Alteration of plasma HDL cholesteryl ester composition with transgenic expression of a point mutation (E149A) of human LCAT

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Abstract **We have previously identified a single amino acid mutation (hE149A) in human LCAT that increases its in vitro reactivity with phosphatidylcholine species containing *sn*-2 arachidonate (Wang et al. 1997. *J. Biol. Chem.* 272: 280–286). The purpose of the present study was to determine whether in vivo overexpression of hE149A compared with human wild-type LCAT (hLCAT-wt) would be sufficient to enrich the steady state composition of plasma HDL cholesteryl esters (CE) with long chain (>18 carbon) polyunsaturated fatty acyl species. Transgenic lines with 20-fold overexpression of hLCAT were created and studied between 12 and 16 weeks of age while consuming a chow diet. Transgenic overexpression of hE149A compared with hLCAT-wt significantly enriched HDL with CE species containing 20:4 (45%) and 22:6 n-3 (108%), at the expense of those containing 18:2, without a significant change in the plasma HDL concentration, particle size, or phospholipid fatty acyl composition. Removing the contribution of endogenous mouse LCAT by crossing the transgenic mice into the mouse LCAT knockout background resulted in even greater changes in HDL CE composition, with a 2.4-, 5-, and 5-fold increase in 20:4, 20:5 n-3, and 22:6 n-3 cholesteryl esters in the hE149A mice compared with hLCAT-wt Tg mice, respectively. Our results demonstrate that in vivo expression of hE149A significantly enriches HDL cholesteryl esters in 20- and 22-carbon fatty acyl species without affecting HDL concentration or size. Furthermore, the data suggest that endogenous mouse LCAT in hLCAT transgenic mice contributes to the plasma HDL CE pool out of proportion to its mass, presumably because the hLCAT transgene is poorly activated by mouse apolipoprotein A-I.**—Furbee, J. W., Jr., O. Francone, and J. S. Parks. **Alteration of plasma HDL cholesteryl ester composition with transgenic expression of a point mutation (E194A) of human LCAT. *J. Lipid Res.* 2001.** 42: **1626–1635.**

Supplementary key words mouse • polyunsaturated • apolipoprotein A-I

LCAT is a 65-kDa glycoprotein that is secreted into plasma by the liver and catalyzes the transacylation of fatty acids from the *sn*-2 position of phospholipid molecules to the 3β hydroxyl group of cholesterol, generating cholesteryl ester (CE) and lysolecithin (1–3). LCAT is responsible for most, if not all, of the cholesterol esterification that occurs in human plasma (2). Most LCAT activity and mass in plasma is associated with HDL; however, a relatively small amount of LCAT is also found both on apolipoprotein B (apoB) lipoproteins and in the lipoprotein-free fraction of plasma (4, 5). The major apolipoprotein of HDL, apolipoprotein A-I (apoA-I), is a potent activator of the LCAT enzyme (6). LCAT is responsible for the maturation of nascent, discodal HDL particles into mature, CE-rich spherical lipoprotein particles by esterifying free cholesterol into CE to form a neutral lipid core (7). This process also plays a critical role in reverse cholesterol transport, a process by which excess cholesterol in peripheral tissues is transported to the liver for excretion via bile (8, 9).

The type of dietary fat consumed by humans (10–12) or animals appears to play an important role in the development of atherosclerosis. Polyunsaturated fat diets result in a lowering of total plasma and LDL cholesterol concentrations as well as a paradoxical lowering of HDL cholesterol concentrations and an increase in the proportion of polyunsaturated CEs in plasma. In both mouse (13) and monkey (14, 15) models, animals fed a diet enriched in polyunsaturated fatty acids develop less atherosclerosis than animals fed a saturated fat diet.

These studies agree with epidemiological data in humans suggesting that diets rich in polyunsaturated fatty acids also lower the risk of coronary heart disease (16, 17). Thus, increasing the polyunsaturated fatty acyl composition of plasma CEs may be beneficial in preventing atherosclerosis development.

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; CE, cholesteryl ester; EC, esterified cholesterol; FC, free cholesterol; FPLC, fast protein liquid chromatography; LDLr, LDL receptor; PAPC, 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; PL, phospholipid; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; rHDL, recombinant HDL; Tg, transgene or transgenic; TPC, total plasma cholesterol.

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LCAT from rodents and primates has different phospholipid substrate fatty acyl specificities (18, 19). Human and nonhuman primate LCAT preferentially uses oleic and linoleic acid, whereas the murine and rat forms of LCAT prefer to use longer chain (>18 carbon) polyunsaturated fatty acids (i.e., arachidonic acid) (19). In work previously done in our lab (20), site-directed mutagenesis was used to narrow the region responsible for the fatty acyl specificity difference between primates and rodents to a single amino acid, 149. Changing the glutamic acid residue at position 149 of the human sequence to an alanine, which is present at that position in both mouse and rat LCAT, resulted in an activation of the human enzyme toward phospholipid substrates containing arachidonic acid in the sn-2 position, with little change in the activity of the mutant enzyme toward substrates containing oleate. These in vitro results suggested that expression of the mutant form of the human enzyme (hE149A) in vivo would enrich plasma in CEs with polyunsaturated fatty acids and possibly protect against atherosclerosis development.

The purpose of this study was to generate transgenic mice overexpressing the mutant hE149A LCAT and compare the HDL CE composition, concentration, and particle size distribution with mice overexpressing a similar level of human wild-type LCAT. Our hypothesis was that overexpression of hE149A LCAT would enrich plasma HDL CE with long-chain polyunsaturated fatty acids compared with human wild-type LCAT, without significantly affecting plasma HDL concentration or particle size distribution. If this hypothesis proved correct, then these animals could subsequently be used to test the hypothesis that CE fatty acyl composition, per se, is an important determinant of atherosclerosis.

MATERIALS AND METHODS

Creation and screening of hLCAT E149A transgenic mice

The human LCAT gene (hLCATg) (EC 2.3.1.43) was kindly provided by Dr. John McLean (Genentech) and Dr. Jake Lusis (UCLA). PCR amplification was used to generate the hE149A mutation in a 1.13-kb fragment flanked by *BssH*II and *Bcl*I restriction sites, which were subsequently used to introduce the mutant fragment into the LCAT gene. Briefly, a mutant forward oligonucleotide (5′-CT GTG CGC GCC GCC CCC TAT GAC TGG CGG CTG GCG CCC GGT GA-3′) (primer 1) and a reverse oligonucleotide (5′-AGA TTG AGA CTG CGG CTA TGA TC-3′) (primer 2) were used to generate a fragment of the hLCATg between restriction sites *BssH*II (position 2230; Genbank X04981 and *Bcl*I (position 3360) using the human wild-type LCAT gene as template for the PCR. The conditions of the PCR were as follows: 5 min at 95°C followed by 40 s at 95°C, 60 s at 55°C, and 90 s at 72°C, repeated 30 cycles and concluding with 10 min at 72°C. The mutant PCR fragment was sequentially restriction digested with *BssH*II and *Bcl*I and gel isolated. The restricted PCR fragment was ligated into the *BssH*II/*Bcl*I restriction-digested, gel-isolated pUC/hLCAT vector to generate the hE149A construct. The ligated hE149A construct was used to transform DH5α *Escherichia coli*, which were plated on Luria broth (LB) agar plates with 50 μg/ml ampicillin and cultured overnight in a 37°C incubator. Single colonies were picked and grown in 3 ml of LB broth with 100 μg/ml ampicillin overnight in a shaking 37°C water bath. A Promega miniprep kit was used to isolate the plasmid DNA from the cultures. A unique *Kas*I site was introduced into the LCAT gene by the hE149A point mutation and was used to screen plasmids. Plasmid DNA from individual colonies was sequenced to confirm the presence of the E149A mutation and to verify that no spurious mutations were introduced into the fragment by PCR.

