LCAT Modulates Atherogenic Plasma Lipoproteins and the Extent of Atherosclerosis Only in the Presence of Normal LDL Receptors in Transgenic Rabbits

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Abstract—Elevated low density lipoprotein cholesterol (LDL-C) and reduced high density lipoprotein cholesterol (HDL-C) concentrations are independent risk factors for coronary heart disease. We have previously demonstrated that overexpression of an enzyme with a well established role in HDL metabolism, lecithin:cholesterol acyltransferase (LCAT), in New Zealand White rabbits not only raises HDL-C concentrations but reduces those of LDL-C as well, ultimately preventing diet-induced atherosclerosis. In the present study, the human LCAT gene (hLCAT) was introduced into LDL receptor (LDLr)–deficient (Watanabe heritable hyperlipidemic) rabbits to (1) investigate the role of the LDLr pathway in the hLCAT-mediated reductions of LDL-C and (2) determine the influence of hLCAT overexpression on atherosclerosis susceptibility in an animal model of familial hypercholesterolemia. Heterozygosity or homozygosity for the LDLr defect was determined by polymerase chain reaction, and 3 groups of hLCAT-transgenic (hLCAT+) rabbits that differed in LDLr status were established: (1) LDLr wild-type (LDLr+/+), (2) LDLr heterozygotes (LDLr+/−), and (3) LDLr homozygotes (LDLr−/−). Data for hLCAT+ rabbits were compared with those of nontransgenic (hLCAT−) rabbits of the same LDLr status. