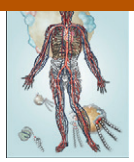

Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases

Lecithin:cholesterol acyltransferase: old friend or foe in atherosclerosis?

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Abstract **Lecithin:cholesterol acyltransferase (LCAT) is a key enzyme that catalyzes the esterification of free cholesterol in plasma lipoproteins and plays a critical role in high-density lipoprotein (HDL) metabolism. Deficiency leads to accumulation of nascent preβ-HDL due to impaired maturation of HDL particles, whereas enhanced expression is associated with the formation of large, apoE-rich HDL_1 particles. In addition to its function in HDL metabolism, LCAT was believed to be an important driving force behind macrophage reverse cholesterol transport (RCT) and, therefore, has been a subject of great interest in cardiovascular research since its discovery in 1962. Although half a century has passed, the importance of LCAT for atheroprotection is still under intense debate. This review provides a comprehensive overview of the insights that have been gained in the past 50 years on the biochemistry of LCAT, the role of LCAT in lipoprotein metabolism and the pathogenesis of atherosclerosis in animal models, and its impact on cardiovascular disease in humans.**—Kunnen, S. and M. Van Eck. **Lecithin:cholesterol acyltransferase: old friend or foe in atherosclerosis?** ***J. Lipid Res.*** **2012.** 53: **1783–1799.**

Supplementary key words cholesterol • high density lipoprotein • LCAT

In 1935, Sperry was the first to recognize that when human plasma was incubated at 37°C, a marked esterification of free cholesterol occurred (1). He attributed this to enzymatic activity, as the effect was abolished when the plasma was heated to 55–60°C. Subsequent work by Glomset led in 1962 to the identification of the lecithin:cholesterol acyltransferase (LCAT) enzyme, which accounts for the synthesis of most of the cholesterol esters in plasma (2). Four years later, Glomset identified LCAT as an important driving force behind the reverse cholesterol transport (RCT) pathway (3), a process that describes the HDL-mediated removal of excess cholesterol from macrophages in the arterial wall and subsequent delivery to the liver for biliary excretion. Interest in the enzyme increased even further when in 1967 the first family with three sisters with familial LCAT deficiency was described (4). To date, approximately 60 isolated cases and 70 small families with partial or complete LCAT deficiency have been described with 86 different molecular defects in the LCAT gene (5) (http://www.hgmd.org). In addition, numerous animal models lacking or overexpressing LCAT, including mice (6–10), hamsters (11), rabbits (12), and monkeys (13) have been generated to gain better insight in the complex role of LCAT in modulating lipoprotein metabolism, RCT, and atherosclerosis.

LCAT STRUCTURE AND FUNCTION

The human LCAT gene is localized in the q21–22 region of chromosome 16. It consists of 6 exons separated by 5 introns and encompasses a total of 4.2 kb (14, 15). In 1986, it was sequenced and cloned for the first time (16, 17). The gene encodes for a polypeptide chain, consisting of 416 amino acid residues with an apparent molecular mass of approximately 60 kDa. LCAT is a glycoprotein with four N-glycosylation (Asn20, 84, 272, and 384) and two O-glycosylation sites (Thr407 and Ser409) (18). The carbohydrate content is ~25% of its total mass, with the majority being N-linked (19). Removal of the carbohydrate moieties of isolated human LCAT by neuraminidase is associated with a 60% increase in the enzymatic activity (20). However, inhibition of glycosylation in Chinese hamster ovary (CHO) cells reduced the enzymatic activity without

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Abbreviations: CETP, cholesterol ester transfer protein; cIMT, carotid intima-media thickness; CVD, cardiovascular disease; FED, fish-eye disease; FLD, familial LCAT deficiency; Lp-X, lipoprotein X; ndHDL, nascent discoidal HDL; RCT, reverse cholesterol transport; SR-BI, scavenger receptor BI.

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affecting LCAT protein secretion (19). The biological significance of the two O-glycosylation sites is largely unclear. Site-directed mutagenesis studies in transfected COS-6 cells by substitution of Asn with Thr showed that N-linked glycosylation at Asn272 is indispensible for secretion of active LCAT, whereas Asn84 is critical for its full activity but not for intracellular processing (21). In another study by Karmin and colleagues, the effect of substitution of the Asn residues with Gln was investigated in COS-1 cells (22). These studies showed that glycosylation at all four sites is required to generate the full-size mature LCAT protein, but deletion of only one of the N-linked glycosylation sites does not affect intracellular processing and secretion. The pattern of N-linked glycosylation, however, has profound effects on the catalytic activity of the enzyme. Substitution of Asn84 or Asn272 with Gln led to 82% and 62% decrease in activity, respectively, whereas replacement of Asn384 led to substantially increased activity (21). Furthermore, N-glycosylation is important for determining substrate specificity toward native HDL and LDL (23).

No X-ray structure of the LCAT protein has been published. By use of selective chemical modification and stoichiometric analysis, it was shown that a single serine and a single histidine mediate lecithin cleavage and that the two free cysteines participate as transient fatty acyl acceptors in cholesterol esterification (24–26). The first structural model of LCAT was proposed by Yang and colleagues in 1987 based upon the primary structure, chemical modification, homology with other proteins, and enzymatic assays (27). Furthermore, 3D modeling based on its homology with other members of the αβ-hydrolase superfamily has shown that LCAT contains a catalytic triad consisting of three amino acid residues, serine (Ser), aspartic acid (Asp), and histidine (His) at positions 181, 345, and 377 (28–30). Recently, using a combination of several new and improved fold-recognition methods, Holleboom and colleagues confirmed this model with, according to the new protein nomenclature, residues Ser205, Asp369, and His401 forming the catalytic triad of LCAT (31). LCAT contains six cysteine residues, of which four are located at the active site of the protein and are used to form two disulfide bridges (Cys50–Cys74 and Cys313–Cys356) (27, 32). The disulfide-linked Cys50–Cys74 residues span the lid region of LCAT that covers the catalytic site of LCAT and opens upon binding to lipoprotein surfaces (33).

The human LCAT protein is synthesized primarily by the liver, although it is also expressed in small amounts in the testes and in astrocytes in the brain, where it is involved in the esterification of cholesterol in glia-derived apoE-containing lipoproteins (15, 27, 34, 35). The plasma concentration of LCAT is about 6 μg/ml and varies little in adult humans with age, gender, and smoking (36). The half-life of human LCAT in plasma has been estimated to be 4–5 days (37) LCAT reversibly binds to lipoproteins and is primarily found on HDL, which likely prevents its rapid clearance from the circulation (38). ApoAI is the most potent activator of LCAT, which enables it to convert free cholesterol into cholesteryl esters on HDL by a transesterification reaction involving the transfer of a fatty acid at the *sn*-2 position of phosphatidylcholine (or lecithin) to the free hydroxyl group of cholesterol (15, 39). During this reaction, lecithins are converted into lysophosphatidylcholines. The transfer process occurs in multiple steps. First, apoAI "activates" the phospholipid-cholesterol bilayer by concentrating the lipid substrates near LCAT and presenting it in an optimal conformation to LCAT. The conformation of these apoAI complexes is affected by the fluidity of the lipid bilayer (40, 41). Furthermore, the binding of LCAT to the apoAI bilayer is influenced by the size and charge of the HDL particles (42). The second step involves the cleavage of the *sn*-2 ester bond of lecithin, leading to the release of a fatty acyl (24). This step is mediated by the phospholipase activity of LCAT and depends on the lecithin composition (42). The last step includes the transacylation of the fatty acyl moiety to the sulfur atom of a cystein residue forming a thioester, which subsequently donates its fatty acyl to the 3β-hydroxy group of the cholesterol molecule, thereby forming cholesteryl ester (24). In addition to apoAI, other apolipoproteins, such as apoAII, apoAIV, apoCI–III, and apoE, can activate LCAT, although less efficiently (43). Two distinct types of LCAT activity can be distinguished: α and β. α-Activity describes the enzymatic activity of LCAT toward cholesterol bound to apoAI-containing lipoproteins (e.g., HDL particles). β-Activity constitutes the enzymatic activity of LCAT toward cholesterol bound to apoB-containing lipoproteins (e.g., VLDL and LDL particles) (44). The equilibrium dissociation constants (Kd) for the interaction of pure human LCAT with LDL, HDL_2, HDL_3, and reconstituted discoidal HDL (rHDL) are as follows: rHDL = $HDL_3 \leqslant HDL_2 <$ LDL with relative reactivities (app. V_{max}/app. K_m) of 100, 16, 1, 6%, respectively (45). Hence, only a minority of LCAT in the circulation is bound to apoB-containing lipoproteins.