To verify that the mutant gene construct resulted in the predicted phenotype, we double digested the pUC/hE149A gene with *Eco*RI and *BsrG*I, and the genomic fragment was gel isolated and subcloned into pCMV5 that had been double digested with *Eco*RI and *Acc65*I. The pCMV/hE149A gene construct was amplified, purified, and transfected into COS-1 cells using the DEAE/Dextran method (21). Media from cells transfected with the pCMV/hE149A gene were used for exogenous LCAT assays with recombinant HDL (rHDL) substrate containing either 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 1-palmitoyl-2-acahidonoyl-*sn*-glycero-3-phosphocholine (PAPC) as the phospholipid (see below) (20).

The pUC/hE149A gene construct was restriction digested with *Bam*HI and *Ssp*I, and the 5,287-bp LCAT gene fragment was gel isolated and purified for microinjection. The transgenic (Tg) mice were generated in the C57BL/6J-SJL hybrid background (denoted as hybrid from here on) at the NICHD Transgenic Mouse Development Facility (N01-HD-5-322) at the University of Alabama, Birmingham, by Dr. Carl Pinkart. Founder mice were screened for hE149A Tg expression by PCR and exogenous LCAT activity assay. Crosses between founder mice and C57Bl/6J mice were performed to check for germ-line transmission of the hE149A transgene. hLCAT wild-type (wt) Tg mice in the C57Bl/6J background were obtained from O.F. and were matched to plasma exogenous LCAT activity of hE149A transgenic animals using rHDL substrate with POPC (see below). The generation of hLCAT-wt Tg mice has been described previously (22).

Genomic DNA was isolated from tail clips using proteinase K digestion, as described previously (23), and PCR was used to screen founder animals for the presence of the transgene. The PCR conditions were 5 min at 95°C followed by 60 s at 95°C, 60 s at 61°C, and 60 s at 72°C, repeated 35 cycles and concluding with 10 min at 72°C. PCR primers were designed in regions of the human LCAT gene that had low sequence identity with murine LCAT. These primers were at the 5′ and 3′ regions of exon 6 (5′-GGA TGT TTC CCT CTC GCA TG-3′) (primer 3), (5′-GGG GAT GCA GGG GGA CCC TG-3′) (primer 4), respectively, and generated a 465-bp fragment. Human and murine genomic DNA samples were included in all PCR screens as positive and negative controls, respectively.

Generation of LCAT Tg mice lacking endogenous mouse LCAT

To determine the contribution of endogenous mouse LCAT to the plasma HDL CE fatty acyl pool of LCAT transgenic animals, we crossed the hLCAT-wt Tg and hE149A Tg animals into the mouse LCAT knockout (mLCAT−/−) and LDL receptor knockout (LDLr−/−) background. The LDLr−/− background was introduced for subsequent atherosclerosis studies and is not the focus of the present results. The LDLr−/− mice were purchased from Jackson Labs and are in the C57Bl/6J background, whereas the mLCAT−/− mice were provided by O.F. and are in a hybrid background (C57Bl/6J and 129). The LDLr−/−, mLCAT−/−, and hLCAT Tg (or hE149A Tg) mice were created in a two-step breeding process. In the first step, LDLr knockout (i.e., LDLr−/−, mLCAT+/+) mice were crossed with mouse LCAT knockout (LDLr+/+, mLCAT−/−)

mice to generate double heterozygotes (LDLr+/−, mLCAT+/−). The F1 generation was then intercrossed to generate LDLr−/−, mLCAT−/− mice. In the second step, the LCAT transgene, either wild type or mutant, was crossed into the double knockout background. To accomplish this, LCAT Tg mice were crossed with LDLr−/−, mLCAT−/− mice to generate the LDLr+/−, mLCAT+/−, LCAT Tg mice. The F1 mice were then backcrossed to the double knockout mice to generate LDLr−/−, mLCAT−/−, LCAT Tg mice (hLCAT-wt or hE149A).

At each step of the breeding protocol, pups were screened by total plasma cholesterol (TPC) and exogenous LCAT activity assays. PCR of genomic DNA was also used to confirm LDLr−/− and mLCAT−/− genotypes. PCR reactions contained (final concentration) buffer (13 mM ammonium sulfate, 50 mM Tris, pH 8.8, 5 mM $MgCl_2$, 50 mM mercaptoethanol, and 5 μM EDTA), 100 μg BSA, 7.7% DMSO, 0.1 μM forward and reverse primers, 800 μM dNTPs, 1.25 U taq polymerase, and genomic DNA in a total volume of 26 μl. The primers used to screen for the LDLr knockout allele were LDLr A, 5′-CTCCCTAGGAT GACTTCCGA-3′, LDLr B, 5′-TCTGAAGCTTGTCCTTGCAGTC-3′, and LDLr neo, 5′-GATTGGGAAGACAATAGCAGGCATGC-3′. Primers LDLr A + LDLr B resulted in a 380-bp band for the wild-type allele, and primers LDLr B + LDLr neo resulted in a 200-bp band for the targeted allele. The primers used for the LCAT knockout allele were LCAT 1F, 5′-TTTGGACCAGCTCT GCTACGT-3′, LCAT 3R, 5′-AGCTTGGCTTCTAGCCGATTCC-3′, and Neo RLT, 5′-TGCGCTGACAGCCGGAACAC-3′. Primers LCAT 1F + LCAT 3R gave a 1.5-kb wild-type band, whereas primers LCAT 1F + Neo RLT resulted in a 1.1-kb band from the targeted allele. The PCR conditions were 3 min at 94°C followed by 30 s at 93°C, 30 s at 61°C, and 2 min at 65°C repeated 40 cycles. PCR products were analyzed on 0.8% TAE agarose gels.

Animal care and handling

The mice in this study were housed at the Wake Forest University School of Medicine. All protocols and procedures were approved by the animal care and use committee of the Wake Forest University School of Medicine. All mice used in this study were male, between 12 and 16 weeks of age, and were fed a chow diet. Unless indicated otherwise, 10 animals per group were studied. Whole blood was obtained by either bleeding from the tail vein or retro-orbital plexus after a 4- to 6-h fast. Tail bleeds were done by restraining animals in a plastic restraint device (Harvard Apparatus). No anesthesia was used because the amount of discomfort to the mouse from this procedure is minimal. A small segment of tail (1–2 mm) was amputated, and the blood was collected in a 50-μl heparanized microhematocrit capillary tube (Fisher Scientific Co.). EDTA and sodium azide were added to whole blood at final concentrations of 0.5 and 1.0 μM, respectively, and the blood was centrifuged at 10,000 rpm for 15 min at 4°C. Plasma was isolated and transferred to a clean tube. Experiments were either run immediately or the plasma was stored at −70°C until needed. A retro-orbital bleed was performed when larger volumes of plasma were needed. The animals were anesthetized using a one-to-one combination of ketamine (50 mg/ml) and xylazine (10 mg/ml) at 0.5 μl per gram body weight. The injection was given in the thigh muscle of the back leg. The animal was given about 15 min to become fully sedated, and orbital bleeding was performed by inserting a glass capillary tube into the retro-orbital plexus. Up to 250 μl of whole blood was drawn from the animal. The animal's eye was treated with an ophthalmic ointment (Lacri-Lube) to keep the eyes lubricated, and the mouse was placed in a heated cage to speed recovery, which took 30 to 120 min. Plasma was then processed and stored as described above.

Lipid, lipoprotein, LCAT activity analysis

Plasma total cholesterol (Wako), free cholesterol (Wako), phospholipid (Wako), and triglyceride (Roche) concentrations were determined by enzymatic assay. Protein concentrations were determined by the Lowry method (24), using BSA as a standard.

rHDL were synthesized by a cholate dialysis procedure (25) using human apoA-I isolated from plasma (26, 27) and two different phospholipids, POPC and PAPC. The starting molar ratio of apoA-I to cholesterol to phospholipid was 1:5:80, with a trace amount of [^{3}H]cholesterol (50,000 dpm/μg of cholesterol) added to quantify CE formation.

Exogenous LCAT assays were performed as described previously (25). Briefly, assays were performed using saturating amounts of rHDL substrate (1.2 μg cholesterol), 0.6% BSA, 2 mM β-mercaptoethanol, and 2 μl of mouse plasma as a source of LCAT. After incubation at 37°C for 5 min, the lipids were extracted from the reaction mixture using the Bligh-Dyer method (28), and free and esterified cholesterol were separated by TLC using a neutral solvent system. Lipid bands were visualized with iodine vapor, the CE and free cholesterol bands were excised, and radioactivity was quantified by scintillation counting. LCAT activity was expressed as nanomoles of CE formed per milliliter of plasma per hour.

Endogenous LCAT assays were preformed as previously described (25). Briefly, 5×10^5 cpm of [^{3}H]cholesterol was added to each reaction tube, and the solvent was evaporated. Tris-buffered saline (TBS) (10 mM Tris, pH 8, 140 mM NaCl, 0.01% NaN_3, 0.01% EDTA) was used to dilute 10 μl of plasma to 50 μl before being added to the cholesterol-coated tube. The samples were incubated overnight at 4°C with vigorous shaking. The samples were split in two; half was incubated at 37°C for 1 h, and the remainder of the sample was maintained at 4°C to serve as the control. After incubation, samples were diluted to 0.5 ml with TBS buffer and processed by lipid extraction and TLC, as described above. Endogenous LCAT activity was expressed as nanomoles of cholesteryl ester formed from free cholesterol in plasma, determined by enzymatic assay, per milliliter of plasma per hour.

Plasma VLDL/LDL and HDL were separated using Superose 6 (1 × 30 cm) and Superose 12 (1 × 30 cm) columns in series. Before sample injection, plasma was diluted one to one with phosphate buffer (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4°C to spin out insoluble material. Lipoproteins were eluted at 0.5 ml/min with TBS, pH 7.4, and the elution profile was monitored by total cholesterol enzymatic assay. For some samples, exogenous LCAT was assayed, as described above, using fast protein liquid chromatography (FPLC) column fractions as the source of enzyme.

Lipoprotein phospholipid and CE fatty acid analysis

Plasma VLDL/LDL and HDL separated by FPLC were extracted by using the Bligh-Dyer method (28). CE and phospholipid bands were isolated by TLC using a neutral solvent system. The CE and phospholipid bands were visualized with primulin (29), and the silica gel from each band was scraped into a 13 × 100-mm tube. One milliliter of 0.5 N NaOH, in methanol, was added to each sample, and the sample was incubated in a boiling water bath for 5 min. The tubes were then cooled to room temperature, and 1 ml of 14% boron trifluoride, in methanol, was added to each sample. The tubes were then incubated in a boiling water bath for 5 min. Methylated fatty acids were extracted with isooctane and injected onto a gas-liquid chromatography (glc) fatty acid column (Chrompak CP-SIL88 FAME column) to determine the relative amounts of specific fatty acids in the sample. Conditions for the glc operation have been given in detail previously (30).

Gradient gel electrophoresis

One microliter of mouse plasma was separated on 4–30% nondenaturing gradient gels (31) for 1,300 V-h using a Pharmacia gel apparatus. The protein was transferred from the gel to a nitrocellulose membrane in a Biolabs transblot cell for 8 h at 35 V. The membrane was stained with 0.2% Ponceau Red, and the location of the Pharmacia broad range standards was marked. Western blots were developed with a polyclonal goat anti-mouse apoA-I (Biodesign International) primary antibody (1:1,000 dilution) and a secondary sheep anti-goat antibody conjugated to alkaline phosphatase (Vector Laboratories; 1:2,000 dilution). The diameter of HDL particles was estimated using an R_f versus log diameter plot generated from the standards on the gel.

Data analysis

The Statview program was used to statistically analyze data using unpaired *t*-tests or ANOVA factorial analysis with Fisher's least significant difference test used for post-hoc analysis.

RESULTS

The hE149A gene was expressed in COS-1 cells to confirm that the mutant construct had the expected increased reactivity with PAPC-containing rHDL compared with those containing POPC. Using media from transfected COS-1 cells as a source of LCAT, the PAPC to POPC cholesterol esterification activity ratio was 0.51 for the hLCAT-wt construct and 1.37 for the hE149A gene. These results were similar to what has been seen previously for transfections with hLCAT and hE149A cDNA constructs (20).

Multiple lines of transgenic mice containing the hE149A gene were generated by pronuclear microinjection of the linearized LCAT gene into C57Bl/6J-SJL mouse embryos. The gene consisted of 562 bp of 5′ untranslated region, 4,200 bp of hE149A gene, and 525 bp of 3′ untranslated region (**Fig. 1A**). Site-directed mutagenesis was used to replace an A with a C at nucleotide 2260 (Genbank X04981) in the human wild-type gene, resulting in a codon switch from glutamic acid to alanine at amino acid 149 (Fig. 1B). Integration of the transgene in founder mice was confirmed by PCR. Fig. 1C shows the results of a PCR using primers 3 and 4 (see Materials and Methods for sequence). The PCR screen was specific for the transgene because no amplification product was observed from the endogenous mouse LCAT gene (Fig. 1C, C57Bl/6). The expected 465-bp product was observed when human genomic DNA (Fig. 1C, human), three different founders (Fig. 1C, human E149A Tg lines 1–3), and pUC/hLCAT plasmid were used as template for PCR. An exogenous LCAT activity assay with transgenic mouse plasma was also used to screen founder mice and determine the level of LCAT overexpression (Fig. 1D). Germline transmission of the transgene was confirmed by crossing hE149A Tg founder animals with C57Bl/6J mice. Two lines were established and expanded with exogenous LCAT activities of 1,807 and 435 nmol CE formed/ml plasma/h using POPC rHDL on initial screen of the founders. The higher activity line was chosen for further study, and an hLCAT-wt Tg line was chosen as a control that exhibited similar exogenous LCAT activity using POPC rHDL. All transgenic mice in this study were heterozygotes for the transgene locus.

