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

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Abbreviated title:
LCAT: old friend or foe in atherosclerosis?
Abbreviations

Apo  Apolipoprotein
CETP  Cholesterol Ester Transfer Protein
cIMT  Carotid intima-media-thickness
CVD  Cardiovascular Disease
FED  Fish Eye Disease
FLD  Familial LCAT deficiency
HDL  High-density lipoprotein
LCAT  Lecithin:Cholesterol Acyltransferase
LDL  Low-density lipoprotein
Lp-X  Lipoprotein X
ndHDL  Nascent discoidal HDL
RCT  Reverse Cholesterol Transport
SR-BI  Scavenger Receptor BI
VLDL  Very-low-density lipoprotein
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Abstract
Lecithin:cholesterol acyltransferase (LCAT) is a key enzyme which 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 pre-β HDL due to impaired maturation of HDL particles, while enhanced expression is associated with the formation of large, apoE-rich HDL₁ 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 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.
Introduction

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 3 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, Human Gene Mutation Database. 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, reverse cholesterol transport, and atherosclerosis.

LCAT structure and function

The human LCAT gene is localized in the q21-22 region of chromosome 16 and consists of 6 exons separated by 5 introns and encompasses a total of 4.2 kilobases (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 60kD. 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 CHO cells
reduced the enzymatic activity without 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, while 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 a 82% and 62% decrease in activity, while replacement of Asn384 led to a substantially increased activity (21). Furthermore, N-glycosylation is important for determining substrate specificity towards 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 colleague’s 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). ApoA-I is the most potent activator of LCAT, which enables it to convert free cholesterol into cholesteryl esters on HDL by a transistification 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, apoA-I “activates” the phospholipid-cholesterol bilayer by concentrating the lipid substrates in the vicinity of LCAT and presenting it in an optimal conformation to LCAT. The conformation of these apoA-I complexes is affected by the fluidity of the lipid bilayer (40,41). Furthermore, the binding of LCAT to the apoA-I 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 apoA-I also other apolipoproteins, such as apoA-II, apoA-IV, apoCI-III and apoE can activate LCAT although less efficiently (43). Two distinct types of LCAT activity can be distinguished: α- and β-activity. The α-activity describes the enzymatic activity of LCAT towards cholesterol bound to apoAI-containing lipoproteins (e.g. HDL). β-activity constitutes the enzymatic activity of LCAT towards cholesterol bound to apolipoprotein-B containing lipoproteins (e.g. VLDL and LDL particles) (44). The equilibrium dissociation constants (Kd) for the interaction of pure human LCAT with LDL, HDL2, HDL3, and reconstituted discoidal HDL (rHDL) are as follows: rHDL = HDL3 <= HDL2 < LDL with relative reactivities (app Vmax/app Km) 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 or not?

Already in 1966, 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 peripheral tissues, including macrophages in the arterial wall, and subsequent delivery to the liver for biliary excretion (Figure 1).

The first step of the RCT pathway involves production of apoA-I in the liver or intestine, which is then released into the plasma (46). Interaction with ATP-binding cassette (ABC) A1 (ABCA1) 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 HDL3 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 particles with cholesteryl ester, they transform into larger HDL2 particles (46). Several studies indicate that LCAT activity decreases upon enlargement of the HDL particle, particularly on large apoE-rich HDL1 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 re-uptake 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). In addition to its essential role in the first step of the RCT pathway, LCAT is therefore also 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 the ABC-transporters ABCA1 and ABCG1 is not and hence 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 extrahepatic tissues despite the accumulation of large of large cholesteryl ester-rich HDL particles in the circulation (60). Fifth, 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). Fourth, 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, while HDL from compound heterozygotes (n=3) and a homozygote even appeared to induce more oxidation (68). Furthermore, HDL of LCAT heterozygotes, compound heterozygotes and 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, also the HDL-associated PAF-AH activity was 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 if 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, since 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). Since 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 for long, LDL receptor-mediated uptake of LDL was 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 that 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). Since 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 if LCAT deficient patients suffer more frequently from adrenal insufficiency. However, carriers of LCAT mutations do 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 adipocytes ER stress, but the mechanistic link with the enzymatic action of LCAT needs further investigation. Currently it is unknown if LCAT deficient
(female) patients are more insulin sensitive and/or protected against obesity. An early study did show 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 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 21st 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 2 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 e.g. aggregation responses to different agonists and adhesion under flow are awaited.

