In vivo contribution of LCAT to apolipoprotein B lipoprotein cholesteryl esters in LDL receptor and apolipoprotein E knockout mice

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Abstract **Previous studies have indicated that LCAT may play a role in the generation of cholesteryl esters (CE) in plasma apolipoprotein B (apoB) lipoproteins. The purpose of the present study was to examine the quantitative importance of LCAT on apoB lipoprotein CE fatty acid (CEFA) composition. LCAT$^{-/-}$ mice were crossed into the LDL receptor (LDLr)$^{-/-}$ and apoE$^{-/-}$ background to retard the clearance and increase the concentration of apoB lipoprotein in plasma. Plasma free cholesterol was significantly elevated but total and esterified cholesterol concentrations were not significantly affected by removal of functioning LCAT in either the LDLr$^{-/-}$ or apoE$^{-/-}$ mice consuming a chow diet. However, when functional LCAT was removed from LDLr$^{-/-}$ mice, the CEFA ratio (saturated + monounsaturated/polyunsaturated) of plasma LDL increased 7-fold because of a 2-fold increase in saturated and monounsaturated CE, a 40% reduction in cholesteryl linoleate, and a complete absence of long chain (>18 carbon) polyunsaturated CE (20:4, 20:5n-3, and 22:6n-3), from 29.3% to 0%. Removal of functional LCAT from apoE$^{-/-}$ mice resulted in only a 1.6-fold increase in the CEFA ratio, due primarily to a complete elimination of long chain CE (7.7% to 0%). Our results demonstrate that LCAT contributes significantly to the CEFA pool of apoB lipoprotein and is the only source of plasma long chain polyunsaturated CE in these mice.—** Furbee, J. W., Jr., O. Francone, and J. S. Parks. **In vivo contribution of LCAT to apolipoprotein B lipoprotein cholesteryl esters in LDL receptor and apolipoprotein E knockout mice. *J. Lipid Res.* 2002.** 43: **428–437.**

Supplementary key words mouse • high density lipoprotein • low density lipoprotein • VLDL • chylomicron remnant • ACAT • polyunsaturated fatty acids • lecithin:cholesterol acyltransferase

Two enzymes are responsible for the synthesis of cholesteryl esters (CE) in vertebrates, LCAT and ACAT. ACAT is an intracellular enzyme that uses fatty acyl-CoA and cholesterol as substrates for CE synthesis (1, 2). Two forms of the enzyme have been identified, ACAT-1 and ACAT-2, which have different tissue distributions, and perhaps different physiological functions. ACAT preferentially uses oleoyl-CoA for the synthesis of CE but can use other available fatty acids enriched in tissues by diet. Although ACAT is an intracellular enzyme, it can contribute to the plasma pool of CE. ACAT in the liver and intestine synthesizes CEs that are secreted in TG-rich lipoproteins, such as chylomicrons and VLDL, which circulate in the plasma compartment. Plasma VLDL is metabolized to LDL, which are CE-rich particles with a prolonged residence time in plasma, and whose concentration has been positively associated with the extent of coronary artery atherosclerosis. LCAT is synthesized predominantly by the liver and secreted into plasma where it uses phosphatidylcholine (PC) and cholesterol in lipoprotein particles as substrate for CE synthesis (3, 4). The preferred plasma substrate particle for LCAT is HDL, which contains >80% of the plasma pool of the apolipoprotein A-I (apoA-I), the best activator of the enzyme. However, other apolipoproteins can activate LCAT in vitro, albeit, to a much lesser extent (5). The CE species generated by LCAT are predominantly polyunsaturated because LCAT uses the *sn*-2 fatty acyl group of PC, which is enriched in PUFA relative to the *sn*-1 position. The cholesterol esterification by LCAT performs several important physiological functions, including HDL particle maturation as well as maintenance of normal plasma HDL concentrations and particle structure (6). HDL also plays an important role in the process of reverse cholesterol transport, whereby excess cholesterol in peripheral tissues is transported back to the liver for excretion, and may help explain the inverse association between HDL concentrations and the prevalence of atherosclerotic heart disease (7, 8).

It has been hypothesized that LCAT is responsible for

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; apoE, apolipoprotein E; CE, cholesteryl ester(s); CEFA, cholesteryl ester fatty acid; EC, esterified cholesterol; FC, free cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LDLr, LDL receptor; PC, phosphatidylcholine; PL, phospholipid; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; TPC, total plasma cholesterol; VLDL-C, VLDL cholesterol.

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the synthesis of most of the CE in the plasma of individuals with normal plasma cholesterol concentrations. This conclusion was based on the marked reduction (~20% of normal) of CE in the plasma of individuals with familial LCAT deficiency (6, 9). However, the contribution of ACAT versus LCAT to the plasma CE pool of individuals who have hyperlipoproteinemia and are at increased risk for development of coronary heart disease is less clear. Although it is generally accepted that LCAT is the source of CE in plasma HDL, the contribution of LCAT to the generation of CE in apoB lipoproteins is poorly understood. Pathological states that lead to over-production or delayed clearance of apoB lipoproteins result in the accumulation of CE-enriched lipoproteins in plasma. Studies in non-human primates have shown that when plasma LDL concentrations increase following consumption of an atherogenic diet, LDL particles become larger and enriched in saturated and monounsaturated CE (10). This enrichment appears to be the result of hepatic ACAT, as strong associations have been observed between hepatic ACAT activity, hepatic CE secretion, and CE enrichment of LDL, in general, and cholesteryl oleate, in particular (11, 12). Several studies in non-human primates have now shown that LDL size is highly correlated with the development of coronary artery atherosclerosis (10, 13, 15), and a recent study has demonstrated that large LDL are an independent predictor of coronary events in patients who have had a myocardial infarction (14). Although the exact mechanism for the increased atherogenic nature of large LDL is unknown, the increased content of saturated and monounsaturated CE at the expense of polyunsaturated CE appears to be a key feature (10, 12). Several studies in man have also shown that the species of CE in circulating plasma LDL are strongly correlated with development of coronary heart disease, with less disease in individuals with a higher cholesteryl linoleate to cholesteryl oleate ratio in plasma LDL (16–18). These data taken together indicate that the types of CEs in LDL as well as the concentration of LDL in plasma are important in the pathogenesis of atherosclerosis.