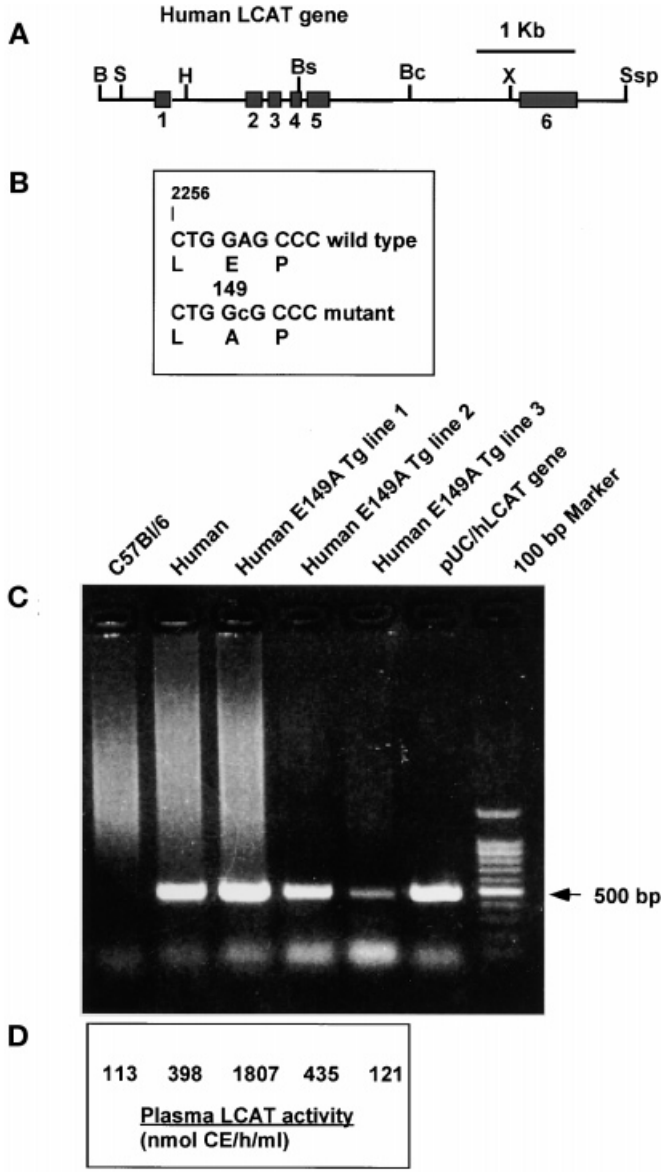

Fig. 1. Construction and screening of hE149A transgenic (Tg) mice. A: Schematic of hE149A transgene construct used to create hE149A Tg mice. The construct used for microinjection contained 562 bp of 5′ untranslated sequence followed by the 4,200-bp gene and 525 bp of 3′ untranslated sequence. B, *Bam*HI; S, *Sse*I; H, *Hind*III; Bs, *BssH*II; Bc, *Bcl*I; X, *Xba*I; and Ssp, *Ssp*I restriction sites. Exons are shown schematically as black rectangles, with exon number indicated beneath. B: Illustration of the mutation site of the hE149A transgene construct. A point mutation (A to C) at nucleotide 2260 (Genbank X04981) created by PCR mutagenesis resulted in a change of glutamic acid 149 in the wild-type enzyme to an alanine. C: PCR screen to identify founder animals using human LCAT exon 6-specific primers (see Materials and Methods for details). PCR amplification products were separated on 0.8% agarose gels. From left to right, the first five lanes contain samples that used the indicated genomic DNA as template for the PCR screen. The sixth lane represents a positive control sample in which the hLCAT-wt gene in pUC was used as template for the PCR. The seventh lane contains a 100-bp marker. The primer pair was predicted to amplify a 465-bp product. D: Exogenous LCAT cholesterol esterification assay of plasma. LCAT assays were performed using POPC rHDL as substrate and the indicated plasma samples as a source of LCAT (see Materials and Methods for details). Values are reported as nanomoles of CE formed per milliliter of plasma per hour for three hE149A Tg founder mice, a C57Bl/6 control mouse, and a human control sample.

Figure 2 summarizes the plasma exogenous LCAT cholesterol esterification activity for the study animals. Because the hE149A Tg animals were in a hybrid background, nontransgenic littermates (referred to as hybrids) were used as controls. The hLCAT-wt Tg animals had been backcrossed into the C57Bl/6J background, so C57Bl/6J mice were used as controls. Exogenous LCAT reactions were conducted with rHDL substrates containing POPC or PAPC. When POPC rHDL was used as substrate, the wild-type and hE149A LCAT transgenic mice had similar levels of exogenous activity, 2,424 ± 412 versus 2,127 ± 197 nmol CE/ml plasma/hr (mean ± SD; n = 10 per group), respectively, which was elevated ~19- to 21-fold over the hybrid (113 ± 11) and C57Bl/6 (113 ± 19) nontransgenic controls (Fig. 2A). The activity observed with PAPC rHDL substrate was 2-fold higher in the hE149A Tg mice (3,177 ± 550 nmol CE/ml plasma/h) compared with the hLCAT-wt Tg mice (1,444 ± 216 nmol CE/ml plasma/h; $P > 0.0001$). The exogenous LCAT activity for both nontransgenic control mice with PAPC rHDL was ~400 nmol CE/ml plasma/h. The PAPC to POPC cholesterol esterification ratio for hLCAT-wt Tg mice was low, 0.60 ± 0.08, whereas the ratio for C57Bl/6 and hybrid mice was much higher, 3.84 ± 1.00 and 3.53 ± 0.45, respectively (Fig. 2B). This outcome reflected the much stronger preference of hLCAT for POPC relative to PAPC compared with mouse LCAT, which was the opposite. The activity ratio for hE149A Tg mice (1.50 ± 0.26) was intermediate to that of the other mouse lines due to a preference for POPC that was similar to that of hLCAT as well as an increased preference for PAPC. The PAPC/POPC cholesterol esterification ratio observed in vivo for hLCAT-wt and hE149A Tg mice was similar to that reported previously for COS-1 cells transfected with the corresponding cDNA constructs (0.51 and 1.78, respectively) (20).

