoprotein changes in the two animal models suggested that the absence of CETP activity in mice may be responsible for the presence of the large HDL fraction and the increased aortic lesions. To test this hypothesis, Foger et al. [34] examined the effect of a concurrent overexpression of CETP in the LCAT transgenic mice in atherosclerosis and found that CETP expression partially attenuated the increase in atherosclerosis seen in the LCAT transgenic mice.

The enzymatic action of LCAT is a critical determinant of the lipid composition of circulating lipoproteins. A tenfold overexpression of the human LCAT gene in mice fed an atherogenic diet (15% of calories from palm oil, 1.0% cholesterol, and 0.5% cholic acid) resulted in a twofold increase in the ratio of saturated+monounsaturated to polyunsaturated CE species in the apoB-containing lipoproteins. However, there was no difference in aortic atherosclerosis determined on the basis of aortic CE content despite a doubling of the saturation index of the apoB-containing particles [31].

In a different experimental system, a short-term overexpression of the LCAT genes appears beneficial when administered to the LDL receptor/leptin double deficient mice. This double mutant serves as a model for combined severe hyperlipidemia, insulin resistance, and leptin deficient-obesity. Furthermore, the mice were also found to have reduced serum platelet-activating factor acetylhydrolase (PAF-AH) activities and paraoxonase 1 (PON1) activities, two oxidative stress markers. Meanwhile, the plasma titre

of oxidized LDL autoantibody, homing of the macrophages and aortic atherosclerosis were increased. Adenovirus-mediated gene transfer of LCAT resulted in a reduction of the titre for the autoantibody to the oxidized LDL and the aortic plaque volume in only 6 weeks. Unlike previous transgenic models, the short-term overexpression of the LCAT gene in this experiment, despite a 64% raise in the LCAT activity, did not result in any significant change in HDL-C, the plasma FC/CE ratio, or the ability of the plasma to increase efflux of cholesterol from cells [35]. Therefore, the observed reduction in atherosclerosis as a result of LCAT-gene transfer was attributed to LCAT playing a significant role in modulating the oxidative stress.

Collectively, hepatic overproduction of LCAT in mouse models have resulted in mixed results in the context of its role in modulating atherogenicity. In the specific situations where beneficial effects were observed, correction of the elevated oxidative stress appeared to be the major mechanism. In the rabbit models, the observed cardioprotective effects of LCAT overproduction may be attributable to the decrease in the proatherogenic apoB-containing lipoprotein particles and the increase in HDL-C. However, changes in oxidative stress markers have not been reported in the rabbit models.

LCAT Knockout Mouse Models

In patients with complete LCAT deficiency, there is no significant increase in the risk of atherosclerosis in spite of the disruption of the RCT pathway and a marked reduction in plasma levels of HDL-C and apoA-I. Plasma very-low-density lipoprotein (VLDL) and LDL fractions are both altered morphologically and compositionally. The apoB-containing lipoproteins are relatively TG-enriched and CE-poor and there is a high prevalence of fasting HTG in LCAT deficient subjects. However, how these dyslipidemic changes might modulate atherogenicity of the lipoproteins remain to be fully elucidated.

A mouse model for LCAT deficiency has been generated by homologous recombination of the murine LCAT gene [36, 37]. The lipoprotein abnormalities in these mice showed remarkable resemblance to those seen in LCAT deficient humans, especially in the changes in HDL. In the homozygous knockout mice (LCAT–/–), plasma CE was markedly reduced to 9.4% of control and there was a 11.7-fold increase in the FC/CE ratio. Both HDL-C and apoA-I levels were reduced in the LCAT deficient mice in a gene dose-dependent manner but the reduction in CE content being dramatically more so than the FC content. The HDL fraction (d = 1.063–1.21 g/mL) was characterized by the presence of particles with a wide distribution in sizes but dominated by a small, 7.6-nm peak. Electron microscopy revealed rouleaux formation from the disc-shaped HDL particles, remarkably similar to those seen in humans [38]. In the d < 1.063 g/mL fractions, vesicle-like particles have also been detected [36, 37]. The presence of compositionally and morphologically altered particles in the LDL and VLDL range is consistent with a direct action of LCAT on these particles.