LCAT: MAIN DRIVING FORCE BEHIND REVERSE CHOLESTEROL TRANSPORT?

Already in 1966, Glomset identified LCAT as an important driving force behind the RCT pathway (3), a process that describes the HDL-mediated removal of excess cholesterol from peripheral tissues, including macrophages from the arterial wall, and subsequent delivery to the liver for biliary excretion (**Fig. 1**).

The first step of the RCT pathway involves production of apoAI in the liver or intestine that is then released into the plasma (46). Interaction with ATP-binding cassette transporter (ABC)A1 on primarily the liver and intestine induces the formation of nascent discoidal HDL (ndHDL) particles that can stimulate cholesterol efflux from macrophages in the arterial wall (47, 48). Upon association of cholesterol with the ndHDL particle, it is esterified by LCAT, leading to partitioning of the cholesterol esters into the core of the particle and conversion of the ndHDL into a more mature HDL_3 particle. This particle subsequently is able to induce efflux of cellular cholesterol via ABCG1 and SR-BI (47, 48). Upon further enrichment of the HDL

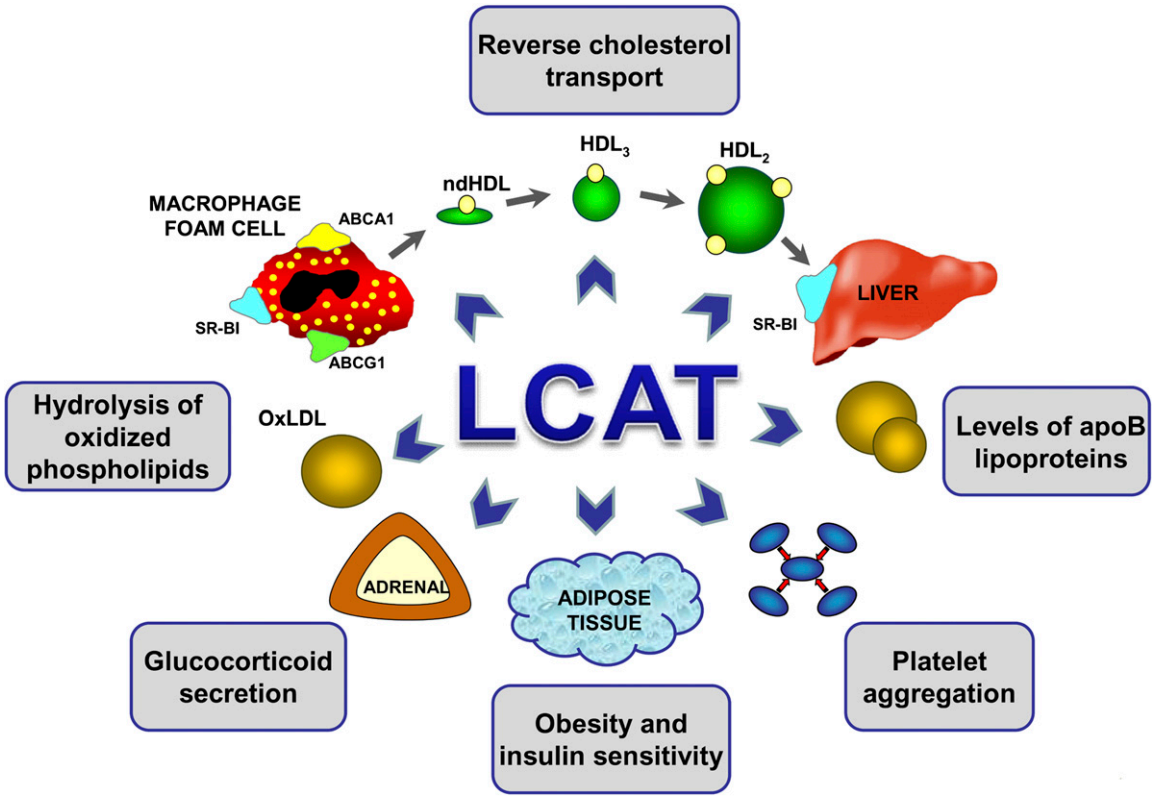

Fig. 1. LCAT functions potentially affecting the pathogenesis of cardiovascular disease. LCAT is considered an important driving force behind the RCT pathway, a process that describes the HDL-mediated removal of excess cholesterol from peripheral tissues, including macrophages in the arterial wall, and subsequent delivery to the liver for biliary excretion. Upon association of cholesterol with ndHDL particles, it is esterified by LCAT, leading to partitioning of the cholesterol esters into the core of the particle and conversion of the ndHDL into mature HDL_3 or HDL_2 particles. Upon esterification of cholesterol in HDL, LCAT maintains the gradient of free cholesterol between the cellular membrane and the surface of the HDL particle, which is thought to generate a continuous flow of cholesterol from the cell to lipoproteins and prevent the transfer of cholesterol back to the cell. In addition to its essential role in the first step of the RCT pathway, LCAT is suggested to enhance the delivery of cholesterol to the liver. Furthermore, LCAT directly or indirectly interferes with several other physiological processes that might affect the development of atherosclerosis, including HDL antioxidant function by hydrolyzing oxidized phospholipids in oxidized LDL (oxLDL), adrenal steroidogenesis, insulin sensitivity and protection against obesity, and platelet function.

particles with cholesteryl ester, they are transformed into larger HDL_2 particles (46). Several studies indicate that LCAT activity decreases upon enlargement of the HDL particle, particularly on large apoE-rich HDL_1 particles (49–52). This might be a direct effect of the fact that LCAT is subject to product inhibition (45), but it has also been suggested that sphingomyelin enrichment of HDL prevents binding of LCAT to the lipoprotein (49, 53). Importantly, upon esterification of cholesterol in HDL, LCAT maintains the gradient of free cholesterol between the cellular membrane and the surface of the HDL particle, which is thought to generate a continuous flow of cholesterol from the cell to lipoproteins and prevent the transfer of cholesterol back to the cell (54–56). The latter (i.e., prevention of reuptake of cholesterol by the cell) is nowadays considered the most important pathway via which LCAT stimulates HDL-mediated efflux. Importantly, it is also postulated that the effect of LCAT on the flux of cholesterol may depend both on the type and metabolic status of the cells, and on the environment of HDL in the extracellular medium (57). Therefore, in addition to its essential role in the first step of the RCT pathway, LCAT is suggested to enhance the delivery of cholesterol to the liver (57).

However, there are several reasons to question whether LCAT is the main driving force for the RCT pathway. First, LCAT-deficient patients only show accumulation of cholesterol in specific cells/organs, including erythrocytes, kidney, and cornea (58). Second, passive diffusion was previously considered the predominant mechanism of cellular cholesterol efflux, but now facilitated transport via ABCA1, ABCG1, and SR-BI is recognized to be essential (47, 48). Although cellular cholesterol efflux via SR-BI is dependent on the cellular free cholesterol gradient (59), active ATP-powered transport via ABCA1 and ABCG1 is not and, hence, is less likely to be dependent on LCAT activity. Third, adenoviral-mediated overexpression of murine LCAT in livers of C57Bl/6 mice did not stimulate cholesterol efflux from extraheptic tissues despite the accumulation of large cholesteryl ester-rich HDL particles in the circulation (60). Fourth, human LCAT overexpression in mice does not enhance macrophage RCT in vivo, and LCAT-deficient mice display largely preserved macrophage RCT despite marked reductions in plasma HDL cholesterol (61). Fifth, serum from carriers of LCAT gene mutations has the same capacity as control serum to decrease the cholesterol content of cholesterol-loaded macrophages

due to a greater cholesterol efflux capacity via ABCA1 (62, 63). Finally and most importantly, the role of LCAT as an atheroprotective factor is under debate.

OTHER FUNCTIONS OF LCAT POTENTIALLY AFFECTING CVD

LCAT also transesterifies and hydrolyzes platelet-activating factor and oxidized phospholipids with long chains in the *sn*-2 position (64–66). Thus, LCAT is expected to contribute to the antioxidant/anti-inflammatory properties of HDL (67). In agreement, in contrast to HDL from healthy volunteers, HDL from LCAT heterozygotes (n = 12) is unable to inhibit the oxidation of LDL, whereas HDL from compound heterozygotes (n = 3) and a homozygote appeared to induce more oxidation (68). Furthermore, HDL of LCAT heterozygotes, compound heterozygotes, and a homozygote had 50% ($P = 0.040$), 166% ($P < 0.0001$), and 1280%, respectively, higher levels of malondialdehyde than controls, indicating increased oxidation. The HDL-associated LCAT activity was decreased by 39% in heterozygotes, and 94% and 80% in the compound heterozygotes and homozygote, respectively. In the latter groups, however, the HDL-associated PAF-AH activity was also reduced by 54%. Isolated human HDL contains some 50 different proteins (69) and a complex range of 100 small peptides (70). Considering that the consequences of LCAT deficiency for the complete HDL proteome are unknown, it is currently unclear whether the effects of LCAT deficiency on the antioxidant properties of HDL are a direct effect of the impaired LCAT function or due to other anomalies of the HDL proteome. However, because oxidation of plasma lipoproteins is an important event in the formation of atherogenic particles, impaired LCAT function is anticipated to induce the pathogenesis of atherosclerosis.