In recent years, many transgenic and gene-targeted mouse models have been developed to study the pathogenesis of atherosclerosis (19). A common feature of nearly all of these models is an increased concentration of apoB lipoproteins in plasma. Two of the most frequently used models for atherosclerosis studies, the LDL receptor $(LDLr)^{-/-}$ and $apoE^{-/-}$ mice, have plasma accumulation of LDL and apoB-48 remnant particles, respectively (20–22). Because mice have no functioning CETP (23), it is assumed that nearly all CE in the apoB lipoproteins of these mice were generated by liver and/or intestinal ACAT. Although LCAT is found associated with plasma LDL, its contribution to the apoB lipoprotein CE pool is thought to be minor (24, 25). However, a recent atherosclerosis study in apoB-100 transgenic, $LDLr^{-/-}$ mice found a high percentage of polyunsaturated CE species in LDL, ranging from 65% on a chow diet to 34% on a saturated, palm oil diet (26). These results raised the intriguing question of whether, in the absence of CETP, the LDL CE were derived primarily from liver ACAT or from direct synthesis by LCAT on LDL particles. We hypothesized that LCAT was an important physiological source of plasma CE in apoB lipoproteins of $LDLr^{-/-}$ and $apoE^{-/-}$ mice. Given the preferred fatty acyl specificity of mouse LCAT for long chain (>18 carbons) fatty acids (27), the LCAT contribution to the apoB lipoprotein CE pool would significantly increase the long-chain polyunsaturated CE species and presumably decrease the atherogenicity of the apoB particles. To test our hypothesis, we generated two lines of double knockout mice ($LDLr^{-/-}$ $LCAT^{-/-}$ and $apoE^{-/-}$ $LCAT^{-/-}$) that had elevated plasma concentrations of apoB lipoproteins and also lacked functioning mouse LCAT, and compared these lines to $LDLr^{-/-}$ and $apoE^{-/-}$ mice with functioning LCAT. Our results demonstrate that plasma LCAT contributes significantly to the cholesterol ester fatty acid (CEFA) pool of apoB lipoproteins in $LDL^{-/-}$ and $apoE^{-/-}$ mice and is the only source of long-chain polyunsaturated CE.

MATERIALS AND METHODS

Generation of $LDLr^{-/-}$ and $apoE^{-/-}$ mice lacking endogenous mouse LCAT

$LCAT^{-/-}$ mice (75% C57Bl/6, 25% 129/Ola background) were obtained from Pfizer, Inc. (28), $apoE^{-/-}$ mice (100% C57Bl/6 background) (29) were kindly provided by Dr. Nobuyo Maeda (University of North Carolina-Chapel Hill), and $LDLr^{-/-}$ and C57Bl/6J mice were purchased from Jackson Labs (Bar Harbor, ME). The $LDLr^{-/-}$ $LCAT^{-/-}$ and $apoE^{-/-}$ $LCAT^{-/-}$ mice were created in a two-step breeding process. In the first step, LDL receptor knockout (i.e., $LDLr^{-/-}$, $LCAT^{+/+}$) mice were crossed with LCAT knockout ($LDLr^{+/+}$ $LCAT^{-/-}$) mice to generate double heterozygotes ($LDLr^{+/-}$ $LCAT^{+/-}$). In the second step, the F1 generation was then intercrossed to generate $LDLr^{-/-}$ $LCAT^{-/-}$ mice that were ~90% in the C57Bl/6 background. The $apoE^{-/-}$ $LCAT^{-/-}$ mice were generated in a similar fashion.

At each step of the breeding protocol, pups were screened by TPC and exogenous LCAT activity assays. PCR of genomic DNA was also used to confirm $LDLr^{-/-}$, $LCAT^{-/-}$, and $apoE^{-/-}$ genotypes. Primers and conditions for PCR have been described previously (30). The PCR conditions for the screening of $apoE^{-/-}$ mice were similar and used the following primers: EKO-F, 5′-GTC TCG GCT CTG AAC TAC ATA G-3′ and EKO-R, 5′-GCA AGA GGT GAT GGT ACT CG-3′. The primers generated a 600 bp band for the wild type allele and a 1600 bp band for the targeted allele. 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 transgenic barrier facility. 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 14 and 20 weeks of age, and were fed a chow diet. Unless indicated otherwise, five 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 as described previously (30).

Lipid, LCAT activity, and lipoprotein analysis

Plasma total cholesterol (Wako), free cholesterol (FC) (Wako), phospholipid (PL) (Wako), and TG (Roche) concentrations

were determined by enzymatic assay (31). Protein concentrations were determined by the Lowry method (32), using BSA as a standard.

Recombinant HDL was synthesized by a cholate dialysis procedure as described previously using a starting molar ratio of 1:5:80 [human apo A-I/cholesterol/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)] with a trace amount of [^{3}H]cholesterol (50,000 dpm/μg of cholesterol) added to quantify CE formation (33).

Exogenous LCAT assays were performed as described previously using 2 μl of mouse plasma as a source of enzyme and an incubation time of 30–60 min at 37°C (33). LCAT activity was expressed as nmols of CE formed per ml plasma per h.

Plasma apoB lipoproteins and HDL were separated from 100–150 μl plasma using a Superose 6 (1 × 30 cm) and Superose 12 (1 × 30 cm) fast protein liquid chromatography (FPLC) columns in series as described previously (30). The elution profile was monitored by total cholesterol enzymatic assay.

Plasma apolipoproteins were analyzed by SDS polyacrylamide gradient gel electrophoresis. Lipoproteins from plasma (200 μl) were isolated by ultracentrifugation at d = 1.21 g/ml using a Beckman TLA 100.2 rotor operated at 1 × 10^5 rpm for 18 h (15°C). The top fraction (lipoproteins) was isolated by tube slicing and the samples were extensively dialyzed against 0.05% EDTA and 0.05% sodium azide. Fifteen micrograms of protein, determined by the Lowry assay as described above, were lyophilized and loaded on a 4–16% SDS polyacrylamide gradient gel in SDS sample buffer. The gel was run at 75 V for 30 min, then 150 V for 2 h (10°C), stained with 0.2% Coomassie blue in 50% methanol, 10% acetic acid, and destained with 50% methanol, 10% acetic acid.

Lipoprotein PL and CEFA analysis

Plasma apoB lipoproteins and HDL were separated by FPLC and the lipids were extracted (34). CEs and PLs were isolated from the lipid extract by TLC, fatty acids were methylated, and relative amounts of fatty acids were quantitated after separation on a gas-liquid chromatography fatty acid column (Chrompak CP-SIL88 FAME column), as described previously (30).

Gradient gel electrophoresis

One μl of mouse plasma was separated on 4–30% nondenaturing gradient gels (35) and transferred to nitrocellulose as described previously (30). Standards were visualized with 0.2% Ponceau Red, and Western blots for mouse apoA-I were developed using a goat anti-mouse apoA-I antibody (Biodesign International, Saco, ME).

Data analysis

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

RESULTS

All mice used in this study were male, 14 to 20 weeks of age, and were fed a rodent chow diet. Exogenous LCAT cholesterol esterification activity with a POPC recombinant HDL substrate is summarized in **Fig. 1**. The LDLr$^{-/-}$, apoE$^{-/-}$, and C57Bl/6J mice had similar levels of plasma LCAT activity (58 vs. 58 vs. 63 nmol CE /ml/ h), whereas the three LCAT deficient lines had no measurable LCAT activity above background.