In summary, in addition to its role in reverse cholesterol transport, LCAT directly or indirectly interferes with several other physiological processes that might affect the development of atherosclerosis (Figure 1). Future studies delineating the exact contribution of the effects of LCAT on HDL antioxidant/antiinflammatory function, adrenal steroidogenesis, insulin sensitivity and protection against obesity, and platelet function are expected to shed more light on the importance of these processes as compared to its role in reverse cholesterol transport for the pathogenesis of cardiovascular disease.
Familial LCAT deficiency (FLD) and Fish-eye disease (FED)

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 while heterozygotes typically have HDL cholesterol levels that are half of normal HDL cholesterol (82,83). Two important LCAT deficiency syndromes can be distinguished: 1) Familial LCAT deficiency (FLD) and 2) Fish-eye disease (FED) (Figure 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, while also lipid alterations were found in erythrocytes (85). Years later it was established that homozygozity 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 FED or Fish-eye disease, a less severe syndrome as compared to FLD. The name refers to a symptom that FED as well as FLD patients often develop: their 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, while 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 also allows the classification of heterozygous mutations, that led to confusion in the previous FLD/FED classification system (93,94). Intermediate phenotypes, i.e. 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: 1) LCAT activity towards HDL analogues; 2) cholesterol esterification rate (endogenous lipoproteins); 3) ratio of plasma FC to EC; 4) plasma LCAT concentration; and 5) clinical symptoms of the disease. For the in vitro experiments, two criteria were proposed: 1) specific activity of mutant protein towards HDL analogues and native LDL and 2) activity of mutant protein towards 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 that 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 analogues, but activity against LDL or other apoB-containing lipoproteins is preserved. The fifth and last class contains three mutations that the group of Kuivenhoven et al. was unable to categorize; this category was therefore 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, Human Gene
Mutation Database. [http://www.hgmd.org](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 a 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 reverse cholesterol transport 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 which 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, as compared to 75% in normal atheromas. In 1982, Carlson showed that FED patients did not suffer from premature atherosclerosis inspite 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) have increased LCAT levels compared to 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 are characteristic of coronary atherogenesis. Ever since then, the role of LCAT in the pathogenesis of atherosclerosis has been under debate.

**LCAT and atherosclerosis in human FED and FLD patients**

Throughout the years 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 near 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, also a remarkable increase in serum triglycerides was 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 2 homozygous LCAT deficient patients (108,109), but it is unknown if triglyceride absorption or synthesis are 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 case with FLD, who developed severe vascular disease, as evidenced by lower limb peripheral arterial obstruction with necrosis of two toes of his left
foot and angina at rest with trival occlusive coronary artery disease at coronarography (110). Eventually this patient died at the age of 42 after a right femoral-axillo artery by-pass 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 cases 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 < 5th percentile had CAD, while no significant increase in risk was observed in mutation carriers with HDLc >5th percentile (100).

The effects of LCAT deficiency on cIMT have been studied using ultrasound imaging in 3 relatively large studies. In 2004, Ayyobi and colleagues published a 25 year follow-up study of a Canadian kindred with 2 homozygous (average age 42 at the end of the study) and 9 heterozygous (average age 39) FLD patients (109). Over the 25 years of the study there had been no vascular events or death 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 75th 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+/-.296 mm versus expected 0.662+/-.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+/-.13 versus 0.591+/-.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 non-family controls (63). Unadjusted average cIMT was 0.50 (0.44-0.56) mm in carriers of 2 mutant alleles and 0.62 (0.57-0.67) mm in carriers of 1 mutant LCAT allele as compared to 0.65 (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 as 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 a 10% higher normalized wall index and a 20% higher mean wall area and total wall volume. In addition, 50% of the carriers as compared to 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$^3$ vs. 3 mm$^3$ in carriers vs. noncarriers, respectively). Despite the encouraging results from this particular study, it should be taken into account that due to the rarity of the disorder the number of subjects investigated is still 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 the Copenhagen City Heart Study and the Copenhagen General Population Study, a common variant, LCAT S208T, 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, previously a single-nucleotide polymorphism (SNP), P143L, in exon 4 of the LCAT gene was discovered 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 regards 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 or concentration in patients with cardiovascular disease (table 2). Unfortunately, also 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 and 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, while 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 or 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 further mechanistic studies will hopefully provide the mechanistic reason in the near future. Importantly, the findings in CVD patients do not support the presumed anti-atherogenic function of LCAT and in females LCAT, if anything, might be pro-atherogenic.
LCAT and atherosclerosis in animal studies