LCAT knockout mice have been studied to explore the role of LCAT in atherosclerosis as well as to elucidate the possible mechanism for the paradoxical absence of accelerated atherosclerosis in LCAT deficient humans. A recent study by Lambert et al. [39] reported significant reductions in aortic atherosclerosis attributable to LCAT deficiency in a number of different dyslipidemic backgrounds, including wild type, LDL receptor knockout (LDLR–/–), and CETP transgenic mice, fed a cholate-containing, high cholesterol/high fat diet, and in apoE$^{-/-}$ background fed a chow diet. In this study, the authors observed a uniform reduction in the level of apoB-containing lipoproteins in the LCAT-deficient mice across all dyslipidemic backgrounds examined and proposed that the apoB-containing particle levels play a key role in modulating atherosclerosis risk in LCAT deficiency.

A study by Furbee et al. [40] using apoE$^{-/-}$xLCAT–/– and LDLR–/–xLCAT–/– mice fed a cholate-free, atherogenic diet yielded opposite results regarding the impact of LCAT deficiency on atherosclerosis. In this study, LDLR–/–xLCAT–/– double knockout mice and their LDLR–/–xLCAT+/+ single knockout control have similar apoB-containing lipoprotein levels but the LDL–CE in the former are more enriched in the saturated, and putatively more proatherogenic, fatty acids. On the other hand, the apoE$^{-/-}$xLCAT–/– double knockout mice have not only more saturated LDL–CE, but also higher levels of apoB-containing lipoproteins. The reason for the disparate findings between these two studies are not apparent. However, the difference in the composition of the diets used in the studies, and the methods of aortic lesion quantification may play a key role.

More recently, Ng et al. [41] reported the effect of LCAT deficiency on atherosclerosis using the hyperlipidemic apoE$^{-/-}$ as background. The apoE$^{-/-}$xLCAT–/– and apoE$^{-/-}$xLCAT+/+ mice were fed a regular chow diet and the aortic atherosclerosis lesions were compared at 8–9 months of age. The lipoprotein profile by Fast Protein Liquid Chromatography (FPLC) revealed, in the apoE$^{-/-}$xLCAT–/– double knockout mice, a marked reduction in HDL-C and a modest reduction in apoB-containing particles in all VLDL and LDL/IDL (intermediate-density lipoprotein) fractions. These findings are in agreement with those reported by Lambert et al. [39]. In their investigation of the mechanism of the impact of LCAT on atherosclerosis, the authors first observed a significant increase in two independently determined oxidative stress markers, namely the plasma F2-isoprostane level and the aortic superoxide production rate, in both the LCAT–/– mice and the apoE$^{-/-}$ mice. The marked increase in oxidative stress status in the LCAT–/– mice is consistent with a previous study showing that, in this model, there is a significant reduction in plasma PON1 activity and PAF-AH activity, in part due to the marked reduction in plasma HDL level [42]. Surprisingly, the oxidative stress markers in the apoE$^{-/-}$xLCAT–/– were completely normalized to the same level as the wild-type mice despite persistent severe hyperlipidemia. This normalization of the oxidative markers was found associated with a 50% reduction in aortic atherosclerosis lesion size. Intriguingly, the complete normalization of the two

oxidative markers was also found to be associated with a restoration of the total plasma level of PON1 arylesterase activity to that of the wild-type mice, despite a persistent severe HDL deficiency. Detailed FPLC fractionation revealed that the PON1 arylesterase activity is no longer restricted to the HDL fractions but is broadly redistributed to all the non-HDL fractions, the latter accounting for nearly 50% of the total plasma PON1 activity.

The mechanism by which PON1 redistribute in the plasma of the LCAT deficient mice remains unclear. A recent study by Sorenson et al. [43] demonstrated that PON1 can bind to phospholipid vesicles via its hydrophobic N-terminal signal sequence peptide and remains active enzymatically without apoA-I. The authors further demonstrated that HDL-associated PON1 can be transferred to these PL vesicles. The physical characteristics of such synthetic vesicles are not dissimilar to that of LpX, a FC- and PL-rich vesicles frequently found in LCAT deficient humans as has been shown to be present in modest quantity in the apoE$^{-/-}$xLCAT–/– mice. However, the possibility of an association of PON1 with other apoB-containing lipoproteins cannot be ruled out. Similarly, the role of the retained PON1 in the observed dramatic reversal of oxidative markers is also unknown. In light of the previous reports on the impact of PON1 on oxidative stress, especially the *in vivo* murine models [44–46], it is conceivable that the paradoxical normalization of the oxidative markers in the apoE$^{-/-}$xLCAT–/– double knockout mice may, at least in part, be attributed to the redistribution and retention of serum PON1. Similar PON1 arylesterase activity redistribution profile by FPLC is also detected in the LDLR–/–xLCAT–/– double knockout mice (Ng, unpublished data). More importantly, PON1 activity has been detected in the non-HDL fractions from a subject homozygous for an LCAT mutation but is absent from his unaffected siblings (Ng, Connelly, and Frohlich, unpublished data).