Recently, evidence was provided that SR-BI-mediated uptake of cholesterol from HDL by the adrenal is essential to acquire cholesterol for the production of glucocorticoids, both in mice and in humans (71, 72). Adrenals from LCAT-deficient mice are severely depleted of cholesterol stores, similarly as previously reported for SR-BI knockout mice (10). As LCAT is not expressed in adrenal (27), the most likely cause for the reduced lipid content of the adrenal is the severe depletion of plasma HDL cholesterol. In humans, LDL receptor-mediated uptake of LDL was long considered the primary pathway for delivery of cholesterol from the circulation to the adrenal. However, recently we found that subjects with an SR-BI c.889C→T missense gene variant, which leads to a proline-to-serine substitution at the highly conserved position 297 (P297S), showed attenuated adrenal steroidogenesis, indicating that also in humans SR-BI-mediated uptake of lipoproteins, likely HDL, is essential for adrenal function (71). Because glucocorticoids have important anti-inflammatory properties, altered glucocorticoid production in response to inflammation in the arterial wall might influence the progression of the disease. It is currently unknown whether LCAT-deficient patients suffer more frequently from adrenal insufficiency. However, carriers of LCAT mutations display lower total urinary 17-ketogenic steroids and 17-hydroxycorticoids (73).

LCAT deficiency in mice is associated with enhanced insulin sensitivity (74, 75). Furthermore, recently it was reported that LCAT-deficient mice, especially females, are protected against high-fat high-sucrose (HFHS) diet-induced obesity (76). These protective metabolic phenotypes are associated with protection against diet-induced hepatic and adipocyte endoplasmic reticulum (ER) stress, but the mechanistic link with the enzymatic action of LCAT needs further investigation. Currently it is unknown whether LCAT-deficient (female) patients are more insulin sensitive and/or protected against obesity. An early study showed that both fractional and molar LCAT rates were positively correlated with obesity in women but not in men (77). However, another more recent study found increased plasma LCAT in obese individuals of both sexes (78). Furthermore, the plasma LCAT activity level was shown to be positively related to insulin resistance in association with a higher body mass index (BMI) in a group of 32 Dutch men (79). In the IMPROVE study with individuals at high risk for developing cardiovascular disease, no link between LCAT quartiles and BMI or occurrence of diabetes was found (80). Considering that obesity is the epidemic of the twenty-first century and is a prominent risk factor for cardiovascular disease, the link between LCAT, insulin resistance, and obesity warrants further investigation.

Acute coronary events are not the result of progressive growth of the lesion but, rather, of lesion disruption and superimposed thrombus formation in which platelets are key elements. Platelets from two LCAT-deficient patients did not show alterations in the cholesterol: phospholipid ratio (81), indicating that impaired cholesterol esterification does not raise free cholesterol in the platelets. However, binding of thrombin, a strong activator of platelet aggregation, was elevated in platelets from LCAT-deficient patients (n = 2). In agreement, one patient showed increased platelet thrombin-induced aggregation responses, but aggregation in another was decreased. The effects of LCAT deficiency on platelet activation are thus inconclusive, and studies in more patients and characterizing the different aspects of platelet function, including, for example, aggregation responses to different agonists and adhesion under flow, are awaited.

In summary, in addition to its role in RCT, LCAT directly or indirectly interferes with several other physiological processes that might affect the development of atherosclerosis (Fig. 1). Future studies delineating the exact contribution of the effects of LCAT on HDL antioxidant/anti-inflammatory function, adrenal steroidogenesis, insulin sensitivity and protection against obesity, and platelet function are expected to shed more light on the importance of these processes compared with LCAT's role in RCT for the pathogenesis of cardiovascular disease.

FAMILIAL LCAT DEFICIENCY AND FISH-EYE DISEASE

LCAT deficiency syndromes are rare metabolic disorders with an autosomal recessive mode of inheritance (82) that arise as a consequence of either a defect in the enzyme or defects in its synthesis and/or secretion. Individuals with deleterious mutations on both alleles present with HDL deficiency, whereas heterozygotes typically have HDL cholesterol levels that are half of normal HDL cholesterol (82, 83). Two important LCAT deficiency syndromes can be distinguished: *i*) familial LCAT deficiency (FLD) and *ii*) fish-eye disease (FED) (**Fig. 2**). FLD was reported for the first time in 1967 in a Norwegian family. In this family, three adult sisters showed extremely low plasma LCAT activity, reduced levels of cholesteryl esters, and reduced plasma LCAT concentration (4). The clinical features included proteinuria, normochromic anemia, corneal opacity (84), and turbid milky plasma. Furthermore, foam cells were found in the bone marrow and kidney, and lipid alterations were found in the erythrocytes (85). Years later, it was established that homozygosity for a single nucleotide substitution in codon 252 of exon 6 in the gene, leading to the exchange of a methionine (ATG) for a lysine residue (AAG), was responsible for the LCAT deficiency in this family (86). In vitro experiments showed that this mutation led to the production of a fully inactive LCAT enzyme (87). In general, FLD is characterized by HDL deficiency and lipid changes in both VLDL and LDL levels. Important clinical features are corneal opacification, mild anemia, and progressive loss of renal function (82).

Partial LCAT deficiency leads to fish-eye disease, a less severe syndrome compared with FLD. The name refers to a symptom that FED as well as FLD patients often develop: the eyes resemble the eyes of boiled fish due to age-dependent opacification of the cornea (88). Other characteristics of FED are low HDL cholesterol, elevated triglyceride (TG) levels, and multiple lipoprotein abnormalities (88–91). The most significant difference between the two LCAT deficiency syndromes is that patients with FLD suffer from a complete lack of LCAT activity, whereas patients with FED have a partial reduction in LCAT activity (88, 89). The latter have LCAT with a reduced ability to esterify cholesterol bound to HDL, but esterification of cholesterol bound to other lipoproteins (e.g., VLDL and LDL) is normal. Thus, the α-activity of LCAT is lost, while its β-activity is preserved (92).

In 1997, Kuivenhoven et al. proposed a new classification system for natural LCAT mutations based on the different biochemical and clinical phenotypes observed in LCAT-deficient patients, as well as the biochemical characteristics of mammalian cell cultures expressing the mutants of the LCAT gene (44). Importantly, this new system allows the classification of heterozygous mutations that led to confusion in the previous FLD/FED classification system (93, 94). Intermediate phenotypes due to mutations not causing a total loss of LCAT activity (FLD) but causing more than "just" partial loss of LCAT activity against HDL (FED) are also taken into account (95, 96). For the in vivo classification, five criteria were proposed: *i*) LCAT activity toward HDL analogs; *ii*) cholesterol esterification rate (endogenous lipoproteins); *iii*) ratio of plasma FC to EC;

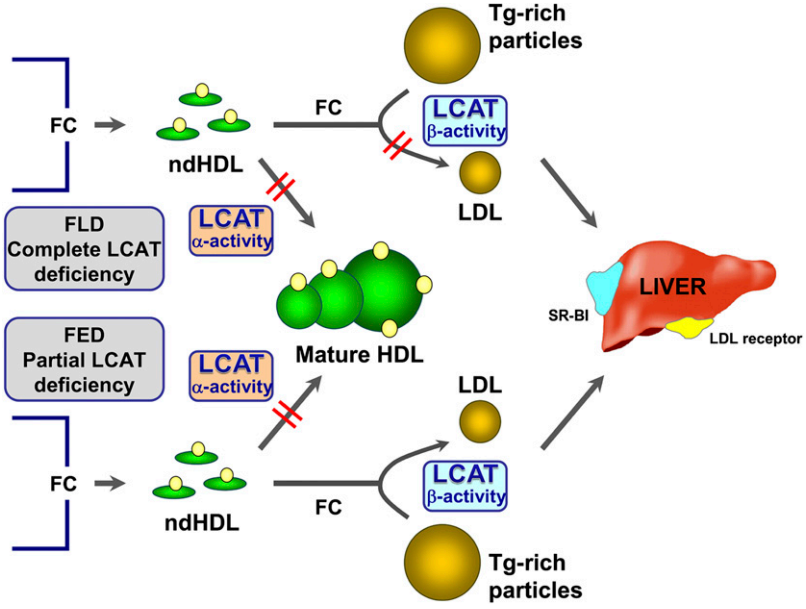

Fig. 2. LCAT deficiency syndromes. Two important LCAT deficiency syndromes can be distinguished: *i*) familial LCAT deficiency (FLD) and *ii*) fish-eye disease (FED). The most significant difference between the two syndromes is that patients with FLD suffer from a complete lack of LCAT activity, whereas patients with FED have a partial reduction in LCAT activity. Both FED and FLD patients have a reduced ability to esterify cholesterol bound to HDL (i.e., impaired α-activity). In contrast to FLD, in FED esterification of cholesterol bound to other lipoproteins like VLDL and LDL (β-activity of LCAT) is preserved. Adapted from Hill (87).