To order to gain better insight in the complex role of LCAT in modulating lipoprotein metabolism, reverse cholesterol transport, 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. 1) 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 ug/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 1.5-2-fold in the transgenic mice, indicating that in vivo other factors may limit full activation of the human enzyme in mice. 2) 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 CETP under control of the natural promoter and ~33.7 nmol/ml/h under control of the mouse albumin enhancer and promoter, as compared to 23.0 nmol/ml/h in nontransgenic controls. Co-expression of human apoAI or human apoAI and apoAII increased LCAT activity further. 3) 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 4431 nmol/ml/h in the transgenics. Strikingly, the endogenous LCAT activity was only half of that of controls (53 μmol/ml/h in transgenics as compared to 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 3 studies, overexpression of human LCAT increased total cholesterol, primarily due to an increase in the amount of cholesterol esters transported by HDL. As a result of the
enhanced esterification of free cholesterol in HDL, also abnormally large HDL particles accumulated in the circulation of these animals, which were rich in apoE (118). No effects were seen on serum triglycerides in the Vaisman study, while Francone et al reported a 2-fold lower triglyceride content of HDL in human LCAT transgenics. Mehlum and colleagues found a similar, but non-significant 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, while 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) (119). Plasma non-HDL cholesterol levels were not significantly different, nor apoB levels. α-LCAT activity under these conditions was 1283 and 2908 nmol/ml/h and endogenous LCAT activity was increased 2-3-fold as compared to control animals. Feeding the Francone LCAT transgenic mice an atherogenic diet, containing 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 (120). 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 (121). α-LCAT activity was 9-fold higher in transgenics as compared to controls (1552 vs 166 nmol/ml/h). Triglyceride levels were 2-fold lower in the LCAT transgenics, while 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 (121). 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, while HDL cholesterol was induced 2-fold (122). In contrast to the findings on chow, no effect on serum triglycerides were found on the atherogenic diet. Of note, α-LCAT activity was 1380 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 (119). As mice are naturally resistant to atherosclerosis, the average lesion size in the animals was small, with a maximum lesion size of ~25000 μm² in the human LCAT transgenic mice with 240 copies of the transgene. In the Francone mice on the FVB background, a ~2.5-fold nonsignificant increase in lesion size was observed (120). 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 atherogenic feeding, indicating that over-expression of human LCAT had no effect on atherosclerosis in this mouse model (121). 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 (122). Also 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 (123). Importantly, LCAT overexpression did not protect against atherosclerosis, and in the mice produced by Vaisman and colleagues even led to increased atherosclerosis in absence of effects on apoB-containing lipoproteins. The abnormally large apoE-rich HDL particles that accumulate in the transgenic animals (LCAT Tg HDL) of Vaisman and colleagues had a normal efflux capacity, but displayed a significantly reduced ability to transfer its cholesterol esters to the liver (119). Interestingly, adenovirus-mediated overexpression of SR-BI failed to normalize the plasma clearance and liver uptake of LCAT-Tg HDL cholesterol esters (124), indicating that the ability of SR-BI to facilitate the uptake of cholesterol esters from the LCAT-Tg HDL is impaired. The observed increase in atherosclerotic lesion development in the Vaisman human LCAT transgenic mice is thus, at least in part, the result of an impaired SR-BI mediated uptake by the liver, similar as previously shown for SR-BI knockout mice (125). In humans, CETP offers an alternate route for delivery of HDL cholesterol esters to the liver after transfer to apoB-containing lipoproteins (126). Cross-breeding of the LCAT transgenic mice with simian CETP transgenic mice (127) reduced total cholesterol and HDL cholesterol levels both on the chow diet as well as on the atherogenic diet (124). The levels, however, remained significantly higher as compared to control animals.
Furthermore, expression of CETP normalized the plasma clearance of cholesterol esters from LCAT Tg HDL (124), as previously also shown upon expression of CETP in SR-BI knockout mice (128,129). Importantly, atherosclerotic lesion size was reduced by 41% upon expression of CETP in the human LCAT transgenic mice (124). However, in CETP/LCAT double transgenic animals lesions were still ~1.9-fold bigger as compared to lesions in control and single CETP transgenic animals, indicating that also 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 of time 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 weeks diet-feeding period and that at the end of the study all mice showed signals of hepatic steatosis and had gall bladders full of gall stones (120).