Table 1 summarizes the plasma lipid concentrations for all study animals. The C57Bl/6J and LCAT$^{-/-}$ mice were

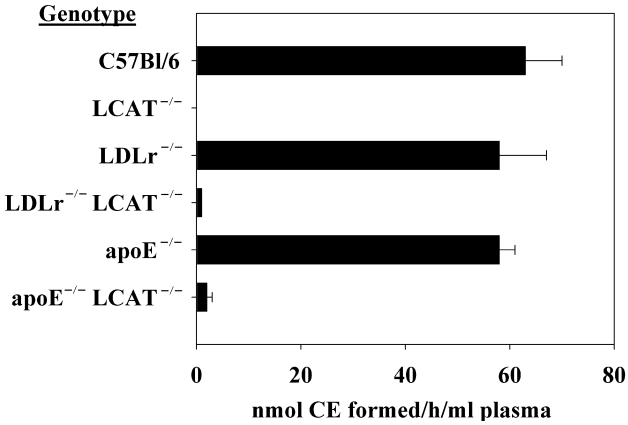

Fig. 1. Exogenous LCAT activity in plasma of mice of the indicated genotype was measured using recombinant HDL substrate particles containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), [^{3}H]cholesterol, and apolipoprotein A-I (apoA-I). Reactions were performed using a saturating concentration of substrate cholesterol (3 μM) and an incubation time of 30–60 min. Values (mean ± SD; n = 5 per group) are expressed as nmol of cholesteryl ester (CE) formed/h/ml of plasma.

included for comparative purposes but are not discussed herein. Compared with the LDLr$^{-/-}$ mice, the apoE$^{-/-}$ mice had significantly higher plasma concentrations of total cholesterol (2.4-fold), FC (2.8-fold), esterified cholesterol (EC) (2.3-fold), and PL (1.3-fold). When the LDLr$^{-/-}$ LCAT$^{-/-}$ mice were compared with the LDLr$^{-/-}$ mice, there was no difference in total plasma cholesterol (TPC) concentrations, but there were significant increases in plasma FC (1.9-fold) and TGs (2.8-fold) concentrations. Similar results were observed when apoE$^{-/-}$ LCAT$^{-/-}$ mice were compared with apoE$^{-/-}$ mice, with no change in TPC, and increases in FC (1.4-fold) and TGs (2.5-fold). When apoE$^{-/-}$ LCAT$^{-/-}$ mice were compared with LDLr$^{-/-}$ LCAT$^{-/-}$ mice, there was an ~2-fold increase in all plasma lipid constituents measured. The most striking result from loss of functional LCAT was a 9-fold increase in the FC/EC ratio in LCAT$^{-/-}$ mice compared with the C57Bl/6 controls. However, in the LDLr$^{-/-}$ LCAT$^{-/-}$ and apoE$^{-/-}$ LCAT$^{-/-}$ mice, there was a more modest increase (1.7–2.7-fold) in the FC/EC ratio that was not statistically different from those of the LDLr$^{-/-}$ and apoE$^{-/-}$ mice, respectively.

Figure 2A and **B** show FPLC cholesterol (FPLC-C) elution profiles of plasma from each of the mouse genotypes included in the study. Figure 2A shows the FPLC-C profile for C57Bl/6J, LCAT$^{-/-}$, LDLr$^{-/-}$, and LDLr$^{-/-}$ LCAT$^{-/-}$ mice. The C57Bl/6J mice have nearly all of their plasma cholesterol distributed in the HDL fraction, but in LCAT-deficient mice there was very little HDL cholesterol (HDL-C) present, and the little cholesterol that was present in plasma was distributed in the VLDL size range (fractions 27–30). The elution profile for the LDLr$^{-/-}$ mice showed nearly equal distribution of plasma cholesterol in the HDL and LDL fractions. However, when LCAT was removed from LDLr$^{-/-}$ mice, there was no de-

TABLE 1. Plasma lipid and lipoprotein concentrations

Genotype	TPC	FC	EC	FC/EC	PL	TG
C57Bl/6	82 ± 13	24 ± 4	58 ± 10	0.41 ± 0.03	169 ± 56	57 ± 30
LCAT$^{-/-}$	48 ± 10	35 ± 5	13 ± 7	3.63 ± 2.68	114 ± 21	88 ± 42
LDLr$^{-/-}$	186 ± 15	55 ± 11	131 ± 21	0.44 ± 0.13	221 ± 23	85 ± 25
LDLr$^{-/-}$ LCAT$^{-/-}$	200 ± 46	105 ± 23	94 ± 27	1.17 ± 0.32	222 ± 38	238 ± 167
ApoE$^{-/-}$	449 ± 110	153 ± 26	296 ± 96	0.55 ± 0.15	279 ± 29	190 ± 47
ApoE$^{-/-}$LCAT$^{-/-}$	465 ± 136	212 ± 34	254 ± 104	0.92 ± 0.26	322 ± 60	469 ± 210
P values						
LDLr$^{-/-}$ vs. apoE$^{-/-}$	<0.0001	<0.0001	0.0002	NS	0.048	NS
LDLr$^{-/-}$ vs. LDLr$^{-/-}$ LCAT$^{-/-}$	NS	0.0007	NS	NS	NS	0.048
ApoE$^{-/-}$ vs. apoE$^{-/-}$ LCAT$^{-/-}$	NS	0.0001	NS	NS	NS	0.0009
ApoE$^{-/-}$ LCAT$^{-/-}$ vs. LDLr$^{-/-}$ LCAT$^{-/-}$	<0.0001	<0.0001	0.0003	NS	0.0009	0.0044

Blood was obtained from 14–20 week old chow fed mice of the indicated genotype and measurements were made as described in Materials and Methods. Values are the mean ± SD (n = 5 per group) and are expressed as mg/dl, except for the FC/EC ratio. *P* values for statistical comparisons between mice were derived from analysis of variance 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.

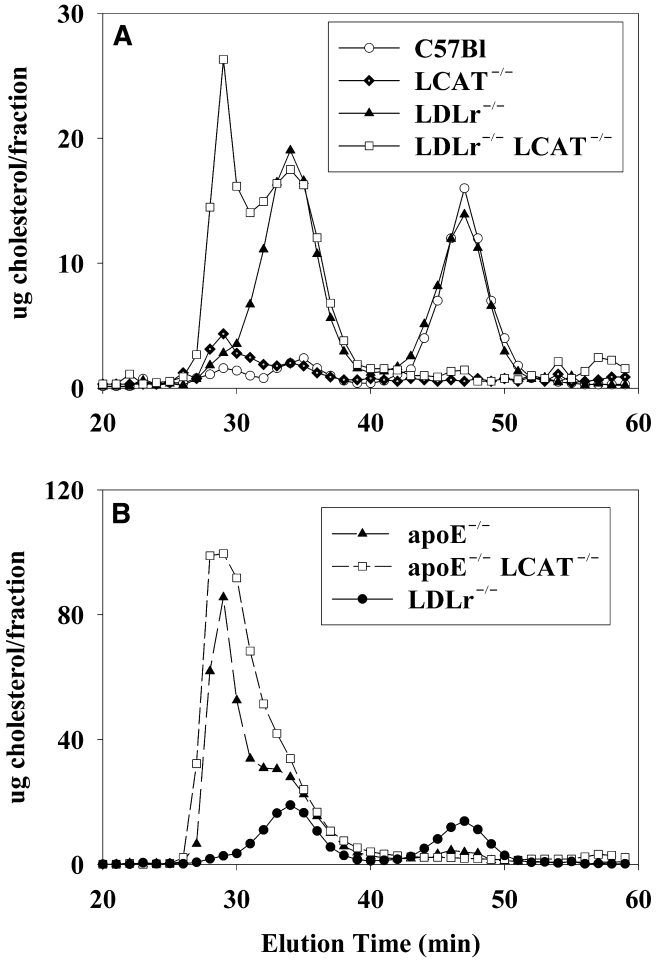

Fig. 2. A: FPLC cholesterol (FPLC-C) elution profile of plasma from C57Bl/6J, LCAT$^{-/-}$ LDLr$^{-/-}$, and LDLr$^{-/-}$ LCAT$^{-/-}$ mice. One hundred μl of plasma from individual animals were injected onto a Superose 6 (1 × 30 cm) and a Superose 12 (1 × 30 cm) column connected in series with a flow rate of 0.5 ml/min. One minute fractions were collected and total cholesterol was measured using an enzymatic assay. B: FPLC cholesterol elution profile of plasma from apoE$^{-/-}$, apoE$^{-/-}$ LCAT$^{-/-}$, and LDLr$^{-/-}$ (shown for reference) mice. Experimental conditions are the same as in A.