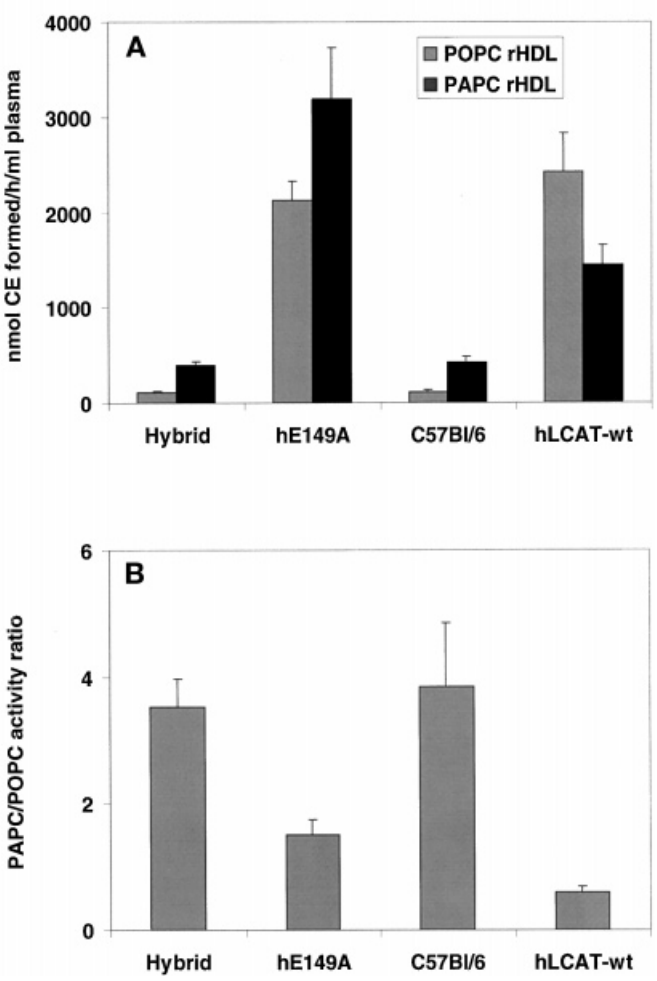

Fig. 2. Exogenous LCAT activity in plasma of transgenic and control mice. Exogenous LCAT activity was measured using recombinant HDL particles containing either 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC) phospholipid substrate. Reactions were performed using a saturating concentration of cholesterol (3 μM) and an incubation time of 5 min. Values (mean ± SD; n = 10) are expressed as nanomoles of CE formed per milliliter of plasma per hour (A) or as a PAPC/POPC activity ratio (B). Hybrid refers to nontransgenic littermate controls for the hE149A-Tg mice.

Endogenous LCAT assays were performed by incorporating [^{3}H]cholesterol into plasma lipoproteins and measuring esterification rates. The endogenous cholesterol esterification rate was similar among the four groups of mice (hybrid = 29 ± 8, hE149A Tg = 33 ±7, C57Bl/6 = 45 ± 17, and hLCAT-wt = 38 ± 11 nmol CE formed/ml plasma/h).

Table 1 summarizes the plasma lipid concentrations of transgenic (wild-type and hE149A) and control mice. hLCAT-wt Tg mice had a significant increase in plasma total cholesterol (32%), esterified cholesterol (39%), and HDL cholesterol (26%) compared with C57Bl/6J mice; however, phospholipid, triglyceride, and VLDL + LDL cholesterol (i.e., apoB lipoprotein) concentrations were similar to control mice. The hE149A Tg mice had significant increases in plasma total cholesterol (40%), esterified cholesterol (48%), and HDL cholesterol (60%) compared with nontransgenic littermate hybrid mice. The hE149A Tg mice had significant increases in phospholipid concentration (24%) and a decrease in triglycerides (43%) compared with controls. The free to esterified cholesterol ratio was lower in both transgenic lines, 0.29 for hE149A Tg and 0.30 for hLCAT-wt Tg, when compared with hybrid and C57Bl/6J control mice (0.37). There was no statistically significant difference for any of the lipid and lipoprotein values measured in Table 1 when the hLCAT-wt Tg and hE149A Tg mice were compared with each other.

Figure 3A shows an FPLC cholesterol elution profile of plasma injected onto Superose 6 and Superose 12 columns in series. The profiles demonstrate that HDL cholesterol concentration was increased in both LCAT transgenic animals, in agreement with data in Table 1, whereas no significant change in HDL particle size was detected. Furthermore, most of the cholesterol in plasma was distributed in the HDL fraction, whereas only a small proportion was in the apoB particle size range. The similarity in HDL particle size was also observed with 4–30% nondenaturing gradient gel electrophoresis (Fig. 3B). All animals had monodispersed HDL particles that averaged 10.2 nm in diameter.

TABLE 1. Plasma lipid and lipoprotein concentrations in LCAT transgenic and control mice

Genotype	TPC	FC	EC	FC/EC	PL	TG	HDL-C	VLDL + LDL-C
Hybrid	89 ± 21	23 ± 3	66 ± 19	0.37 ± 0.09	158 ± 23	46 ±18	62 ± 9	14 ± 5
hE149A	125 ± 20	27 ± 3	98 ± 20	0.29 ± 0.07	196 ± 26	26 ± 9	99 ± 22	25 ± 3
C57Bl/6	90 ± 7	24 ± 2	66 ± 6	0.37 ± 0.04	166 ± 22	40 ± 18	77 ± 7	17 ± 2
hLCAT-wt	119 ± 17	27 ± 3	92 ± 16	0.30 ± 0.06	174 ± 23	26 ± 10	98 ± 13	19 ± 9
	P values							
Hybrid vs. hE149A	0.001	0.006	0.002	0.03	0.003	0.005	0.008	0.06
C57Bl/6 vs. hLCAT-wt	<0.001	0.017	<0.001	0.013	NS	NS	0.003	NS
hE149A vs. hLCAT-wt	NS	NS	NS	NS	NS	NS	NS	NS

Blood was obtained from 12-to 16-week-old chow-fed mice of the indicated genotype, and measurements were made as described in Materials and Methods. Hybrid refers to nontransgenic littermate controls for the hE149A-Tg mice. Values are the mean ± SD (n = 10 per group) and are expressed as milligrams per deciliter, except for the FC/EC ratio. *P* values for statistical comparisons between hybrid versus hE149A Tg, C57Bl/6 versus hLCAT-wt, and hE149A versus hLCAT-wt Tg mice were derived from ANOVA and Fisher's least significant difference test. NS, not significant at $P = 0.05$. TPC, total plasma cholesterol; FC, plasma free cholesterol; EC, plasma esterified cholesterol; PL, plasma phospholipid; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; VLDL+LDL-C, very low density and low density lipoprotein cholesterol.

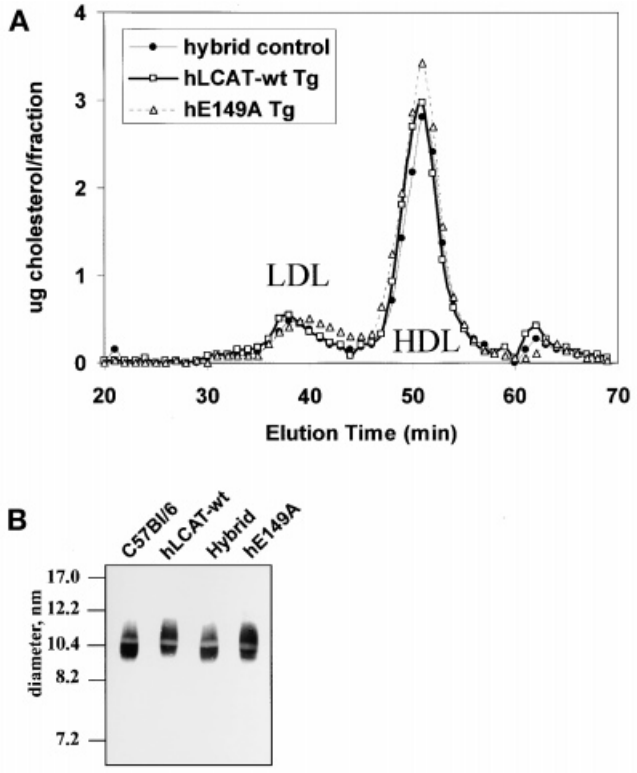

Fig. 3. A: FPLC cholesterol elution profile of transgenic and control mouse plasma. One hundred microliters of plasma were injected onto a Superose 6 (1 × 30 cm) and a Superose 12 (1 × 30 cm) column connected in series with a flow rate of 0.5 ml/min. One-minute fractions were collected, and total cholesterol was measured using an enzymatic assay. The relative positions of LDL and HDL are shown. B: Western blot of mouse plasma (1 μl) separated by 4–30% nondenaturing gel electrophoresis and developed with anti-mouse apolipoprotein A-I (apoA-I) antiserum, as described in Materials and Methods. Genotype of mouse from which the plasma was obtained is shown at the top of the blot. Hydrated diameters of the protein standards run on the gel are shown on the left side of the blot.

After separating the lipoprotein classes by FPLC, the CE and phospholipid fatty acyl composition was determined for the HDL fraction. **Table 2** shows the results of these analyses. The fatty acyl composition of the plasma HDL phospholipid pool, which serves as substrate for the LCAT reaction, was similar for all four groups of mice. However, the composition of HDL polyunsaturated CEs was affected by transgenic LCAT expression. Compared with C57Bl/6 control mice, the hLCAT Tg mice had a significantly greater percentage of C18:2 (i.e., cholesteryl 18:2) and a significant 40–50% reduction of C20:4 and C22:6. There was no statistically significant difference in the HDL CE fatty acyl composition between hybrid controls and hE149A Tg mice. However, mice expressing the hE149A Tg had a 45% increase in C20:4, a 108% increase in C22:6, and a 17% decrease in C18:2 compared with the hLCAT-wt Tg mice. These data show that transgenic expression of hE149A resulted in an enrichment of plasma HDL in polyunsaturated CE that could not be explained by an enrichment in polyunsaturated fatty acids in the HDL phospholipid substrate pool. This enrichment occurred with no apparent difference in plasma lipid or lipoprotein concentrations between the hLCAT and hE149A LCAT Tg animals. Differences in genetic background between hE149A and hLCAT-wt transgenic mice could also have affected the fatty acid composition of HDL CE.

The relatively high percentage of C20:4 in HDL CEs of hLCAT Tg mice, 18.1 ± 3.3%, was surprising given that these animals expressed hLCAT 21-fold over control animals. This result suggested that mouse LCAT contributed to the plasma HDL CE pool out of proportion to its mass in plasma. To test this hypothesis, we analyzed the HDL CE fatty acid composition of hLCAT and hE149A Tg mice that were crossed into the mouse LCAT knockout background (mLCAT−/−) to eliminate the contribution of endogenous mouse LCAT. These animals had also been

TABLE 2. Plasma HDL phospholipid and cholesteryl ester fatty acyl percentage composition

Genotype	16:0	16:1	18:0	18:1	18:2	20:4	20:5	22:6	Other
HDL phospholipid									
Hybrid	23.4 ± 2.6	0.2 ± 0.4	17.5 ± 1.5	13.0 ± 6.8	22.6 ± 4.6	8.4 ± 1.2	0.6 ± 0.5	11.1 ± 3.9	3.3 ± 0.9
HE149A	20.8 ± 3.1	0.0 ± 0.0	20.2 ± 2.1	8.6 ± 2.1	21.9 ± 2.2	11.6 ± 2.5	1.2 ± 0.2	13.2 ± 2.0	2.5 ± 0.6
C57Bl/6	25.4 ± 2.4	0.5 ± 0.4	19.1 ± 3.0	13.1 ± 6.7	20.7 ± 2.2	8.3 ± 0.9	0.7 ± 0.4	8.2 ± 1.3	4.1 ± 2.0
hLCAT-wt	19.6 ± 4.0	0.1 ± 0.3	20.8 ± 3.7	7.8 ± 4.7	26.6 ± 6.3	10.1 ± 3.3	0.8 ± 0.7	12.0 ± 2.0	2.3 ± 1.5
HDL cholesteryl ester									
Hybrid	3.6 ± 2.1	2.1 ± 2.0	0.2 ± 0.5	5.8 ± 0.6	45.0^{a} ± 6.0	$28.2^{a,b}$ ± 4.5	4.7 ± 0.7	9.2^{a} ± 3.2	1.3 ± 1.2
HE149A	4.5 ± 1.0	1.5 ± 0.9	0.2 ± 0.4	7.3 ± 2.6	44.2^{a} ± 4.9	26.2^{b} ± 3.8	5.4 ± 0.7	9.8^{a} ± 2.1	1.0 ± 1.4
C57Bl/6	4.1 ± 0.2	2.1 ± 0.8	0.6 ± 0.6	4.5 ± 0.7	41.1^{a} ± 5.2	33.7^{a} ± 5.1	4.7 ± 0.7	7.5^{b} ± 0.8	1.7 ± 1.5
hLCAT-wt	6.5 ± 2.7	2.6 ± 2.6	0.2 ± 0.5	8.1 ± 2.2	53.4^{b} ± 5.6	18.1^{c} ± 3.3	5.4 ± 1.0	4.7^{c} ± 1.2	0.9 ± 1.1

Plasma phospholipid and cholesteryl ester fatty acyl percentage compositions (mean ± SD; n = 5 per group) were measured on 12- to 16-week-old chow-fed mice of the indicated genotype, as described in Materials and Methods. Hybrid refers to nontransgenic littermate controls for the hE149A-Tg mice. ANOVA and Fisher's least significant difference test were used to identify statistically significant differences among groups. Values with unlike superscripts are statistically different ($P < 0.05$).

crossed into the LDL receptor knockout (LDLr−/−) background for future atherosclerosis studies. Plasma lipid and lipoprotein concentrations for these animals consuming a chow diet are shown in **Table 3**. The values were similar between the two groups of animals. LDLr−/− mice were included in Table 3 for comparative purposes. Compared with LCAT transgenic mice with functional mLCAT and LDL receptors (Table 1), the LCAT transgenic animals in the double knockout background had statistically significant increases in TPC, free cholesterol (FC), esterified cholesterol (EC), phospholipid (PL), TG and VLDL + LDL-C, reflecting the absence of active LDL receptors. However, the double knockout background did not affect the plasma concentration of HDL-C. There was a trend toward an increased FC/EC ratio for LCAT transgenic animals in the double knockout background, which was statistically significant for the hE149A mice, compared with those with active mLCAT and LDL receptors.

Table 4 shows the results of the HDL CE fatty acid analysis comparing LCAT transgenic animals in the double knockout background with those with active mLCAT. Elimination of functional mouse LCAT resulted in a statistically significant increase in both hLCAT-wt and hE149A Tg lines, respectively, in the percentage of HDL C18:1 (58% and 73%) and C18:2 (24% and 21%) as well as a significant decrease in C20:4 (62% and 39%), C20:5n-3 (85% and 28%), and C22:6n-3 (81% and 55%). Comparing the two LCAT transgenic lines in double knockout background, the hE149A animals had a significantly greater percentage of C20:4 (2.4-fold), C20:5n-3 (5-fold), and C22:6n-3 (5-fold) than did the hLCAT-wt mice. Thus, endogenous mouse LCAT disproportionately affected plasma HDL CE composition compared with the overexpressed human LCAT transgenes. In addition, when mLCAT was absent, the hE149A Tg resulted in a 3-fold enrichment of CEs containing 20- and 22-carbon fatty acyl chains compared with the hLCAT-wt Tg. The genetic background of the hE149A and hLCAT-wt transgenic mice (hybrid vs. C57Bl/6J) may also have contributed to the observed alteration in HDL CE composition.