**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), while Ng and colleagues generated a mouse lacking exon 1 (10). In both cases the homozygous LCAT knockout mice had no detectable α-HDL activity, while 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 (gender unknown; 10). The size and levels of the α-HDL were significantly reduced in the LCAT knockout mice, while 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 (9,130). 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 (131). This particle also accumulates in cholic liver disease and has been shown to have anti-oxidant properties (131). 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 (130). 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 feeding the cholate-containing atherogenic diet (Table 4). Using the same diet, 99% smaller lesions were observed in the CETP transgenic background, while on the LDL receptor knockout background 35% smaller lesions were observed (130). 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%), while 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 as compared to 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 pro-atherogenic apoB-containing lipoproteins.
When Ng and colleagues cross-bred their LCAT knockout mice to the apoE deficient background also a 52% reduction in atherosclerosis was found at 8–9 months of age when fed regular chow diet (132). 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 its 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, containing 0.1% cholesterol and 10% calories from palm oil a significant increase in aortic cholesterol accumulation was found, indicative of enhanced lesion development (133). 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 (134). These findings by Furbee (133) and Lee et al (134) are thus opposite to that observed in the studies of Lambert et al. (130) and Ng et al. (132).

A possible explanation for the discrepancy between the studies are the differences in the plasma lipid responses with decreased apoB lipoproteins in the Lambert (130) and Ng (132) studies, and increased or unchanged apoB lipoprotein concentrations in the study of Furbee (133) and Lee (134) colleagues.

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

**LCAT and atherosclerosis in rabbit models**

Unlike mice, rabbits do 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 38-1436 copies of the transgene with
an LCAT mass of 1.9-5.4 ug/mL and α-LCAT activity of 219-3217 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, while the fractional catabolic rate was decreased, indicating that LCAT overexpression reduces apoAI catabolism (135).

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 (136). Plasma LCAT activity was 101 ± 11 nmol/ml/h in controls and 1593 ± 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 as compared to controls. The reduction in total cholesterol was the consequence of a 2.6-fold decrease in non-HDL cholesterol levels, while 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 lesion (Table 3). In marked contrast, only 5±1% of the aortic surface was covered by lesion in the LCAT transgenic rabbits (136). 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 pro-atherogenic 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 (137). 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 that lacks functional LDL receptors due to an amino acid deletion in the cysteine-rich ligand-binding domain of the protein (138,139). 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 were ~5-fold lower in animals lacking the LDL receptor as
compared to controls. Analysis of atherosclerosis at 22 months of age showed that both in WHHL control rabbits and in WHHL rabbits overexpressing LCAT 84±3% of the surface of the aorta was covered with lesion. 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 thus well be that different effects would have been found when 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. While most of the mouse studies suggest an 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 reverse cholesterol transport, such as CETP and the LDL receptor. Of note, viral overexpression of LCAT in non-human primates also resulted in an anti-atherogenic profile characterized by increased HDL cholesterol and decreased levels of apoB-containing lipoproteins, similar as observed in the transgenic rabbits. Since rabbits and non-human 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 (140). Moreover, a preliminary report indicated that subcutaneous injection of recombinant LCAT stimulated reverse cholesterol transport and attenuated atherosclerosis progression in New Zealand White rabbits (141). 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 (142). 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 >20-fold higher as compared to the physiological concentration of LCAT. The mice had marked increases in HDL cholesterol and particle size, while 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 (143,144). LCAT from the transduced adipocytes improved the abnormal HDL particles from an FED patient in vitro (143). Furthermore, LCAT could be steadily detected in adipocyte transplanted mice at four weeks after transplantation (130). Lastly, the therapeutic potential of a small molecule activator of LCAT, compound A, is being explored for the treatment of atherosclerosis (145). 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, while 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 anti-atherogenic 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 in the future 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. During the years many studies have been performed investigating the role of LCAT in atherosclerosis, both in animal models and humans, but 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, do suggest that LCAT might be beneficial for atherosclerosis. The anti-atherogenic effects in rabbits are probably, at least partly, due to the combined effect of a marked increase in HDL cholesterol and the lowering of pro-atherogenic apoB-containing lipoproteins. Unfortunately, studies investigating LCAT deficient FED and FLD patients and individuals with CVD were also 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 pro-atherogenic in women, a finding which does clearly not support the presumed anti-atherogenic function of LCAT.

All the studies performed during the last 50 years have made it clear that LCAT does play a role in the pathogenesis of atherosclerosis. Unfortunately, it remains unclear what that exact 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 might prove valuable tools to establish if therapeutic targeting of LCAT is a promising therapeutic strategy to reduce cardiovascular risk.
Acknowledgment

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References


the lecithin: cholesterol acyltransferase gene. Evidence for expression in brain and testes as well as liver. J. Biol. Chem. 264: 21573-21581.


Figure Legends

Figure 1: LCAT functions potentially affecting the pathogenesis of cardiovascular disease
LCAT is considered an important driving force behind the reverse cholesterol transport (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 nascent discoidal HDL (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 therefore also 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.

Figure 2: LCAT deficiency syndromes
Two important LCAT deficiency syndromes can be distinguished: 1) Familial LCAT deficiency (FLD) and 2) 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, while 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).
Table 1. Effects of LCAT loss-of-function mutations on the development of atherosclerosis in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>FED/FLD</th>
<th>Atherosclerosis related effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hovig, 1973 (103)</td>
<td>FLD</td>
<td>Lipid deposition in renal arteries and veins</td>
</tr>
<tr>
<td>Carlson, 1982 (89)</td>
<td>FED</td>
<td>No signs of premature atherosclerosis</td>
</tr>
<tr>
<td>Funke, 1991 (146)</td>
<td>FED</td>
<td>No signs of atherosclerosis in FED patients</td>
</tr>
<tr>
<td>Kastelein, 1992 (147)</td>
<td>FED</td>
<td>No signs of atherosclerosis in FED patients</td>
</tr>
<tr>
<td>Kuivenhoven, 1995 (96)</td>
<td>FED</td>
<td>Premature coronary artery disease observed in homozygous male probands</td>
</tr>
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<td>Kuivenhoven, 1996 (148)</td>
<td>FED</td>
<td>Patient with premature coronary artery disease in the absence of other risk factors</td>
</tr>
<tr>
<td>Homma, 2001 (149)</td>
<td>FLD</td>
<td>FLD patient associated with marked atherosclerosis</td>
</tr>
<tr>
<td>Ayyobi, 2004 (109)</td>
<td>FLD</td>
<td>Heterozygozity for LCAT deficiency associated with increased cIMT</td>
</tr>
<tr>
<td>Calabresi, 2005 (150)</td>
<td>FLD+FED</td>
<td>No signs of premature cardiovascular disease</td>
</tr>
<tr>
<td>Hovingh, 2005 (83)</td>
<td>FED</td>
<td>Increased cIMT in heterozygotes</td>
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<tr>
<td>Scarpioni, 2008 (97)</td>
<td>FLD</td>
<td>Severe vascular disease with peripheral arterial obstruction and occlusive coronary artery disease</td>
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<tr>
<td>Calabresi, 2009 (110)</td>
<td>FED+FLD</td>
<td>Decreased cIMT</td>
</tr>
<tr>
<td>Duivenvoorden, 2011 (111)</td>
<td>FED+FLD</td>
<td>Carriers of LCAT gene have increased carotid atherosclerosis compared with controls</td>
</tr>
<tr>
<td>Tietjen, 2012 (100)</td>
<td>FED+FLD</td>
<td>Increased risk for CAD with HDL&lt;5th percentile</td>
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</table>