JOURNAL OF LIPID RESEARCH ASBMB

iv) plasma LCAT concentration; and *v*) clinical symptoms of the disease. For the in vitro experiments, two criteria were proposed: *i*) specific activity of mutant protein toward HDL analogs and native LDL and *ii*) activity of mutant protein toward heat-inactivated plasma (44). The classification system describes five classes. The first class contains null mutations of the LCAT gene. This means that patients in this class display a total loss of catalytic activity of LCAT and that they have the clinical phenotype of FLD. The second class contains missense mutations that cause complete or nearly complete loss of catalytic activity of the LCAT gene. The third class contains both missense mutations and minor deletions in the LCAT gene that are responsible for an intermediate phenotype, meaning that there is either partial loss of activity against LDL or combined partial loss of activity against both HDL and LDL. This class thus also includes patients who show FED symptoms but do not develop all the symptoms of the FLD syndrome. The fourth class contains the mutations responsible for the typical symptoms of the FED syndrome. This class thus includes missense mutations that result in specific loss of activity against HDL analogs, but activity against LDL or other apoB-containing lipoproteins is preserved. The fifth class contains three mutations that the group of Kuivenhoven et al. was unable to categorize; therefore, this category was named "unclassified mutations."

To date, approximately 60 isolated cases and 70 small families with partial or complete LCAT deficiency have been described with 86 different molecular defects in the LCAT gene (5) (http://www.hgmd.org). LCAT deficiency is a rare disorder. In general, the prevalence of LCAT mutations in subjects with low HDL cholesterol has been estimated at 2–16% (97–100) in Finnish and Caucasian Canadian patients, respectively. In the recently published results of the Copenhagen City Heart Study, in which the regulatory and coding regions of LCAT were resequenced in individuals with the 2% lowest (n = 180) plasma HDL cholesterol levels, no carriers of loss-of-function mutations in LCAT were identified, indicating that these are extremely rare in the general population (101). In the Netherlands, however, functional mutations in LCAT were found in almost one third (29%) of patients with low HDL cholesterol, thus constituting a common cause of low HDL cholesterol in referred patients in this country (31). Interestingly, a recent genome-wide association study (GWAS) in more than 100,000 individuals of European ancestry identified a single-nucleotide polymorphism (SNP) in LCAT as the strongest marker of isolated variation in HDL cholesterol levels (102).

LCAT AND ATHEROSCLEROSIS IN HUMAN STUDIES

As LCAT was considered the main driving force in the RCT pathway, it was soon thought that the enzymatic activity of LCAT could be involved in the protection against atherosclerotic lesion formation. In 1973, Hovig and Gjone demonstrated lipid deposition in renal arteries and veins of patients with FLD (103). Furthermore, the spleen contained numerous lipid-laden cells that are assumed to be partly responsible for the splenomegaly found in FLD patients. Notably, only 35% of the total cholesterol was esterified in arterial lipid depositions of FLD patients compared with 75% in normal atheromas. In 1982, Carlson showed that FED patients did not suffer from premature atherosclerosis in spite of the extremely low HDL cholesterol levels (89). This was surprising, as FED patients have very low levels of HDL, and it thus was expected that these patients would have an increased risk for atherosclerosis. Four years later, it was demonstrated that patients with atherosclerosis (one- to three-vessel disease) had increased LCAT levels compared with healthy controls (clinical out-patients or hospital personnel) and patients without atherosclerosis (zero-vessel disease) (104). Furthermore, LCAT activity was augmented with increased severity of coronary atherosclerosis. The results of this study suggest that increased, rather than decreased, plasma LCAT activity is characteristic of coronary atherogenesis. Since then, the role of LCAT in the pathogenesis of atherosclerosis has been under debate.

LCAT and atherosclerosis in human FED and FLD patients

Atherosclerosis susceptibility has been extensively investigated in FED and FLD patients (see **Table 1** for overview). Strikingly, in several of the studies no differentiation was made between the phenotypic differences of FED and FLD patients. LCAT activity is clearly reduced in FED and FLD patients, with the largest effects in patients with a homozygous LCAT mutation. FLD and FED patients present with nearly complete HDL deficiency. However, heterozygotes for LCAT mutations typically present with 35–50% reductions in HDL cholesterol levels. Furthermore, in several studies, homozygous LCAT deficiency was associated with a reduction in total cholesterol and LDL-C, although the findings differ largely between the individual studies. In a number of the studies (but not all) a remarkable increase in serum triglycerides was also found, especially in homozygous carriers of an LCAT mutation. Low HDL cholesterol levels are frequently associated with elevated levels of triglyceride-rich lipoproteins in the general population (105). Conversely, postprandial and fasting hypertriglyceridemia lead to low HDL (106, 107). The direct cause of the increased triglycerides in some LCAT-deficient patients has not been extensively studied. One study by Frohlich and colleagues showed that postheparin lipoprotein lipase activity was reduced in two homozygous LCAT-deficient patients (108, 109), but it is unknown whether triglyceride absorption or synthesis was affected in these patients.

Considering the low HDL cholesterol levels and often increased triglyceride levels, one would expect that carriers of LCAT mutations would be at increased risk for developing cardiovascular disease. Indeed, cases have been described indicating dramatically increased atherosclerotic burden. Scarpioni and colleagues described in 2008 an interesting patient with FLD, who developed severe vascular disease, as evidenced by lower limb peripheral arterial

TABLE 1. Effects of LCAT loss-of-function mutations on the development of atherosclerosis in humans

Study	FED/FLD	Atherosclerosis-Related Effect	
(103)	FLD	Lipid deposition in renal arteries and veins	Antiatherogenic?
(89)	FED	No signs of premature atherosclerosis	?
(145)	FED	No signs of atherosclerosis in FED patients	?
(146)	FED	No signs of atherosclerosis in FED patients	?
(96)	FED	Premature coronary artery disease observed in homozygous male probands	Antiatherogenic
(147)	FED	Patient with premature coronary artery disease in the absence of other risk factors	Antiatherogenic
(148)	FLD	FLD patient associated with marked atherosclerosis	Antiatherogenic
(109)	FLD	Heterozygosity for LCAT deficiency associated with increased cIMT	Antiatherogenic in heterozygotes
(149)	FLD+FED	No signs of premature cardiovascular disease	?
(83)	FED	Increased cIMT in heterozygotes	Antiatherogenic
(97)	FLD	Severe vascular disease with peripheral arterial obstruction and occlusive coronary artery disease	Antiatherogenic
(110)	FED+FLD	Decreased cIMT	Proatherogenic
(111)	FED+FLD	Carriers of LCAT gene mutations have increased carotid atherosclerosis compared with controls	Antiatherogenic
(100)	FED+FLD	Increased risk for CAD with HDL< 5^{th} percentile	Antiatherogenic if HDL < 5^{th} percentile

obstruction with necrosis of two toes of his left foot and angina at rest with trivasal occlusive coronary artery disease at coronarography (110). Eventually this patient died at the age of 42 after a right femoral-axillo artery bypass and a thigh amputation. This is a single case illustrating that LCAT-deficient patients can suffer from premature atherosclerosis. As indicated in Table 1, cardiovascular disease (CVD) and increased carotid intima-media thickness (cIMT), a surrogate marker of CVD, have been reported in several other FLD and FED patients. However, many patients do not show an apparent increased risk for developing clinically apparent disease. Very recently, Tietjen et al. reported that 16 out of 38 (42.1%) LCAT loss-of-function mutation carriers from Dutch and Canadian descent with HDLc < 5^{th} percentile had CAD, whereas no significant increase in risk was observed in mutation carriers with HDLc > 5^{th} percentile (100).