tectable HDL-C, a large increase in cholesterol in the VLDL fraction, and little change in LDL cholesterol (LDL-C). Figure 2B shows the FPLC-C elution profile for apoE$^{-/-}$ and apoE$^{-/-}$ LCAT$^{-/-}$ mice, with the profile of the LDLr$^{-/-}$ sample shown in Fig. 2A included for reference. The apoE$^{-/-}$ mice have a decreased concentration of HDL-C compared with C57Bl/6J or LDLr$^{-/-}$ mice (see below), and elevated concentrations of VLDL-C and LDL-C present in plasma. The apoE$^{-/-}$ LCAT$^{-/-}$ mice have no detectable HDL-C, and increases in both VLDL-C and LDL-C compared with apoE$^{-/-}$ mice. Thus, LDLr$^{-/-}$ mice accumulate mostly LDL-sized particles in plasma, whereas apoE$^{-/-}$ mice accumulate larger VLDL-sized or remnant particles. When functioning LCAT is removed from either model, there is an absence of HDL and an increase in VLDL-C.

To examine plasma apolipoprotein composition, lipoproteins from study mice were isolated by ultracentrifugation of plasma and apolipoproteins were separated by 4–16% SDS-PAGE as described in Materials and Methods. The results are shown in **Fig. 3**. Lipoproteins from C57Bl/6 mice (lane 2) had predominantly apoA-I, reflecting the major lipoprotein species (HDL) in plasma, with low levels of apoE, apoB-48, and apoB-100. When apolipoproteins from LCAT$^{-/-}$ mice (lane 3) were compared with C57Bl/6 mice, there was a moderate decrease in apoA-I, and an increase in apoE, apoB-48, and apoB-100. Lipoproteins from both LDLr$^{-/-}$ (lane 4) and LDLr$^{-/-}$ LCAT$^{-/-}$ mice (lane 5) showed an accumulation of apoB-100, whereas other apolipoprotein bands were similar in intensity to C57Bl/6 mice. The apolipoproteins from apoE$^{-/-}$ (lane 6) and apoE$^{-/-}$ LCAT$^{-/-}$ mice (lane 7) completely lacked apoE and showed an accumulation of apoB-48, but otherwise the apolipoprotein composition was similar to that of C57Bl/6 mice. These results are consistent with previously published work (36) and suggest that LDLr$^{-/-}$ mice are accumulating, in plasma, apoB lipoproteins secreted by the liver, whereas apoE$^{-/-}$ mice are accumulating apoB particles that have been secreted by the small intestine.

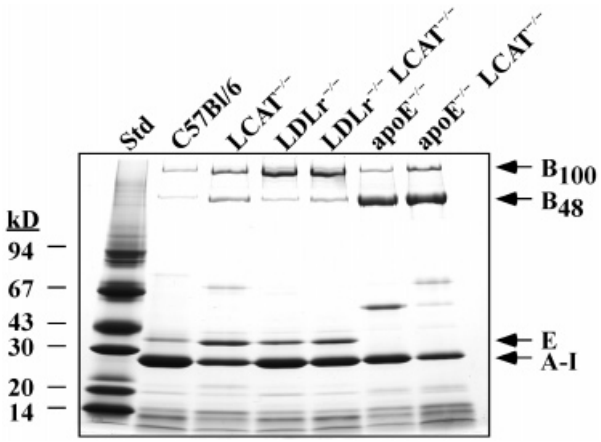

Fig. 3. Four to sixteen percent SDS-polyacrylamide gel separation of plasma lipoproteins floated at a density = 1.21 g/ml in the ultracentrifuge. Plasma was isolated from mice of the indicated genotypes (top of gel) and plasma lipoproteins were isolated as indicated in Materials and Methods. Fifteen micrograms of protein were applied per lane. Std = low molecular weight standard (Pharmacia Biotech). Estimated migration distance of apoB-100, B-48, E, and A-I are indicated on the right side of the gel.

To quantitatively examine the apparent decrease in plasma HDL between the apoE$^{-/-}$ and LDLr$^{-/-}$ mice observed in Fig. 2, we measured the distribution of apoB lipoprotein and HDL-C from the FPLC column runs of 4–5 individual C57Bl/6J, LDLr$^{-/-}$, and apoE$^{-/-}$ mice; and the results are shown in **Fig. 4**. The cholesterol distribution for LCAT$^{-/-}$, LDLr$^{-/-}$ LCAT$^{-/-}$, and apoE$^{-/-}$ LCAT$^{-/-}$ mice was not determined because FPLC-C profiles (Fig. 2) indicated that nearly all cholesterol from these animals was in the apoB lipoprotein particles. The concentration of HDL-C in the C57Bl/6J and LDLr$^{-/-}$ mice was similar, 67 ± 13 versus 61 ± 8 mg/dl, whereas that of apoE$^{-/-}$ mice was 50% lower (37 ± 6 mg/dl). All three genotypes of mice had mono-dispersed HDL particles with an average particle size of 9.4 nm, as determined by apoA-I Western blot of 4–30% non-denaturing gradient gels (**Fig. 5**). Their LCAT$^{-/-}$ counterparts had only HDL particles in the pre-beta to small HDL size range (Fig. 5). The concentration of apoB lipoprotein cholesterol in these mice was markedly affected by genotype, with C57Bl/6J mice having a very low concentration (15 ± 11 mg/dl), LDLr$^{-/-}$ mice having an intermediate concentration (122 ± 14 mg/dl), and the apoE$^{-/-}$ mice having the highest concentration of apoB lipoprotein cholesterol (412 ± 105 mg/dl).