To determine whether the disproportional contribution of mouse LCAT to the HDL CE fatty acyl composition was the result of decreased binding of human LCAT to HDL particles, we subjected whole plasma from C57Bl/6 and hLCAT-wt Tg mice to gel filtration and measured the elution position of cholesterol and LCAT activity. The results are shown in **Fig. 4**. Nearly all of the LCAT activity in the plasma of C56Bl/6 control and hLCAT-wt Tg mice co-eluted on the ascending front of the plasma HDL cholesterol peak. Thus, a 20-fold overexpression of hLCAT-wt Tg did not result in a significant amount of LCAT activity that was lipoprotein free in plasma.

DISCUSSION

The purpose of this study was to determine whether in vivo expression of a point mutation of human LCAT would alter the CE composition of plasma HDL, resulting in more long-chain polyunsaturated CE species. We have

TABLE 3. Plasma lipid and lipoprotein concentrations of LCAT transgenic mice in the mLCAT−/−, LDLr−/− double knockout background

Genotype	TC	FC	EC	FC/EC	PL	TG	HDL-C	VLDL+ LDL-C
LDLr−/−	260 ± 47	90 ± 18	170 ± 56	0.61 ± 0.27	293 ± 46	114 ± 37	85 ± 19	185 ± 42
hE149A Dbl KO	371 ± 52	106 ± 21	266 ± 39	0.40 ± 0.08	267 ± 32	107 ± 21	95 ± 46	276 ± 80
hLCAT-wt Dbl KO	357 ± 42	94 ± 10	263 ± 35	0.36 ± 0.04	285 ± 37	116 ± 48	106 ± 51	251 ± 45
P	NS	NS	NS	NS	NS	NS	NS	NS

LCAT transgenic mice were crossed into the mLCAT−/−, LDLr−/− double knockout (Dbl KO) background, as described in Materials and Methods. Values are mean ± SD (n = 5 per group). Unpaired *t*-test was used to test for statistically significant differences. NS, not significant at $P = 0.05$. LDLr−/− mice are included for comparative purposes but are not included in the statistical analysis.

TABLE 4. Plasma HDL cholesteryl ester fatty acid percentage composition of LCAT transgenic mice with and without endogenous mouse LCAT

Genotype	16:0	16:1	18:0	18:1	18:2	20:4	20:5	22:6	Other
HDL cholesteryl ester									
hE149A Tg	4.5 ± 1.0	1.5 ± 0.9	0.2 ± 0.4	7.3 ± 2.6	44.2 ± 4.9	26.2 ± 3.8	5.4 ± 0.7	9.8 ± 2.1	1.0 ± 1.4
hE149A Tg Dbl KO	7.9 ± 3.3	1.0 ± 1.5	0.7 ± 1.6	12.6 ± 1.9	53.4 ± 4.2	16.1 ± 3.7	3.9 ± 1.4	4.4 ± 0.9	0.0 ± 0.0
hLCAT-wt Tg	6.5 ± 2.7	2.6 ± 2.6	0.2 ± 0.5	8.1 ± 2.2	53.4 ± 5.6	18.1 ± 3.3	5.4 ± 1.0	4.7 ± 1.2	0.9 ± 1.1
hLCAT-wt Tg Dbl KO	8.3 ± 2.4	0.9 ± 1.3	1.9 ± 1.1	12.8 ± 1.6	66.1 ± 5.0	6.8 ± 1.8	0.8 ± 1.1	0.9 ± 1.3	1.3 ± 1.9
	P values								
hE149A Tg vs. hE149A-Tg Dbl KO	0.045	NS	NS	0.009	0.01	<0.001	0.044	<0.001	
hLCAT-wt Tg vs. hLCAT-wt Tg Dbl KO	NS	NS	0.019	0.003	0.001	<0.001	0.001	0.001	
hE149A Tg Dbl KO vs. hLCAT-wt Tg Dbl KO	NS	NS	NS	NS	0.001	0.001	<0.001	0.002	

LCAT transgenic mice were crossed into the mLCAT−/−, LDLr−/− double knockout (Dbl KO) background, as described in Materials and Methods. Plasma HDL cholesteryl ester fatty acyl percentage compositions were then measured and compared with LCAT Tg mice with functional endogenous mouse LCAT. Values are mean ± SD (n = 5 per group). ANOVA and Fisher's least significant difference test were used to identify statistically significant differences among animals, and *P* values for the comparisons are shown. NS, not significant at $P = 0.05$. Data for the hE149A Tg and hLCAT-wt mice are the same as those given in Table 2 and are shown here for ease of comparison.

previously identified a single amino acid mutation (E149A) in human LCAT that increases its in vitro reactivity with rHDL containing PAPC but not those containing POPC. Our goal was to determine whether in vivo overexpression of hE149A would be sufficient to enrich the steady state composition of plasma HDL CE with 20- and 22-carbon polyunsaturated fatty acyl species. Because several studies in man, mice, and nonhuman primates have suggested that a higher content of polyunsaturated CE in plasma results in less atherosclerosis compared with more saturated CE species, we were interested in whether a genetic approach to enriching plasma lipoproteins with polyunsaturated CE would have a similar result. Two novel and significant findings resulted from this study. First, the transgenic overexpression of hE149A compared with hLCAT-wt significantly enriched HDL with CE species containing 20:4, 20:5 n-3, and 22:6 n-3, at the expense of those containing 18:2. This occurred without a significant change in the plasma HDL concentration or HDL phospholipid fatty acyl composition. Second, in spite of a 20-fold overexpression of human LCAT enzymes (hE149A and hLCAT-wt), endogenous mouse LCAT had a significant impact on the fatty acyl composition of HDL CE, suggesting that mouse LCAT contributed to the HDL CE pool out of proportion to its mass. This outcome suggests that mouse apoA-I does not fully activate human LCAT to the extent of mouse LCAT activation. We believe that in vivo overexpression of hE149A is a unique way to test the hypothesis that enrichment of plasma lipoproteins with polyunsaturated CE species is anti-atherogenic, without the confounding changes in plasma lipoprotein concentration and distribution that occurs with feeding polyunsaturated dietary fat.