The effects of LCAT deficiency on cIMT have been studied using ultrasound imaging in three relatively large studies. In 2004, Ayyobi and colleagues published a 25-year follow-up study of a Canadian kindred with two homozygous (average age 42 at the end of the study) and nine heterozygous (average age 39) FLD patients (109). Over the 25 years of the study, there had been no vascular events or deaths in the family, but this might be due to the relatively young age of the studied individuals. In the two homozygotes, the cIMT was above the 75^{th} percentile expected for age and gender (0.721 mm versus expected 0.647 mm). However, the cIMT abnormalities were much more pronounced in the heterozygotes (0.898 ± 0.296 mm versus expected 0.662 ± 0.132), four of whom also had detectable plaques.

In a second study by Hovingh and colleagues, cIMT values were assessed in 47 Dutch heterozygotes for LCAT gene mutations causing FED and 58 family controls (83). Mean cIMT was increased in heterozygotes compared with family controls (0.623 ± 0.13 versus 0.591 ± 0.08 mm), which became statistically significant ($P < 0.0015$) after adjustment for age, gender, and alcohol use.

Third, Calabresi and colleagues investigated cIMT in 30 Italian FLD and FED patients (of which 12 homozygous or compound heterozygous carriers and 28 heterozygous carriers) and 80 matched nonfamily controls (63). Unadjusted average cIMT was 0.50 mm (0.44–0.56 mm) in carriers of two mutant alleles and 0.62 mm (0.57–0.67 mm) in carriers of one mutant LCAT allele compared with 0.65 mm (0.62–0.68 mm) in matched controls.

Thus, in contrast to the studies in both Canadian and Dutch FED subjects, cIMT was decreased in Italian patients with FLD and FED, suggesting that functional LCAT is not required for efficient atheroprotection in this population. These contradictory outcomes are difficult to explain, but may result from differences in the populations studied. In addition, a limitation of cIMT analysis is that carotid ultrasound lacks statistical power to reliably measure arterial wall thickness in small population studies because it provides 2-dimensional longitudinal images, whereas atherosclerosis is a 3-dimensional eccentric developing disease.

In a recent study, Duivenvoorden et al. used 3.0-Tesla carotid magnetic resonance imaging, which allows transverse 3-dimensional imaging of atherosclerosis at high resolution, to investigate the role of reduced LCAT function on atherogenesis (98). In this study, 40 subjects (38 heterozygotes and 2 homozygotes) with mutations in the gene for LCAT were compared with 40 controls (both family members and unrelated individuals) matched for age and cardiovascular risk factors (111, 112). The carriers had 10% higher normalized wall index and 20% higher mean wall area and total wall volume. In addition, 50% of the carriers, compared with only 8% of controls, had atherosclerotic plaque components defined as either lipid-rich tissue or calcified material, and total plaque volume was significantly increased (102 mm^3 versus 3 mm^3 in carriers versus noncarriers, respectively). Despite the encouraging results from this particular study, it should be noted that due to the rarity of the disorder, the number of subjects investigated is relatively small and no conclusions can be drawn on hard cardiovascular end points.

LCAT and atherosclerosis in the general population

Only a few studies examining genetic variants in the general population have been described. Very recently, in

JOURNAL OF LIPID RESEARCH ASBMB

the Copenhagen City Heart Study and the Copenhagen General Population Study, LCAT S208T, a common variant identified in individuals with the lowest 2% HDL cholesterol, was found to be associated with a 13% decrease in HDL cholesterol (n = 85) but not with increased risk of myocardial infarction or other ischemic end points (101). In contrast, in Chinese CVD patients, P143L, a single-nucleotide polymorphism (SNP) in exon 4 of the LCAT gene, was discovered previously in nine males and two females (frequency of 5.8%), which was associated with low HDL cholesterol (113). Furthermore, Pare and colleagues identified an LCAT regulatory SNP, rs2292318, which was associated both with HDL cholesterol and plasma triglyceride levels (114). Interestingly, the minor allele was the "protective" allele with regard to CVD. In a smaller validation sample, no statistically significant association with HDL was found, although the findings were consistent with the direction of the original association.

Several studies also addressed the association between LCAT activity and concentration in patients with cardiovascular disease (**Table 2**). Unfortunately, the results from these studies are far from conclusive. In early studies, either reduced (115) or increased (104) LCAT activity was found in patients with CVD. More recently, in a community-based prospective nested case-control study (PREVEND cohort), an exogenous substrate assay was used to measure plasma LCAT activity in 116 men who developed CVD (cases) and in 111 male controls (116). In this cohort, plasma LCAT activity was found to be 5% higher in cases ($P = 0.027$) in association with higher total cholesterol, non-HDL cholesterol, and triglycerides; thus, high plasma LCAT activity did not predict reduced CVD risk. In the EPIC-Norfolk population study, plasma LCAT levels, which strongly correlate with LCAT activity, were measured in 933 apparently healthy men and women who developed cardiovascular disease and 1,852 matched controls who remained free of CVD during 6-year follow-up (117). Mixed-gender analyses showed no association between plasma LCAT levels and future cardiovascular events. However, high LCAT levels were associated with an increased CVD risk in women, whereas in males an opposite nonsignificant trend was observed. It is possible that the opposite trends in men and women underlie the absence of a relation between LCAT levels and CVD in the mixed-gender studies. In agreement, in a recent study by Calabresi and colleagues (80) gender-specific analysis of 247 European women of the multicenter IMPROVE study showed that low plasma LCAT levels were associated, although with borderline significance, with decreased cIMT in women with high cardiovascular risk. In the entire cohort and in men, no association between cIMT and LCAT levels was found. Currently, it is not clear how low levels of LCAT would protect against CVD in women but not in men. Other gender-specific prospective studies should confirm the findings, and it is hoped that further mechanistic studies provide the mechanistic reason in the near future. Importantly, the findings in CVD patients do not support the presumed antiatherogenic

TABLE 2. LCAT in patients with cardiovascular disease

Study	Study Design	Disease	LCAT Activity (nmol/ml/h)	Atherosclerosis-Related Effect	
(104)	Male CVD patients	Control No lesion Single vessel Double vessel Triple vessel Myocardial infarction	91 84 126 121 125 80	Increased LCAT in subjects with increased atherosclerosis severity	Proatherogenic
(115)	CVD patients	No lesion Single vessel Double vessel Triple vessel	107 55 32 16	Reduced LCAT in subjects with increased atherosclerosis severity	Antiatherogenic
(116)	PREVEND[a]	Control (men) Case (men)	111 116	High plasma LCAT activity does not predict reduced CVD risk, and may attenuate cardioprotection associated with higher HDL cholesterol.	Possibly proatherogenic
		Odds ratio for CAD	LCAT quartiles		
(117)	EPIC-NORFOLK[b]	Men: 1 Women: 1 Men: 0.83 Women: 0.88 Men: 0.75 Women: 1.14 Men: 0.71 Women: 1.35	1 2 3 4	Decreased risk of CAD with increasing LCAT levels in men, while the risk of CAD in women increased with increasing LCAT levels.	Antiatherogenic in men Proatherogenic in women
		cIMTmax (mm)			
(80)	IMPROVE[c]	Men: 1.35 Women: 1.19 Men: 1.37 Women: 1.22 Men: 1.33 Women: 1.23 Men: 1.35 Women: 1.27	1 2 3 4	Low plasma levels of LCAT associated with decreased cIMT in women, but not in men	Proatherogenic in women, but not in men

[a]Prevention of Renal and Vascular End-stage Disease (PREVEND) study of 40,856 inhabitants (age range at recruitment, 28–75 years) in Groningen, The Netherlands.

[b]European Prospective Investigation into Cancer and Nutrition (EPIC-NORFOLK) of over 30,000 inhabitants (age range at recruitment, 45–74 years) in Norfolk, UK.

[c]Prospective, multicentre, longitudinal, observational study of 3,711 subjects (age range, 54–79 years) with at least three vascular risk factors recruited in seven centers in Finland, France, Italy, the Netherlands, and Sweden.

function of LCAT and in females LCAT, if anything, might be proatherogenic.

LCAT AND ATHEROSCLEROSIS IN ANIMAL STUDIES

To gain better insight in the complex role of LCAT in modulating lipoprotein metabolism, RCT, and atherosclerosis, multiple genetically modified animal models have been developed, including transgenic and knockout mice and transgenic rabbits.

LCAT and atherosclerosis in human LCAT transgenic mouse models

In 1995, three independent groups published studies describing the effects of overexpression of human LCAT in mice.