Plasma apoB lipoproteins and HDL, separated by FPLC, were analyzed for PL and CE fatty acyl composition. The results of the apoB lipoprotein analyses for all six groups are shown in **Table 2**. The PL fatty acyl composition was similar among all groups, with only an occasional statistically significant difference as indicated in the upper half of Table 2. However, the apoB lipoprotein CE fatty acyl composition was significantly affected by the genotype of the animals. Compared with the LDLr$^{-/-}$ mice, the CE compositions in the LDLr$^{-/-}$ LCAT$^{-/-}$ had an increase in 16:0 (2.1-fold), 18:0 (2.3-fold), and 18:1 (2.1-fold), whereas there was a decrease in 18:2 (39%). The long chain PUFA, 20:4, 20:5 n-3, and 22:6 n-3, made up 29.3% of the total LDL CEs in the LDLr$^{-/-}$ mice, but these esters were completely absent in the LDLr$^{-/-}$ LCAT$^{-/-}$ mice. The CE compositional differences between

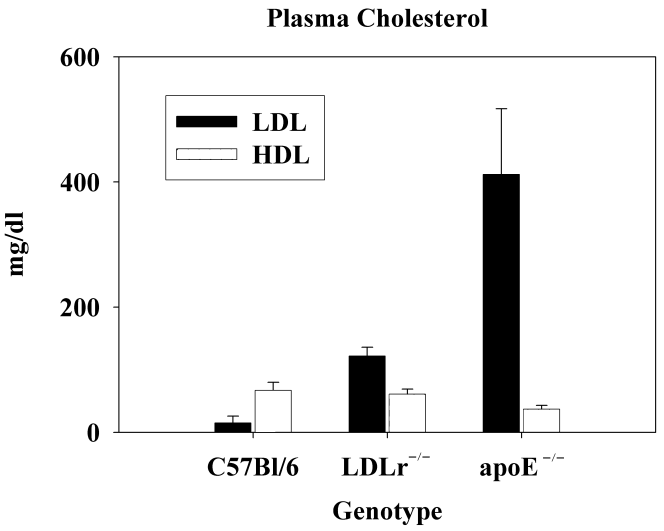

Fig. 4. Cholesterol distribution in apoB lipoproteins (i.e., VLDL and LDL) and HDL was determined for C57Bl/6, LDLr$^{-/-}$, and apoE$^{-/-}$ mice consuming chow. Values are the mean ± SD (n = 5 per group). Analysis of variance and Fisher's least significant difference test were used to identify statistically significant differences among groups. For apoB lipoproteins: C57Bl/6 versus LDLr$^{-/-}$ $P = 0.03$, C57Bl/6 versus apoE$^{-/-}$ $P < 0.0001$, and LDLr$^{-/-}$ versus apoE$^{-/-}$ $P < 0.0001$. For HDL, C57Bl/6 versus LDLr$^{-/-}$ P = not significant, C57Bl/6 versus apoE$^{-/-}$ $P = 0.0005$, and LDLr$^{-/-}$ versus apoE$^{-/-}$ $P = 0.0034$.

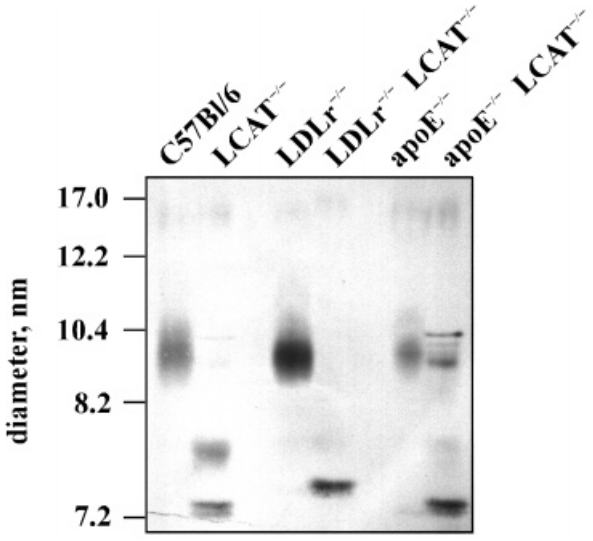

Fig. 5. Western blot of mouse plasma (1 μl) separated on 4–30% non-denaturing gradient gels and developed with a goat anti-mouse apoA-I antiserum as described in the Materials and Methods section. Genotype of mouse from which the plasma was obtained is shown at the top of the blot. Hydrated diameters of the protein standards run on the gel are shown on the left side of the blot.

TABLE 2. Plasma apoB lipoprotein 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
Percent fatty acids in apoB lipoprotein phospholipid									
C57Bl/6	22.1 ± 5.2[a]	0.2 ± 0.4	23.2 ± 2.8	9.9 ± 2.6[a]	21.1 ± 2.5	9.1 ± 1.3[a]	0.0 ± 0.0[a]	10.7 ± 4.2	3.9 ± 0.9
LCAT$^{-/-}$	9.4 ± 4.2	0.0 ± 0.0	25.1 ± 1.3	5.7 ± 1.1	21.0 ± 1.0	21.1 ± 2.0	1.9 ± 0.3	11.6 ± 2.1	4.2 ± 1.3
LDLr$^{-/-}$	17.9 ± 4.3	0.0 ± 0.0	22.3 ± 1.8[b]	8.4 ± 0.3	25.1 ± 2.2	11.1 ± 1.9[b]	0.0 ± 0.0	9.0 ± 1.1[b]	6.1 ± 1.2
LDLr$^{-/-}$ LCAT$^{-/-}$	18.8 ± 2.1	0.0 ± 0.0	25.9 ± 2.4	7.8 ± 1.6	23.6 ± 2.1	15.7 ± 1.9	0.3 ± 0.6	5.9 ± 0.5	2.0 ± 0.1
ApoE$^{-/-}$	22.6 ± 6.4	0.1 ± 0.2	23.8 ± 3.4	9.9 ± 3.5	26.2 ± 5.3	8.2 ± 2.6	0.5 ± 0.9	7.0 ± 4.2	1.7 ± 2.0
ApoE$^{-/-}$ LCAT$^{-/-}$	20.3 ± 7.8	0.0 ± 0.0	25.2 ± 5.0	10.7 ± 5.7	30.7 ± 5.4	7.8 ± 4.2	1.3 ± 1.2	4.0 ± 3.1	0.0 ± 0.0
Percent fatty acids in apoB lipoprotein cholesteryl esters									
CB7Bl/6	12.2 ± 2.0	5.6 ± 2.9[a]	3.9 ± 1.4[a]	26.1 ± 6.4[a]	31.0 ± 7.4[a]	12.1 ± 4.5[a]	2.9 ± 1.9	5.3 ± 2.2[a]	1.8 ± 1.2
LCAT$^{-/-}$	9.4 ± 2.6	1.6 ± 0.7	7.1 ± 1.5	50.0 ± 5.2	19.9 ± 0.7	2.4 ± 1.6	1.1 ± 1.3	2.5 ± 0.7	6.1 ± 1.2
LDLr$^{-/-}$	6.2 ± 1.7[b]	3.5 ± 3.4	2.3 ± 1.3[b]	27.2 ± 2.5[b]	30.3 ± 1.6[b]	15.1 ± 1.8[b]	6.0 ± 0.8[b]	8.2 ± 1.5[b]	1.2 ± 1.8
LDLr$^{-/-}$ LCAT$^{-/-}$	13.2 ± 1.5	6.2 ± 2.0	5.2 ± 0.9	56.0 ± 2.4	18.5 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.1
ApoE$^{-/-}$	15.4 ± 1.3	3.9 ± 0.3	5.8 ± 0.8[c]	31.7 ± 2.3[c]	31.8 ± 1.6	5.4 ± 0.9[c]	0.4 ± 0.8	1.9 ± 0.2	3.7 ± 1.8
ApoE$^{-/-}$ LCAT$^{-/-}$	17.7 ± 2.8	3.0 ± 0.8	8.5 ± 1.2	38.8 ± 2.3	29.3 ± 2.6	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 1.1	1.7 ± 0.8