FED: Fish Eye Disease; FLD: Familial LCAT deficiency
Table 2. LCAT in patients with cardiovascular disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Disease</th>
<th>LCAT Activity (nmol/ml/h)</th>
<th>Atherosclerosis related effect</th>
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<tr>
<td>Wells, 1986 (104)</td>
<td>Male CVD patients</td>
<td>Control</td>
<td>91</td>
<td>Increased LCAT in subjects with increased atherosclerosis severity</td>
</tr>
<tr>
<td></td>
<td>No lesion</td>
<td>84</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Single vessel</td>
<td>126</td>
<td></td>
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<tr>
<td></td>
<td>Double vessel</td>
<td>121</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Triple vessel</td>
<td>125</td>
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<tr>
<td></td>
<td>Myocardial infarction</td>
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<tr>
<td>Šolajić-Božičević, 1994 (115)</td>
<td>CVD patients</td>
<td>No lesion</td>
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<td>Reduced LCAT in subjects with increased atherosclerosis severity</td>
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<td>Single vessel</td>
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<td>Double vessel</td>
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<td>Triple vessel</td>
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<td>Dullaart, 2010 (116)</td>
<td>PREVEND Inhabitants of the city of Groningen, aged 28-75 years when recruited</td>
<td>Control (men)</td>
<td>111</td>
<td>High plasma LCAT activity does not predict reduced CVD risk, and may attenuate cardioprotection associated with higher HDL cholesterol.</td>
</tr>
<tr>
<td></td>
<td>Case (men)</td>
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<tr>
<td>Holleboom, 2010 (117)</td>
<td>EPIC-NORFOLK Inhabitants of the city of Norfolk, aged 45-74 when recruited</td>
<td>Odds ratio for CAD</td>
<td>LCAT quartiles</td>
<td>Decreased risk of CAD with increasing LCAT levels in men, while the risk of CAD in women increased with increasing LCAT levels.</td>
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<td></td>
<td>Men: 1</td>
<td>Women: 1</td>
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<td>Pro-atherogenic in women</td>
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<td>Men: 0.83</td>
<td>Women: 0.88</td>
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<tr>
<td></td>
<td>Men: 0.75</td>
<td>Women: 1.14</td>
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<td>Men: 0.71</td>
<td>Women: 1.35</td>
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<tr>
<td>Calabresi, 2011 (80)</td>
<td>IMPROVE Subjects with high cardiovascular risk</td>
<td>cIMTmax (mm)</td>
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<td>Low plasma levels of LCAT associated with decreased cIMT in women, but not in men</td>
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<td>Men: 1.35</td>
<td>Women: 1.19</td>
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<td>Women: 1.23</td>
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<td>Men: 1.35</td>
<td>Women: 1.27</td>
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</tbody>
</table>