Vaisman and colleagues generated transgenic mice containing the entire human LCAT gene, including 0.851 kb of the 5′ flanking region and 1.134 kb of the 3′ flanking region on a C57Bl/6 background (6). The generated mice contained 15–120 copies of the transgene with an LCAT mass of 11–109 μg/ml and α-LCAT activity of 607–3513 nmol/ml/h. For comparison, α-LCAT activity in control mice was only 32 nmol/ml/h. Interestingly, the endogenous plasma LCAT activity was increased only 1.5- to 2-fold in the transgenic mice, indicating that in vivo other factors may limit full activation of the human enzyme in mice.

Francone et al. generated transgenic mice expressing the human LCAT gene, under control of either the natural or the mouse albumin enhancer and promoter in the FVB background (7). α-LCAT activity was ~27.6 nmol/ml/h in the transgenic mice expressing human cholesterol ester transfer protein (CETP) under control of the natural promoter and ~33.7 nmol/ml/h under control of the mouse albumin enhancer and promoter, compared with 23.0 nmol/ml/h in nontransgenic controls. Coexpression of human apoAI or human apoAI and apoAII increased LCAT activity further.

Mehlum and colleagues generated C57Bl/6 mice containing the full-length human LCAT gene, including 0.1932 kb of the 5′ flanking region and 0.908 kb of the 3′ flanking region (8). About 30 copies of the transgene were integrated into one site. α-LCAT activity was increased from 106 nmol/ml/h in controls to 4,431 nmol/ml/h in the transgenics. Strikingly, the endogenous LCAT activity was only half that of controls (53 μmol/ml/h in transgenics compared with 106 μmol/ml/h in controls), which was attributed to substrate inhibition of LCAT or lack of available free cholesterol in a suitable compartment.

In all three studies, overexpression of human LCAT increased total cholesterol, primarily because of an increase in the amount of cholesterol esters transported by HDL. As a result of the enhanced esterification of free cholesterol in HDL, abnormally large HDL particles that were rich in apoE accumulated in the circulation of these animals (6). No effects were seen on serum triglycerides in the Vaisman study, whereas Francone et al. reported a 2-fold lower triglyceriode content of HDL in human LCAT transgenics. Mehlum and colleagues found a similar but nonsignificant lowering of HDL triglycerides. Furthermore, a highly significant 2-fold lowering of triglycerides was found in VLDL, the predominant transporter of triglycerides in mice on chow diet, whereas triglycerides in LDL were 3-fold lower.

In **Table 3**, an overview is given of the studies investigating the effects of overexpression of human LCAT in mice on atherosclerosis susceptibility. Upon feeding a high-fat, high-cholesterol diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid for 16 weeks, Vaisman LCAT transgenic mice expressing 100 or 240 copies of the transgene had significantly higher plasma levels of total (1.2- to 1.5-fold) and HDL cholesterol (1.6- to 3.3-fold), as well as apoAI (3.8- to 7.6-fold increase) (118). Plasma non-HDL cholesterol levels were not significantly different, nor were apoB levels. α-LCAT activity under these conditions was 1,283 and 2,908 nmol/ml/h, and endogenous LCAT activity was increased 2- to 3-fold compared with control animals. Feeding the Francone LCAT transgenic mice an

TABLE 3. Effects of LCAT overexpression on atherosclerosis susceptibility in mice and rabbits

Study	Animal Model	Background	Diet	Weeks	HDL-C	Non-HDL-C	Atherosclerosis
	Vaisman mouse, 100 copies		15% fat, 1.25% cholesterol,		↑	=	180% ↑
(118)	Vaisman mouse, 240 copies	C57Bl/6	0.5% cholic acid	16	↑↑	=	350% ↑
(120)	Francone mouse	C57Bl/6	15% calories from palm oil, 1.0% cholesterol, 0.5% cholic acid	24	=	=	= (based on analysis of cholesterol content of the aorta)
(119)	Francone mouse	FVB	20% fat, 1.25% cholesterol,	16	↑ (ns)	↑ (ns)	250%↑ (ns)
		FVB/apoAI KO	0.5% cholic acid		↓ (ns)	↑ (ns)	250% ↑
(121)	Mehlum mouse, 30 copies	C57Bl/6	11.5% fat, 1.25% cholesterol, 0.5% cholate	12	↑↑	=	118% ↑ (ns)
				17			318% ↑
				22			114% ↑ (ns)
(122)	Mehlum mouse, 60 copies	C57Bl/6	30% fat, 5% cholesterol, 2% cholate	16	↑↑	↑	=
(12)	Rabbit	NZW	0.3% cholesterol (120g)	17	↑↑	↓	700% ↑
(137)	Rabbit	WHHL (LDLr KO)	Chow	88	↑	=	=

LDLr, LDL receptor knockout; NZW, New Zealand white rabbit; WHHL, Watanabe heritable hyperlipidemic rabbit.

atherogenic diet that contained 20% fat, 1.25% cholesterol, and 0.5% cholic acid for 16 weeks resulted only in 1.2-fold higher total plasma cholesterol, HDL cholesterol, non-HDL cholesterol, and triglyceride levels. No information was provided on LCAT activity in these animals (119). Furbee and Parks backcrossed the Francone mice to the C57Bl/6 background and fed them with a cholic acid-containing atherogenic diet (15% of calories from palm oil, 1.0% cholesterol, and 0.5% cholic acid) for 24 weeks (120). α-LCAT activity was 9-fold higher in transgenics compared with controls (1,552 versus 166 nmol/ml/h). Triglyceride levels were 2-fold lower in the LCAT transgenics, whereas total plasma cholesterol levels were significantly increased (1.15%). FPLC analysis, however, did not show significant differences in the amounts of cholesterol transported by apoB lipoproteins or HDL (120). When the Mehlum LCAT transgenics were fed an atherogenic diet containing 11.5% fat, 1.25% cholesterol, and 0.5% cholic acid for 12 weeks, total cholesterol levels were 1.2-fold higher, and HDL cholesterol was induced 2-fold (121). In contrast to the findings on chow, no effect on serum triglycerides was found on the atherogenic diet. Of note, α-LCAT activity was 1,380 nmol/ml/h under these dietary conditions, which was substantially lower than on chow diet. After 16 weeks of atherogenic diet feeding, the Vaisman LCAT transgenics developed 1.8-fold and 3.5-fold larger atherosclerotic lesions in the animals with 100 and 240 copies of the human transgene, respectively (118). As mice are naturally resistant to atherosclerosis, the average lesion size in the animals was small, with a maximum lesion size of ~25,000 μm^2 in the human LCAT transgenic mice with 240 copies of the transgene. In the Francone mice ob the FVB background, a ~2.5-fold nonsignificant increase in lesion size was observed (119). In the presence of human apoAI, however, a significant 3.4-fold increase in lesion size was found. Furbee and Parks found similar amounts of cholesterol in aortas of Francone LCAT transgenic mice on the C57Bl/6 background after 24 weeks of atherogenic feeding, indicating that overexpression of human LCAT had no effect on atherosclerosis in this mouse model (120). The Mehlum human LCAT transgenics showed a 3.2-fold increase in lesion size after 17 weeks of atherogenic diet feeding but not after 12 or 22 weeks (121). In homozygous human LCAT transgenic mice expressing 60 copies of the transgene, Mehlum and colleagues could not demonstrate an effect of human LCAT expression on lesion development (122). Importantly, LCAT overexpression did not protect against atherosclerosis, and in the mice produced by Vaisman and colleagues, it even led to increased atherosclerosis in absence of effects on apoB-containing lipoproteins. The abnormally large apoE-rich HDL particles that accumulated in the transgenic animals (LCAT-Tg HDL) of Vaisman and colleagues had a normal efflux capacity but displayed a significantly reduced ability to transfer cholesterol esters to the liver (118). Interestingly, adenovirus-mediated overexpression of SR-BI failed to normalize the plasma clearance and liver uptake of LCAT-Tg HDL cholesterol esters (123), indicating that the ability of SR-BI to facilitate the uptake of cholesterol esters from the LCAT-Tg HDL was impaired. The observed increase in atherosclerotic lesion development in the Vaisman human LCAT transgenic mice was thus, at least in part, the result of an impaired SR-BI-mediated uptake by the liver, similar to that previously shown for SR-BI knockout mice (124). In humans, CETP offers an alternate route for delivery of HDL cholesterol esters to the liver after transfer to apoB-containing lipoproteins (125). Cross-breeding of the LCAT transgenic mice with simian CETP transgenic mice (126) reduced total cholesterol and HDL cholesterol levels both on the chow diet and on the atherogenic diet (123). The levels, however, remained significantly higher compared with control animals. Furthermore, expression of CETP normalized the plasma clearance of cholesterol esters from LCAT-Tg HDL (123), as previously shown upon expression of CETP in SR-BI knockout mice (127, 128). Importantly, atherosclerotic lesion size was reduced by 41% upon expression of CETP in the human LCAT transgenic mice (123). However, in CETP/LCAT double transgenic animals, lesions were still ~1.9-fold bigger compared with lesions in control and single CETP transgenic animals, indicating that in the presence of CETP, high expression levels of human LCAT remained proatherogenic in mice.