Plasma apoB lipoprotein PL and cholesteryl ester fatty acyl percentage compositions (mean ± SD; n = 5 per group) were measured on 14–20 week old chow-fed mice of the indicated genotype, as described in Materials and Methods. Student's unpaired *t*-test was used to identify statistically significant differences between C57Bl/6J versus LCAT$^{-/-}$, LDLr$^{-/-}$ versus LDLr$^{-/-}$ LCAT$^{-/-}$, and apoE$^{-/-}$ versus apoE$^{-/-}$ LCAT$^{-/-}$. Superscripted letters indicate statistically different values ($P < 0.05$).

the apoE$^{-/-}$ and apoE$^{-/-}$ LCAT$^{-/-}$ mice were small compared with those in the LDLr$^{-/-}$ and LDLr$^{-/-}$ LCAT$^{-/-}$ mice. There was a 46% and 22% increase in cholesteryl stearate and oleate, respectively, for the apoE$^{-/-}$ LCAT$^{-/-}$ mice compared with the apoE$^{-/-}$ mice. The long chain PUFA made up 7.7% of the total apoB lipoprotein CE in the apoE$^{-/-}$ mice, but these fatty acids accounted for 1.0% of the total in the apoE$^{-/-}$ LCAT$^{-/-}$ mice.

The changes in apoB lipoprotein CEFA composition are easier to visualize as a ratio of saturated + monounsaturated/polyunsaturated CE species. This ratio is highly correlated with the extent of coronary artery atherosclerosis in non-human primates (37). A plot of the apoB lipoprotein CEFA ratio for the animals of this study is shown in **Fig. 6**. There was a 2.6-fold increase in the ratio when LCAT$^{-/-}$ mice were compared with C57Bl/6 controls (2.6 ± 0.3 vs. 1.0 ± 0.4, respectively). In the LDLr$^{-/-}$ background, loss of LCAT resulted in a 7-fold increase in the CEFA ratio (4.4 ± 0.5 vs. 0.6 ± 0.1). However, in the apoE$^{-/-}$ background, the increase in the CEFA ratio was only 1.6-fold higher for the apoE$^{-/-}$ LCAT$^{-/-}$ mice (2.3 ± 0.3) compared with the apoE$^{-/-}$ mice (1.4 ± 0.1). Thus, loss of LCAT function resulted in an increase in the saturation of CE in apoB lipoproteins that was much more pronounced with the accumulation of LDL in plasma than with the accumulation of VLDL-sized apoE remnant particles.

The CEFA ratio for apoB lipoproteins was 2.3-fold higher for apoE$^{-/-}$ mice compared with LDLr$^{-/-}$ mice (see above). To investigate whether this compositional difference was the result of accumulation of VLDL-sized remnants in the plasma of apoE$^{-/-}$ mice (Fig. 2) that were not present in the plasma of LDLr$^{-/-}$ mice, LDL-sized particles were isolated by FPLC from plasma of apoE$^{-/-}$ mice, and CEFA composition was determined and compared with that measured in the entire apoB lipoprotein fraction (**Table 3**). The CEFA composition of the LDL fraction contained significantly less 16:0 and 16:1, and more 20:4 and 20:5 n-3, compared with the entire apoB lipoprotein fraction. However, the CEFA ratio of the LDL isolated from the apoE$^{-/-}$ mice was still greater than that of LDL in the LDLr$^{-/-}$ mice (1.1 ± 0.2 vs. 0.6 ± 0.1). Thus, the difference in apoB lipoprotein CEFA composition between LDLr$^{-/-}$ and apoE$^{-/-}$ mice could not be adequately explained by the accumulation of remnant particles in the plasma of apoE$^{-/-}$ mice.

The HDL PL and CE fatty acyl composition for C57Bl/6J, LDLr$^{-/-}$ and apoE$^{-/-}$ are shown in **Table 4**. The HDL PL fraction from apoE$^{-/-}$ mice was more enriched in 16:0

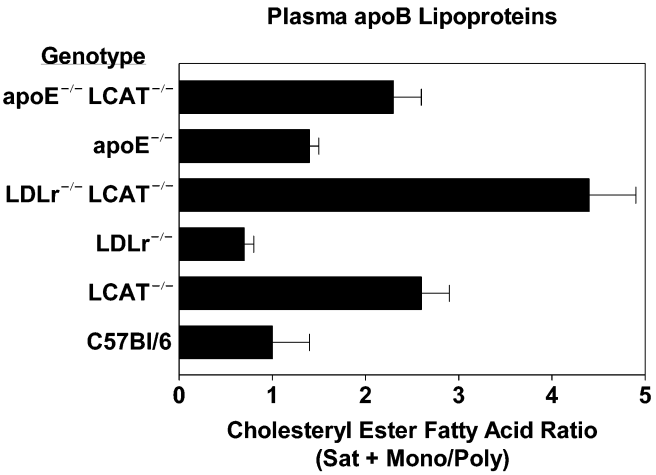

Fig. 6. The apoB lipoprotein CEFA ratio, which is the sum of saturated and monounsaturated CE divided by polyunsaturated CE, was calculated from the data in Table 2. ApoB lipoproteins were isolated from plasma by FPLC, the CE fraction was isolated from the lipoproteins by lipid extraction and TLC, and the fatty acid composition was determined by gas liquid chromatography as described in Materials and Methods. Values represent the mean ± SD (n = 5 per group).

TABLE 3. Plasma apoB lipoprotein and LDL cholesteryl ester fatty acyl percentage composition for apoE$^{-/-}$ mice

Lipoprotein Fraction	Percentage Cholesteryl Esters in Lipoprotein Fractions of ApoE$^{-/-}$ Mice								
	16:0	16:1	18:0	18:1	18:2	20:4	20:5	22:6	Other
VLDL+LDL	15.4 ± 1.3	3.9 ± 0.3	5.8 ± 0.8	31.7 ± 2.3	31.8 ± 1.6	5.4 ± 0.9	0.4 ± 0.8	1.9 ± 0.2	3.7 ± 1.8
LDL	9.1 ± 1.0	2.2 ± 0.6	5.0 ± 1.0	34.5 ± 2.7	33.3 ± 0.6	8.1 ± 0.5	1.9 ± 0.5	2.9 ± 1.0	3.0 ± 0.1
P	0.0004	0.0016	NS	NS	NS	0.017	.027	NS	

Plasma apoB lipoproteins (VLDL+LDL) or LDL were isolated by FPLC from the plasma of 14–20 week old chow apoE$^{-/-}$ mice and cholesteryl ester fatty acyl percentage compositions (mean ± SD; n = 5 VLDL+LDL, n = 3 LDL) were measured as described in Materials and Methods. *P* values for statistical comparisons between VLDL+LDL versus LDL percentage compositions were derived by analysis of variance and Fisher's least significant difference test. NS, not significant.

and 18:0 and contained less 20:4 and 22:6, compared with LDLr$^{-/-}$ mice. Somewhat similar trends were observed in the HDL CEFA composition, with significant increases in 16:0 and 18:1 and decreases in 20:5 and 22:6 for apoE$^{-/-}$, compared with those of LDLr$^{-/-}$ mice. These data show that there is a small enrichment in long-chain polyunsaturated CEs in LDLr$^{-/-}$ mice when compared with apoE$^{-/-}$ mice (85.2% vs. 70.3%), but this difference is much smaller than that observed in the apoB lipoprotein CEFA composition.