Collectively, data to date based on murine models of the impact of LCAT deficiency in atherosclerosis are also mixed but are suggestive of strong gene–gene and possibly gene–diet interactions. They also suggest that oxidative modifications of lipids may play an important role in mediating the effect of LCAT on atherogenesis. In addition to the observed significant alterations in PON1 abundance and distribution as a result of changes in the dyslipidemic background and LCAT gene dose, it is conceivable that other enzymes pertinent to oxidation and perhaps inflammation may also be altered.

The Role of LCAT in the Metabolism of apoB-Containing Lipoproteins

Several lines of experimental evidence suggest that LCAT is active not only on HDL particles, but a small fraction of the total plasma LCAT activity is detected in the LDL particles. In humans, several mutations in the LCAT gene led to a selective loss of transesterification activity in the HDL particles, resulting in clinically a marked reduction in HDL-C levels but relative normal

LDL levels, compositions, and the FC/CE ratio. These subjects with selective LCAT deficiency do not develop renal disease but tend to have more severe clouding of the cornea, hence the term FED [10]. To further address the role of LCAT in the CE content of apoB-containing particles, Furbee et al. [47] examined the lipoprotein compositions of two strains of double knockout mice, namely the apoE$^{-/-}$xLCAT–/– and LDLR–/–xLCAT–/– mice. The removal of functional LCAT in these two strains of hyperlipidemic mice revealed a differential impact on the fatty acid composition of CE in the apoB-containing particles. In the case of LCAT deficiency in the LDLR–/– background, there was a significant loss of linoleate and a complete loss of polyunsaturated fatty acids (PUFA) in the LDL–CE. On the other hand, in the apoE$^{-/-}$ background, LCAT deficiency resulted in only a modest alteration in the LDL–CE fatty acid content. These authors concluded that LCAT contributes to the CE fatty acid pool of apoB-containing lipoproteins and is the only source of plasma long-chain polyunsaturated CE in these mice. In a follow-up study, Zhao et al. [48] found that, in apoE$^{-/-}$xapoA-I–/– mice, the dramatic reduction in plasma cholesterol esterificaton rate (CER) is disproportionate to the more modest reduction in the LCAT mass. However, incubation of the VLDL from these double knockout mice with apoE resulted in a threefold increase in CER whereas a similar incubation with apoA-I only resulted in a modest 80% increase. The authors concluded that apoE is a more significant physiologic activator of LCAT than apoA-I-containing lipoproteins.

The Role of LCAT in Triglyceride and Glucose Metabolism

HTG is a risk marker for CHD based on epidemiologic studies but the pathogenesis of how TG-rich lipoproteins contribute to the atherosclerosis is frequently confounded by other associated risk factors. HTG is a common feature in a number of highly proatherogenic conditions, namely type 2 diabetes mellitus, metabolic syndrome, and familial combined hyperlipidemia [49], in which low HDL-C, insulin resistance, central obesity are frequently present. The dyslipidemia in these conditions share the cardinal feature of a hepatic overproduction of VLDL/apoB as contributor to the HTG, which in turn promotes the generation of small dense LDL and lowering of HDL-C through CETP. HTG has also been documented in LCAT deficient subjects, both in homozygotes and heterozygotes but due to the small number of subjects in this rare syndrome [11, 50, 51], the pathophysiologic relevance and pathogenesis of HTG remain poorly understood. In animal models of either LCAT overexpression or deficiency, an inverse association between the LCAT gene dose and fasting TG levels has begun to emerge. Transgenic mice [29] and rabbits [52] overexpressing the human LCAT gene have been reported to have a modest reduction in fasting TG levels. Gene-targeted mice deficient in

LCAT activities are associated with elevated TG in a gene dose-dependent manner [36, 37]. Collectively, these data are in support of a mechanistic link between LCAT deficiency and HTG. In humans, a partial reduction in postheparin lipase activity has been observed in association with a fasting HTG [53], other possible contributions have not been explored.