A major drawback of all the studies described above is that they were not performed in mouse models susceptible to lesion development, such as the LDL receptor knockout or apoE knockout mouse, and that a cholate-containing atherogenic diet had to be used for long periods to induce some degree of lesion development. Under these conditions, only the effects on the formation of small, initial foam cell lesions can be determined, a condition that is not very relevant for the human situation. Furthermore, it has to be taken into account that cholate-containing diets can have detrimental effects on liver function. For example, in the study of Berti and colleagues using the Francone human LCAT transgenic mice, it was clearly stated that 19% of the mice died during the 16-week diet-feeding period and that at the end of the study, all mice showed signals of hepatic steatosis and had gall bladders full of gallstones (119).

LCAT and atherosclerosis in LCAT knockout mouse models

In 1997, two independent groups published results on a mouse model for human LCAT deficiency generated by targeted disruption of the LCAT gene in mouse embryonic stem cells (9, 10). Sakai and colleagues generated an LCAT knockout mouse in which the disrupted LCAT allele lacked exons 2–5 (9), and Ng and colleagues generated a mouse lacking exon 1 (10). In both cases, the homozygous LCAT knockout mice had no detectable α-HDL activity, and activity in heterozygous mice was reduced to 30–55% of control. In contrast to human FLD patients, there was no evidence of corneal opacities or renal insufficiency in homozygous LCAT knockout mice at the age of 2–3 months. On chow diet, the plasma concentrations of total cholesterol and HDL cholesterol of the LCAT knockout mice were reduced to approximately 24%

and 30%, and 7% and 8.4% in the mice generated by Sakai and Ng, respectively. Plasma apoAI levels were also reduced to 13% and 19%, respectively. Sakai found that triglycerides were increased in males but not in females (9). Ng also found increased triglyceride levels, but this failed to reach statistical significance (mouse gender unknown) (10). The size and levels of the α-HDL were significantly reduced in the LCAT knockout mice, whereas preβ-HDL was increased. LCAT knockout mice of Sakai et al. that were fed an atherogenic diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid displayed not only nearly absent HDL levels but also remarkably lower plasma levels of proatherogenic apoB-containing lipoproteins, probably through upregulation of the LDL receptor and an increase in plasma apoE (9, 129). In addition, some mice accumulated lipoprotein X (Lp-X), an abnormal lipoprotein particle within the LDL density region that is rich in free cholesterol and phospholipids (130). This particle also accumulates in cholic liver disease and has been shown to have anti-oxidant properties (130). The subset of LCAT knockout mice that accumulated lipoprotein X also developed proteinuria and glomerulosclerosis characterized by mesangial cell proliferation, sclerosis, lipid accumulation, and deposition of electron dense material throughout the glomeruli on the atherogenic diet (129). However, no ocular abnormalities were found, even though corneal opacities are associated with FLD and FED in humans.

Despite the largely decreased HDL cholesterol levels, atherosclerotic lesions were 85% smaller after 16 weeks of feeding the cholate-containing atherogenic diet (**Table 4**). Using the same diet, 99% smaller lesions were observed in the CETP transgenic background, whereas on the LDL receptor knockout background, 35% smaller lesions were observed (129). ApoE knockout mice lacking LCAT displayed 51% smaller lesions when fed regular chow. Interestingly, in the mice with the smallest lesions (C57Bl/6 and CETP-Tg mice), the largest reduction in lesion size was seen upon deletion of LCAT (−85 to −99%), whereas the smallest effect was seen in mice with the largest lesions (−35% in LDL receptor knockout). Taken together, these findings might suggest that LCAT deletion has a more pronounced effect on early lesions compared with advanced lesions. This should, however, be confirmed in experiments in which lesion development is followed in time in the same animal model. In all cases, lesion size reduction coincided with a significant decrease in the proatherogenic apoB-containing lipoproteins.

When Ng and colleagues cross-bred their LCAT knockout mice to the apoE-deficient background, a 52% reduction in atherosclerosis was also found at 8–9 months of age when fed regular chow diet (131). Total cholesterol was 1.3-fold lower in these animals due to markedly decreased HDL cholesterol and 23% lower IDL/LDL cholesterol. VLDL, which transports the majority of cholesterol in these animals, was not affected. Interestingly, the LCAT/ApoE double-knockout mice displayed decreased markers of oxidative stress, and the LDL was less susceptible to oxidation, probably as a result of redistribution of paraoxanase 1 from HDL to the abnormal LDL that accumulates in LCAT-deficient animals. However, when Furbee et al. cross-bred the same LCAT knockout mice to the apoE and LDL receptor knockout background and induced lesion development with a mild atherogenic diet, which contained 0.1% cholesterol and 10% calories from palm oil, a significant increase in aortic cholesterol accumulation was found, indicative of enhanced lesion development (132). Furthermore, Lee et al. found a 3-fold increase in atherosclerotic lesion development upon deletion of LCAT in LDL receptor knockout mice that were challenged with a diet containing transmonounsaturated fatty acid-enriched fat (10% of energy) and 0.18% cholesterol for a period of 20 weeks (133). These findings by Furbee (132) and Lee et al. (133) are opposite to those observed in the studies of Lambert et al. (129) and Ng et al. (131). A possible explanation for the discrepancy among the studies are the differences in the plasma lipid responses with decreased apoB lipoproteins in the Lambert (129) and Ng (131) studies, and the increased or unchanged apoB lipoprotein concentrations in the studies of Furbee (132) and Lee et al. (133). In general, it can be concluded that the effect of LCAT deletion in mice on atherosclerotic lesion development correlates more closely with its effects on

TABLE 4. Effects of LCAT deficiency on atherosclerosis susceptibility in mice

Study	Mouse	Background	Diet	Weeks	HDL-C	Non-HDL-C	Atherosclerosis
		C57Bl/6			↓	↓	85% ↓
		CETP Tg	15% fat, 1.25% cholesterol, 0.5% cholic acid		↓	↓	99% ↓
(129)	Exon 2-5 deletion	LDLr KO		16	↓	↓	35% ↓
		ApoE KO	Chow	24–28	↓	↓	51% ↓
(131)	Exon 1 deletion	ApoE KO	Chow	32–36	↓	↓	52% ↓
(132)	Exon 1 deletion	LDLr KO	0.1% cholesterol, 10% calories from palm oil	16	↓	=	200% ↑ (based on analysis of cholesterol content of the aorta)
		ApoE KO			↓	↑	140% ↑ (based on analysis of cholesterol content of the aorta)
(133)	Exon 1 deletion	LDLr KO	0.18% cholesterol, 10% energy from transmonounsaturated fatty acid fat	20	↓	↑	284% (based on analysis of cholesterol content of the aorta)–312% ↑

JOURNAL OF LIPID RESEARCH ASBMB

proatherogenic apoB-containing lipoproteins than with its HDL-lowering effects.

LCAT and atherosclerosis in rabbit models

Unlike mice, rabbits express CETP and can develop spontaneous atherosclerosis. Therefore, rabbits are often thought to be a better model for studying atherosclerosis than mice. In 1996, Hoeg et al. described the generation of transgenic New Zealand White rabbits with a 6.2 kb genomic fragment consisting of the entire human LCAT gene, including 0.851 kb of the 5′ flanking region and 1.134 kb of the 3′ flanking region (12). The generated rabbits contained 38–1,436 copies of the transgene with an LCAT mass of 1.9–54 μg/ml and α-LCAT activity of 219–3,217 nmol/ml/h. For comparison, α-LCAT activity of the control rabbits was 202 nmol/ml/h. Overexpression of human LCAT in the rabbit led to markedly increased concentrations of large HDL particles containing apoE and reduced the concentrations of the apoB-containing VLDL and LDL particles (12). ApoAI pool size was increased, whereas the fractional catabolic rate was decreased, indicating that LCAT overexpression reduces apoAI catabolism (134).