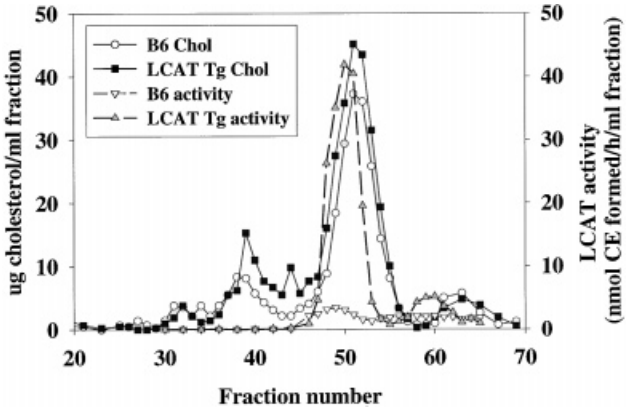

Fig. 4. FPLC cholesterol and LCAT activity elution profile of hLCAT-wt and C57Bl/6 control mouse plasma. Conditions for the FPLC runs are indicated in the legend of Fig. 3. Total cholesterol in each fraction was determined by enzymatic assay, as described in Materials and Methods. For exogenous LCAT assays, 50 μl of each fraction was taken as a source of enzyme with a saturating amount (6.2 μM cholesterol) of POPC rHDL. Incubation times were adjusted from 5–120 min to keep cholesterol esterification <20%. LCAT activities were converted to nanomoles per milliliter fraction per hour for comparative purposes.

Transgenic overexpression of hE149A compared with hLCAT-wt in mice resulted in an enrichment of plasma HDL CE in the fatty acyl species 20:4, 20:5 n-3, and 20:6 n-3 and a relative depletion of those containing 18:2 (Table 4). The difference in the HDL CE fatty acyl composition was even greater when the contribution of endogenous mouse LCAT was eliminated by crossing the LCAT Tg lines into the mouse LCAT knockout background. To our knowledge, only one other study has investigated the effect of transgenic expression of human LCAT on CE fatty acyl composition (32). In that study, mice overexpressing human LCAT had a decrease in C20:4, an increase in C16:0, and no change in C18:2. Data in humans (16, 17, 33), nonhuman primates (14, 34, 35), and mice (13) suggest that enrichment of plasma CE with polyunsaturated fatty acids is less atherogenic than CE species containing saturated or mono-unsaturated fatty acids. Despite the consistency of this finding across studies and species, the mechanism for the decreased atherogenicity of polyunsaturated dietary fat is unknown. Polyunsaturated dietary fat results in a decrease in plasma LDL and HDL concentrations,

the latter being anti-atherogenic and the former being atherogenic. There are also changes in lipoprotein composition and subfraction size distribution that accompany consumption of polyunsaturated fat. Because of the multiple changes that occur in plasma lipoproteins with dietary polyunsaturated fat that confound the interpretation of the atherosclesosis results, we have taken a molecular genetic approach to enrich plasma lipoproteins with polyunsaturated CE. This approach became feasible with the discovery that mutating amino acid 149 of human LCAT from a glutamic acid to an alanine residue increased the reactivity of the enzyme to PC substrates containing *sn*-2 20- to 22-carbon fatty acyl chains but not those containing 18-carbon *sn*-2 fatty acyl chains (20) (Y. Zhao, J. Wang, and J. S. Parks, unpublished observations). Atherosclerosis studies are currently underway to determine whether overexpression of hE149A compared with hLCAT-wt in the mLCAT−/−, LDLr−/− background results in less atherosclerosis.

An interesting, yet perplexing, finding in our study is that a 20-fold overexpression of human LCAT (hE149A or hLCAT-wt) only results in a modest increase in plasma HDL concentration (77 vs. 98 mg/dl for C57Bl/6 and hLCAT-wt, respectively) and no detectable increase in HDL particle size. This outcome is likely related to the level of human LCAT overexpression. Vaisman et al. (36), using several lines of human LCAT transgenic mice, found a correlation between plasma LCAT activity and HDL cholesterol as well as HDL particle size. However, even the highest expressing transgenic line, with a 100-fold overexpression of human LCAT, only resulted in a doubling of HDL cholesterol concentration. Mehlum et al. (32) saw a moderate increase in HDL-C concentration (72 vs. 87 mg/dl) and a moderate increase in HDL size with a 40-fold overexpression of LCAT. One possible explanation for these findings is that most of the human transgene is not associated with mouse HDL particles and thus is unavailable for CE synthesis. However, our FPLC data (Fig. 4) as well as that of others (32) demonstrates that nearly all of the LCAT activity in the plasma of hLCAT-wt Tg mice comigrates in the HDL region and, as such, would be available for cholesterol esterification. Another possibility is that human LCAT does not use mouse HDL PL as efficiently for cholesterol esterification as mouse LCAT. However, this seems unlikely because the fatty acyl composition of mouse HDL PL is similar to that of man (37). Based on these considerations, it is likely that the modest changes in HDL concentration and size with LCAT (wild type or mutant) overexpression are not the result of inefficient binding of LCAT to HDL particles or to phospholipid substrate utilization.

The most likely cause for the modest changes in HDL concentration and size in the face of larger changes in exogenous activity and CE fatty acid composition is that human LCAT is poorly activated by mouse apoA-I. This results in lower than expected in vivo activity for human LCAT and a contribution of mouse LCAT to the HDL CE pool that is out of proportion to its mass in plasma. Although there is little direct experimental evidence to support or refute this hypothesis, Vaisman et al. (36), citing unpublished data, state that there is no difference in plasma LCAT activity when measured with exogenous substrate particles containing mouse versus human apoA-I. Furukawa et al. (38) have shown that rat apoA-I does not activate human LCAT as well as it activates rat LCAT in in vitro assays. We believe that three pieces of data support our hypothesis that human LCAT is poorly activated by mouse apoA-I. First, 20-fold overexpression of the human LCAT transgene did not lead to an increase in endogenous cholesterol esterification, even though the overexpressed LCAT appeared to bind to mouse HDL particles (Fig. 4). Second, elimination of endogenous mouse LCAT by crossing the LCAT transgenic mice into the mouse LCAT−/− background resulted in a significant reduction in the 20- to 22-carbon fatty acyl CE species and an increase in proportion of C18:1 and C18:2 in both transgenic lines (Table 4). Thus, the contribution of mouse LCAT to the HDL CE pool was overrepresented compared with the human LCAT transgenes. The most likely explanation for this observation is that endogenous mouse LCAT was preferentially activated by mouse apoA-I compared with human LCAT. Finally, when the human apoA-I transgene was crossed into the LCAT transgenic animals, HDL cholesterol concentrations increased 4-fold to 300–400 mg/dl, and HDL particles became larger and polydispersed (39). In this situation, mouse apoA-I is displaced from HDL particles and becomes hypercatabolized, resulting in a plasma concentration that is 5–10% of that in control animals not expressing the human apoA-I transgene (40). Thus, in the presence of sufficient human apoA-I in plasma, cholesterol esterification by human LCAT is supported. The less efficient activation of human LCAT by mouse apoA-I may involve decreased stabilization of the enzyme on the HDL surface, decreased presentation of substrate by mouse apoA-I to the active site of human LCAT, or a decreased direct interaction of mouse apoA-I and human LCAT, resulting in a less active conformation of human LCAT. The increase in HDL concentration and size that occurs when human LCAT is overexpressed at very high levels may result from the overwhelming effect of large quantities of human LCAT in plasma that drive the esterification process on apoA-I- as well as apoE-containing HDL.

In summary, we have described a transgenic mouse model that we created to study the role of plasma CE fatty acyl composition on atherosclerosis development. The advantage of this model is that the confounding problems of altered lipoprotein concentration and composition that are usually present in dietary fat modification studies are minimized. We are currently conducting atherosclerosis studies with these mice.

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