CVD: Cardiovascular Disease; CAD: Coronary Artery Disease; PREVEND: Prevention of renal and vascular end-stage disease study of 40,856 inhabitants (age range 28-75 years) of the city of Groningen in The Netherlands; EPIC-NORFOLK: European Prospective Investigation into Cancer and Nutrition of over 30,000 inhabitants (age range 45-74 years) of the city of Norfolk in England; IMPROVE: A prospective, multicentre, longitudinal, observational study of 3711 subjects (age range 54–79 years) with at least three vascular risk factors recruited in seven centres in Finland, France, Italy, the Netherlands, and Sweden.
Table 3. Effects of LCAT overexpression on atherosclerosis susceptibility in mice and rabbits.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Background</th>
<th>Diet</th>
<th>Weeks</th>
<th>HDL-C</th>
<th>Non-HDL-C</th>
<th>Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bérard, 1997 (119)</td>
<td>Vaisman mouse, 100 copies, Vaisman mouse, 240 copies</td>
<td>C57Bl/6</td>
<td>15% fat, 1.25% cholesterol, 0.5% cholic acid</td>
<td>16</td>
<td>↑</td>
<td>=</td>
<td>180% ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑↑</td>
<td>=</td>
<td>350% ↑</td>
</tr>
<tr>
<td>Furbee, 2002 (121)</td>
<td>Francone mouse</td>
<td>C57Bl/6</td>
<td>15% calories from palm oil, 1.0% cholesterol, 0.5% cholic acid</td>
<td>24</td>
<td>=</td>
<td>=</td>
<td>=*</td>
</tr>
<tr>
<td>Berti, 2005 (120)</td>
<td>Francone mouse</td>
<td>FVB, FVB/apoAI KO</td>
<td>20% fat, 1.25% cholesterol, 0.5% cholic acid</td>
<td>16</td>
<td>↑ (ns)</td>
<td>↑ (ns)</td>
<td>250%↑ (ns)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ (ns)</td>
<td>↑ (ns)</td>
<td>250%↑</td>
</tr>
<tr>
<td>Mehlum, 1997 (122)</td>
<td>Mehlum mouse, 30 copies</td>
<td>C57Bl/6</td>
<td>11.5% fat, 1.25% cholesterol, 0.5% cholate</td>
<td>12</td>
<td>↑↑</td>
<td>=</td>
<td>118%↑ (ns)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>=</td>
<td>318%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>=</td>
<td>114%↑ (ns)</td>
</tr>
<tr>
<td>Mehlum, 2000 (123)</td>
<td>Mehlum mouse, 60 copies</td>
<td>C57Bl/6</td>
<td>30% fat, 5% cholesterol, 2% cholate</td>
<td>16</td>
<td>↑↑</td>
<td>↑</td>
<td>=</td>
</tr>
<tr>
<td>Hoeg, 1996 (136)</td>
<td>Rabbit</td>
<td>NZW</td>
<td>0.3% cholesterol (120g)</td>
<td>17</td>
<td>↑↑</td>
<td>↓</td>
<td>700%↑</td>
</tr>
<tr>
<td>Brousseau, 2000 (139)</td>
<td>Rabbit</td>
<td>WHHL (LDLr KO)</td>
<td>Chow</td>
<td>88</td>
<td>↑</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

NZW: New Zealand White Rabbit; WHHL: Watanabe heritable hyperlipidemic rabbit; LDLr: LDL receptor knockout.

*Based on analysis of cholesterol content of the aorta.
Table 4. Effects of LCAT deficiency on atherosclerosis susceptibility in mice.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mouse</th>
<th>Background</th>
<th>Diet</th>
<th>Weeks</th>
<th>HDL-C</th>
<th>Non-HDL-C</th>
<th>Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambert, 2001</td>
<td>Exon 2-5 deletion</td>
<td>C57Bl/6, CETP Tg, LDLr KO, ApoE KO</td>
<td>15% fat, 1.25% cholesterol, 0.5% cholic acid</td>
<td>16</td>
<td>↓</td>
<td>↓</td>
<td>85% ↓</td>
</tr>
<tr>
<td>(130)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99% ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35% ↓</td>
</tr>
<tr>
<td>Ng, 2002</td>
<td>Exon 1 deletion</td>
<td>ApoE KO</td>
<td>Chow</td>
<td>32-36</td>
<td>↓</td>
<td>↓</td>
<td>52% ↓</td>
</tr>
<tr>
<td>(132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furbee, 2002</td>
<td>Exon 1 deletion</td>
<td>LDLr KO</td>
<td>0.1% cholesterol, 10% calories from palm oil</td>
<td>16</td>
<td>↓</td>
<td>=</td>
<td>200% ↑*</td>
</tr>
<tr>
<td>(133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>140% ↑*</td>
</tr>
<tr>
<td>Lee, 2004</td>
<td>Exon 1 deletion</td>
<td>LDLr KO</td>
<td>0.18% cholesterol, 10% energy from transmono-unsaturated fatty acid fat</td>
<td>20</td>
<td>↓</td>
<td>↑</td>
<td>284%*-312% ↑</td>
</tr>
<tr>
<td>(134)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Based on analysis of cholesterol content of the aorta.
Figure 1
Figure 2

Tg-rich particles

LCAT β-activity

LDL

LCAT α-activity

Mature HDL

LDL receptor

SR-BI

LIVER

FC

ndHDL

FLD Complete LCAT deficiency

FED Partial LCAT deficiency

Complete LCAT deficiency

Partial LCAT deficiency

Tg-rich particles

LCAT β-activity

FC

ndHDL

LCAT α-activity

Mature HDL

LDL

LCAT α-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL

LCAT β-activity

Mature HDL

LDL