To study the effects of human LCAT expression on atherosclerosis susceptibility, the rabbits were fed a daily ration of 120 g diet containing 0.3% cholesterol for a period of 17 weeks (12). Plasma LCAT activity was 101 ± 11 nmol/ml/h in controls and 1,593 ± 101 nmol/ml/h in the transgenic rabbits on chow. On the cholesterol diet, LCAT activity remained more than 3-fold that of controls. Total cholesterol and triglyceride levels were 28% and 24% lower, respectively, in the transgenic rabbits compared with controls. The reduction in total cholesterol was the consequence of a 2.6-fold decrease in non-HDL cholesterol levels, and HDL cholesterol was 5-fold higher. Quantification of atherosclerosis showed that aortas of the control group had 35 ± 7% of the surface of the aorta covered with lesions (Table 3). In marked contrast, only 5 ± 1% of the aortic surface was covered by lesions in the LCAT transgenic rabbits (12). Overexpression of human LCAT thus protects against atherosclerosis in rabbits, probably due to the combined effect of the marked increase in HDL cholesterol and the lowering of proatherogenic apoB-containing lipoproteins.

In vivo kinetic experiments confirmed that LCAT dose-dependently increased the catabolism of apoB-containing lipoproteins, which was opposite to what was seen for apoAI catabolism (135). To investigate the importance of the LDL receptor for this enhanced catabolism, the LCAT transgenic rabbit was cross-bred with the Watanabe heritable hyperlipidemic (WHHL) rabbit, which lacks functional LDL receptors due to an amino acid deletion in the cysteine-rich ligand-binding domain of the protein (136, 137). As expected, circulating LDL levels were markedly higher in rabbits lacking the LDL receptor. Interestingly, no lowering of LDL cholesterol was seen upon overexpression of LCAT, which might indicate that the enhanced catabolism of apoB-containing lipoproteins upon overexpression of LCAT is mediated via the LDL receptor. It must, however, be noted that the LCAT activity as well as HDL cholesterol levels was ~5-fold lower in animals lacking the LDL receptor compared with controls (136). Analysis of atherosclerosis at 22 months of age showed that in both WHHL control rabbits and WHHL rabbits overexpressing LCAT, 84 ± 3% of the surface of the aorta was covered with lesions. This lack of protection despite the high HDL cholesterol levels is likely due to the overwhelming presence of apoB-containing lipoproteins in plasma. Furthermore, the massive lesion coverage of the aorta indicates that the disease was in a very advanced stage. It might be that different effects would have been found if the effects were determined at an earlier age.

Comparison between mouse and rabbit studies

The results of the rabbit studies differ significantly from the results shown in the mouse studies. Although most of the mouse studies suggest a unanticipated proatherogenic role for LCAT in the development of atherosclerosis, the rabbit studies largely confirm an antiatherogenic role for LCAT. In both models, it appears that the influence of LCAT on atherosclerosis mostly depends on its effects on proatherogenic apoB-containing lipoproteins and to a lesser extent, if any, on its effects on HDL levels. In addition, the effects found are highly dependent on the presence of additional key proteins involved in RCT, such as CETP and the LDL receptor. Of note, viral overexpression of LCAT in nonhuman primates also resulted in an antiatherogenic profile characterized by increased HDL cholesterol and decreased levels of apoB-containing lipoproteins (13), similar to that observed in the transgenic rabbits. As rabbits and nonhuman primates more closely resemble humans in their lipoprotein metabolism, this might support the theory that raising LCAT functionality might be beneficial for atherosclerosis.

THERAPEUTIC REGULATION OF LCAT

Therapeutic upregulation of LCAT function has gained interest in the recent years, not only as enzyme replacement therapy for LCAT deficiency syndromes but also as a potential new therapeutic strategy for reducing atherosclerosis. Strategies for therapeutically raising LCAT activity include recombinant LCAT protein administration, viral expression of LCAT, and small-molecule activators of LCAT. Intravenous infusion of recombinant LCAT in LCAT knockout mice with or without expression of human apoAI rapidly raised HDL cholesterol and restored other lipid abnormalities (38). Moreover, a preliminary report indicated that subcutaneous injection of recombinant LCAT stimulated RCT and attenuated atherosclerosis progression in New Zealand White rabbits (138). Importantly, a phase 1 clinical trial was announced recently in which the effects of intravenous recombinant human LCAT infusion (ACP-501) in subjects with coronary artery disease will be evaluated (139).

Van Craeyveld et al. investigated the effects of adenoviral LCAT overexpression in liver on established complex fibroatheromatous atherosclerotic lesions induced by feeding

heterozygous LDL receptor knockout rabbits (62.5% New Zealand White and 37.5% Japanese White) a 0.15% cholesterol diet for 420 days (140). Adenoviral LCAT overexpression increased HDL cholesterol 1.9-fold, whereas non-HDL cholesterol was not affected. No significant effects were observed in the intima/media ratio, macrophage, smooth muscle cell, or collagen content compared with baseline at 120 days after gene transfer. However, the intima/media ratio tended to be lower compared with the animals treated with an empty adenovirus, indicating that LCAT overexpression slowed the progression of atherosclerosis. Interestingly, adenoviral LCAT overexpression also induced cholesterol unloading of the lesions, consistent with enhanced RCT from the arterial wall (140). Zhen et al. recently applied the adeno-associated viral vector serotype 8 (AAV8) for liver-directed delivery of human LCAT in heterozygous LDL receptor knockout mice expressing CETP (141). AAV-hLCAT administration resulted in a human LCAT concentration of 300 μg/ml, which declined slightly over the course of the experiment to 220 μg/ml, a level which is estimated to be more than 20-fold higher than the physiological concentration of LCAT. The mice had marked increases in HDL cholesterol and particle size, whereas LDL cholesterol, plasma triglycerides, and plasma apoB were reduced. Kuroda and colleagues have focused on developing a long-lasting LCAT replacement therapy via transplantation of human LCAT gene-transduced autologous adipocytes (142, 143). LCAT from the transduced adipocytes improved the abnormal HDL particles from an FED patient in vitro (142). Furthermore, LCAT could be steadily detected in adipocyte transplanted mice at four weeks after transplantation (129). Lastly, the therapeutic potential of a small-molecule activator of LCAT, compound A, is being explored for the treatment of atherosclerosis (144). Compound A increases LCAT activity with micromolar potency by interacting with the free sulfhydryl group in Cys31 near the catalytic site of LCAT. Cys 31 is a conserved residue in multiple species, and in line, compound A is able to activate LCAT from multiple species, including mouse, hamster, rhesus monkey, and human. Intraperitoneal administration of 20 mg/kg compound A increased HDL cholesterol acutely in C57Bl/6 mice and in high-fat diet-fed Syrian golden hamsters, whereas non-HDL cholesterol and triglycerides were reduced. Also chronic daily administration of 20 mg/kg and 60 mg/kg via oral gavage into high-fat diet-fed Syrian golden hamsters led to a dose-dependent increase in HDL cholesterol. VLDL cholesterol was decreased at the dose of 20 mg/kg, but no further decrease was seen after administration of 60 mg/kg. Gall bladder bile acids at termination were increased 2-fold, indicative of enhanced RCT upon chronic treatment with the LCAT activating compound A. The effects of these studies with respect to generation of a more antiatherogenic lipoprotein profile look promising. However, considering the complex interaction of LCAT with lipoproteins in the circulation, extensive studies on the effects on atherosclerosis susceptibility should be performed to be able to draw any conclusions on the therapeutic applicability of these new strategies.

CONCLUSION

Although a half a century of extensive research has passed since the discovery of LCAT as a key enzyme in the esterification of cholesterol in 1962, it has not (yet) fulfilled the promise as a new therapeutic target for atherosclerosis. Over the years, many studies have been performed investigating the role of LCAT in atherosclerosis in animal models and humans, with many conflicting data as a result. From the animal studies, it can be concluded that the effects of LCAT on lipoprotein metabolism and atherosclerosis largely depend on the animal model used and the presence of additional proteins involved in the RCT pathway, like CETP and the LDL receptor. Studies in rabbits, which more closely resemble humans in their lipoprotein metabolism, however, suggest that LCAT might be beneficial for atherosclerosis. The antiatherogenic effects in rabbits are probably, at least partly, due to the combined effect of a marked increase in HDL cholesterol and the lowering of proatherogenic apoB-containing lipoproteins. Unfortunately, studies investigating LCAT-deficient FED and FLD patients and individuals with CVD were largely contradictory and inconclusive. In FED and FLD patients, both increased and decreased signs of atherosclerosis have been reported. Several recent studies in CVD patients indicate that LCAT might be proatherogenic in women, a finding that clearly does not support the presumed antiatherogenic function of LCAT.

All the studies performed during the last 50 years have made it clear that LCAT plays a role in the pathogenesis of atherosclerosis. Unfortunately, it remains unclear what that role is. Therefore, further investigation is required to establish the exact role of LCAT in the development of atherosclerosis. Newly developed activator(s) of endogenous LCAT and recombinant LCAT infusions in humans will provide valuable information to establish whether targeting of LCAT is a promising therapeutic strategy to reduce cardiovascular risk.

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