DISCUSSION

The purpose of the present study was to determine the quantitative importance of LCAT on apoB lipoprotein CEFA composition using LDLr$^{-/-}$ and apoE$^{-/-}$ mice, two mouse models used extensively in atherosclerosis research. To perform this study, LCAT$^{-/-}$ mice were crossed into the LDLr$^{-/-}$ and apoE$^{-/-}$ backgrounds to retard the clearance and increase the concentration of apoB lipoproteins in plasma. Two novel and significant findings resulted from this study. First, in spite of no significant change in total or esterified plasma cholesterol, removal of functional LCAT in vivo resulted in a significant increase in the saturation index (i.e., CEFA ratio) of apoB lipoprotein CEs in both backgrounds, suggesting that LCAT contributes significantly to plasma pool of CE in LDL, VLDL, and remnant particles, in addition to its well-accepted role in the generation of HDL CE. Second, our results show that plasma LCAT is responsible for the synthesis of nearly all long chain (>18 carbon) CE species in apoB lipoproteins. A corollary of this observation is that mouse ACAT uses long chain PUFA poorly for the intracellular synthesis of CE that are destined for secretion in intestinal or hepatic apoB lipoproteins. Taken together, these results document an important in vivo role for LCAT in the generation of CE in apoB lipoproteins that has previously not been appreciated.

Our results suggest that plasma LCAT makes a significant contribution to the in vivo generation of CE in apoB lipoproteins. LCAT deficiency results in the elimination of spherical HDL, a decrease in plasma total and EC and an increase in plasma TG concentrations in familial LCAT deficiency subjects (6) and gene-targeted mice (38, 39). However, LCAT deficiency in the LDLr$^{-/-}$ or apoE$^{-/-}$ background did not result in a significant decrease in plasma total or EC, but did result in a significant increase in plasma FC concentrations (Table 1). This outcome likely occurred because of the retarded clearance of apoB lipoproteins, which resulted in the accumulation of apoB lipoproteins enriched in ACAT-derived saturated and monounsaturated CE. Studies have shown that ACAT has a broad fatty acyl substrate specificity but in general prefers monounsaturated and saturated fatty acids for the generation of intracellular CE (40, 41). In studies with non-human primates, there is a strong correlation between hepatic ACAT activity and the accumulation of satu-

TABLE 4. 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
Percentage fatty acids in HDL PLs									
C57B1/6	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
LDLr$^{-/-}$	13.9 ± 7.2^a	0.0 ± 0.0	21.1 ± 2.1^a	9.6 ± 1.8	22.7 ± 3.6	12.1 ± 2.1^a	2.0 ± 2.0	13.0 ± 2.4^a	5.7 ± 4.1
apoE$^{-/-}$	23.2 ± 2.9^b	0.0 ± 0.0	28.3 ± 1.2^b	8.7 ± 0.3	21.8 ± 3.0	8.1 ± 1.1^b	2.7 ± 4.7	7.1 ± 0.8^b	0.0 ± 0.0
Percentage fatty acids in HDL CEs									
C57B1/6	3.6 ± 2.1	2.1 ± 2.0	0.2 ± 0.5	5.8 ± 0.6	45.0 ± 6.0	28.2 ± 4.5	4.7 ± 0.7	9.2 ± 3.2	1.3 ± 1.2
LDLr$^{-/-}$	3.9 ± 0.4^a	0.9 ± 1.7	2.1 ± 3.1	7.5 ± 0.9^a	40.7 ± 2.1	28.3 ± 2.6	7.3 ± 0.6^a	8.9 ± 1.3^a	0.5 ± 1.0
apoE$^{-/-}$	8.8 ± 4.0^b	0.0 ± 0.0	4.1 ± 1.3	15.7 ± 1.3^b	39.3 ± 2.3	23.1 ± 2.9	2.6 ± 2.6^b	5.3 ± 1.1^b	1.1 ± 1.6

Plasma PL and CE fatty acyl percentage compositions (mean ± SD; n = 5 per group) were measured on 14–20 week old chow-fed mice of the indicated genotype, as described in Materials and Methods. Unpaired Student's *t*-test was used to identify statistically significant differences between samples from LDLr$^{-/-}$ and apoE$^{-/-}$ mice, whereas values for C57B1/6J mice were included for comparative purposes, but were not included in the statistical analyses. Values with unlike superscripts are statistically different ($P < 0.05$).

rated and monounsaturated CE in plasma LDL particles (11). However, mouse LDL particles are enriched in polyunsaturated CE even when animals are consuming a saturated fat diet (26). Because mice do not have active CETP in plasma to exchange LCAT-derived HDL CE to LDL (23), we hypothesized that plasma LCAT may directly synthesize these polyunsaturated CE in LDL. Although studies have shown that LCAT can use LDL particles to synthesize CE in vitro (42–44), the rate of the reaction is slow compared to that on HDL particles, and the extent to which this reaction contributes to the LDL CE pool in vivo has not been investigated. Our results show that inactivation of LCAT in vivo results in a 2.6-fold increase in the apoB lipoprotein CEFA ratio compared with that of C57Bl/6 controls, a 7-fold increase relative to $LDLr^{-/-}$ controls, and a 1.6-fold increase compared with $apoE^{-/-}$ controls (Fig. 6). The increase in CEFA ratio resulted from both an increase in the percentage of saturated and monounsaturated CE as well as a decrease in the polyunsaturated species (Table 2). Similar results have been observed in the plasma of individuals with familial LCAT deficiency (9). In species with active CETP, the source of long-chain polyunsaturated CE in LDL likely originates through LCAT activity on HDL particles, with subsequent transfer of the LCAT-generated CE to apoB lipoproteins. Whether direct synthesis of CE by LCAT on plasma LDL particles is a significant source of CE when CETP is present in plasma is unknown. However, the redistribution of LCAT from HDL to LDL in hyperlipidemic states where plasma concentrations of HDL are low and LDL are high may result in a greater proportion of LDL CEs produced by LCAT relative to ACAT.

The variation in the increase in the CEFA ratio among these mice likely results from the extent to which particles with ACAT-derived CEs accumulate in plasma and from the reactivity of LCAT with these particles (Figs. 2 and 6). For instance, the smallest increase in the CEFA ratio was observed with $apoE^{-/-}$ versus $apoE^{-/-}$ $LCAT^{-/-}$ mice. $ApoE^{-/-}$ mice accumulate large remnant particles in plasma, which are enriched in ACAT-derived CE and are poorly reactive with LCAT, whereas $LDLr^{-/-}$ mice have an accumulation of LDL particles in plasma that are more reactive with LCAT than remnant particles (Fig. 2). Previous studies in non-human primates have shown a significant correlation between the LDL CEFA ratio and LDL particle size, which is strongly predictive of coronary artery atherosclerosis in this model. Based on these past results, we believe that an increase in the CEFA ratio of apoB lipoproteins is pro-atherogenic, and hypothesize that in vivo inactivation of LCAT will be pro-atherogenic in the $LDLr^{-/-}$ and $apoE^{-/-}$ mice. Atherosclerosis studies are in progress to test this hypothesis.