The possible mechanism of HTG in complete LCAT deficient mice was recently reported by Ng et al. [54] using the LDLR–/–xLCAT–/– double knockout mice, which showed modest fasting HTG when compared with the LDLR–/–xLCAT+/+ single knockout control. FPLC lipid profile showed accumulation of VLDL particles in the double knockout mice and the apoB-containing lipoproteins (VLDL/IDL/LDL fractions) are all relatively TG-enriched. Ultracentrifuge-isolated VLDL fractions are, at least semiquantitatively, better substrate for the exogenous (bovine) lipoprotein lipase. On the other hand, postheparin lipase activity assay revealed a significant isolated reduction in the LPL activity, consistent with those seen in human subjects. More intriguingly, the double knockout mice were found to have a marked increase in hepatic TG production rate, a metabolic alteration not seen in other models of primary severe low HDL syndromes. This was further found to be associated with an upregulation of the hepatic SREBP1 gene, which was further linked to a coordinated upregulation of a number of target genes encoding for enzymes in lipogenesis on the one hand, and a downregulation of PEPCK1, a gene for the rate-limiting enzyme for gluconeogenesis on the other. The change in the fasting PEPCK mRNA level was further found to be associated with a reduction in fasting glucose and fasting insulin levels in the double knockout mice, consistent with the notion of an improvement in hepatic insulin sensitivity and reduced gluconeogenesis. Although both *srebp1* and *pepck1* gene are known as target genes for the transcription factor liver X receptor α (LXRα) [55, 56], survey of the expression levels of a number of other specific LXRα target genes suggests that the SREBP1 upregulation in this LCAT deficiency mouse model is unlikely to be driven by LXR. On the other hand, the LDLR–/–xlcat–/– mouse LDL were recently shown to be markedly depleted of linoleic acid moiety and are virtually completely devoid of other PUFA [47]. In light of the suppressive effect of PUFA on *srebp1* mRNA level shown by many laboratories [57, 58], the authors postulated that the lack of PUFA in LDL in the double knockout mice might have resulted in a reduction in the abundance of PUFA in the liver, leading to less suppression of the SREBP1 gene expression. In short, the findings presented here suggest that LCAT deficiency may constitute a unique metabolic milieu in which an increase in hepatic TG production, possibly through an induction of hepatic SREBP1 expression and the downstream lipogenesis, acts in concert with the reduction in postheparin lipase activity to cause HTG. This pathway represents first to link a primary HDL deficiency state to HTG through hepatic TG overproduction and, putatively through increased lipogenesis, may extend our mechanistic view of the yin/yang relationship between HTG and low HDL-C seen in many proatherogenic states.

Conclusion

LCAT plays a central role in RCT and is a major modulator of plasma HDL-C levels as well as compositions and levels of apoB-containing lipoproteins. However, its role in atherosclerosis continues to be controversial. Studies in LCAT deficient humans with monogenic LCAT gene defects continue to be consistent with the notion of a lack of accelerated atherosclerosis in those with complete LCAT deficiency. On the other hand, based on recent studies with larger cohorts of subjects who are either heterozygotes for CLD mutations or both heterozygotes and homozygotes for FED mutations, suggest that partial LCAT deficiency may be proatherogenic. Data from animal models are also conflicting. LCAT knockout mice have been shown to be proatherogenic and antiatherogenic. Although these studies were carried out by different laboratories using slightly different experimental protocols, the contradictions are not easily reconciled. On the other hand, the significant impact of LCAT deficiency on oxidative status seen in the LCAT knockout mice underscores the complex functional impact of LCAT on not only lipoprotein metabolism but also in oxidative stress, the latter likely plays important roles in atherogenesis. Likewise, the recent discoveries of how LCAT deficiency in the murine model significantly impact on intrahepatic TG, fatty acid, and glucose metabolism further exemplifies the multiplicity in the phenotypic changes in response to a reduction in the enzymatic activity. In light of the common occurrence of partial LCAT deficiency in a number of common proatherogenic disorders like diabetes, renal insufficiency etc., a better understanding of the pathophysiologic impact of this metabolic disorder at the molecular level would enable improved and targeted therapeutic strategies.

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