LCAT deficiency in $LDLr^{-/-}$ or $apoE^{-/-}$ mice resulted in the complete elimination of long-chain polyunsaturated CE species (20:4, 20:5n-3, and 22:6n-3) in plasma apoB lipoproteins compared with their counterparts with active LCAT, whereas the PL pool in the same particles demonstrated a trend toward greater enrichment with these fatty acyl species (Table 2). This finding suggests that all long-chain polyunsaturated CE species in the plasma of these mice are the product of the LCAT reaction. A corollary of this conclusion is that hepatic and intestinal ACAT cannot efficiently synthesize these CE in mice. The reason for this is unknown but may be related to the inability of mouse ACAT to use long chain fatty acyl CoA species efficiently for CE synthesis. Recent results from gene-targeted mice have documented that ACAT2 is the major enzyme responsible for cholesterol esterification in mouse liver and intestine (45). Furthermore, in vitro assays have shown that arachidonyl-CoA is a poor substrate for ACAT2 (41, 46). Although LCAT is capable of synthesizing CE with long-chain PUFA, it does not use these substrates as well as 18 carbon fatty acyl substrates, apparently because the increased number of double bonds decreases the appVmax of the enzyme through some interference at the active site of LCAT (47, 48). Together these observations suggest that ACAT2, which appears to be responsible for the synthesis of CEs that are secreted by the liver or intestine in chylomicron or VLDL particles, does not significantly contribute to the pool of long chain CE in plasma apoB lipoproteins. However, this outcome may be unique to the mouse. Hepatic VLDL particles isolated from non-human primate recirculating liver perfusate, which has very little active LCAT (49), contain 5% of total CE as long-chain polyunsaturated species, which nearly doubles when a diet enriched in n-3 PUFA is fed (50). This is nearly 5–10 times that observed in plasma apoB lipoproteins in the double knockout mice of this study (Table 2), suggesting that nonhuman primate hepatic ACAT may be better able to use long-chain PUFA for CE synthesis.

Our results suggest that less LCAT esterification was occurring in the apoB lipoprotein fraction of the $apoE^{-/-}$ mice compared with that of $LDLr^{-/-}$ mice. There was a 4-fold greater content of long chain polyunsaturated CE in apoB lipoproteins of $LDLr^{-/-}$ mice compared with $apoE^{-/-}$ mice, with little difference in the PL fatty acyl substrate pool (Table 2). We first considered the possibility that this result might arise from the different size distribution of apoB lipoproteins between the $apoE^{-/-}$ and $LDLr^{-/-}$ mice (Fig. 2). Previous studies have shown that VLDL sized particles are less reactive with LCAT than LDL-sized particles (51). Although LDL-sized particles isolated from the plasma of $apoE^{-/-}$ mice were more enriched in cholesteryl arachidonate and cholesteryl eicosapentaenoate compared with the total apoB lipoprotein fraction from the same animals, there was still a 2.3-fold greater enrichment of long-chain polyunsaturated CE in LDL particles from the $LDLr^{-/-}$ mice (Tables 2 and 3). Thus, differences in apoB lipoprotein particle size between the $apoE^{-/-}$ and $LDLr^{-/-}$ mice did not completely explain the greater enrichment of long-chain polyunsaturated CE in the LDL fraction of the $LDLr^{-/-}$ mice.

If the polyunsaturated CE content of LDL particles is less in the $apoE^{-/-}$ mice compared with $LDLr^{-/-}$ mice because of decreased LCAT reactivity, what is the molecular mechanism for this outcome? There are several potential explanations. A trivial explanation is that less LCAT is

present in the plasma of apoE$^{-/-}$ mice compared with that of LDLr$^{-/-}$ mice, but this is not the case based on an exogenous assay of plasma (Fig. 1). Another explanation is that apoE is a physiological activator of LCAT on apoB lipoproteins. It is well known that apoE can activate LCAT in vitro, but the efficiency is ~20% that of apoA-I (52). However, there is little direct experimental evidence to support or refute this possibility. Another possibility is that there is some compositional difference in the apoB lipoproteins in apoE$^{-/-}$ mice that inhibits the LCAT reaction. ApoE$^{-/-}$ mice have an increased sphingomyelin content in whole plasma and apoB lipoproteins compared with wild-type and LDLr$^{-/-}$ mice (53), and increased sphingomyelin content has been demonstrated to inhibit the LCAT reaction in vitro (51). There are also some differences in the apolipoprotein composition between apoE$^{-/-}$ and LDLr$^{-/-}$ mice that include a relatively greater proportion of apoB-48 and apoA-IV in the apoE$^{-/-}$ mice compared with the LDLr$^{-/-}$ mice (Fig. 3). However, it is not obvious how this difference might decrease LCAT reactivity. Further studies will be necessary to provide a more detailed molecular explanation for the decreased polyunsaturated CE content of apoB lipoproteins in apoE$^{-/-}$ mice.

ApoE$^{-/-}$ mice had half the HDL-C concentration when compared with the C57Bl/6J or LDLr$^{-/-}$ mice, whereas HDL particle size, determined by FPLC and apoA-I Western blots, was similar for all three genotypes. We do not believe this finding can be explained by decreased LCAT activity in the apoE$^{-/-}$ mice. First, there was a high percentage of long-chain polyunsaturated CE in the HDL of apoE$^{-/-}$ mice, similar to that of LDLr$^{-/-}$ mice (Table 4). Second, the proportion of apoA-I in plasma was similar for both apoE$^{-/-}$ and LDLr$^{-/-}$ mice (Fig. 3), and thus, was not likely to be limiting in the activation of LCAT. There are two possible explanations for the decreased HDL concentrations in apoE$^{-/-}$ mice. First, the decrease in HDL could be caused by increased catabolism of particles in the apoE$^{-/-}$ mice. However, this seems unlikely because apoE enrichment of HDL particles leads to increased removal by the liver (54); and available data suggest that HDL protein and CE catabolism is slower in apoE$^{-/-}$ mice compared with those of wild-type controls (55). The other possible mechanism is that HDL production is decreased in apoE$^{-/-}$ mice. This option seems more plausible as it is known that the liver secretes nascent HDL particles that contain apoE (49, 56), and hepatic VLDL production is stimulated by overexpression of apoE (57). In addition, peripheral tissues synthesize apoE (58, 59) that can be associated with lipid to become HDL precursor particles. Loss of these sources of nascent HDL in apoE$^{-/-}$ mice may result in decreased HDL production and a reduction in plasma HDL concentrations.

In summary, we have described an important role for LCAT in the generation of CE on apoB lipoproteins of LDLr$^{-/-}$ and apoE$^{-/-}$ mice. Loss of LCAT results in a significantly greater proportion of LDL CE that are saturated or monounsaturated. Because in vivo removal of LCAT results in the depletion of plasma HDL as well as an increased CE saturation index of apoB lipoproteins, we hypothesized that LDLr$^{-/-}$ LCAT$^{-/-}$ and apoE$^{-/-}$ LCAT$^{-/-}$ mice would have more atherosclerosis than their counterparts with active LCAT.

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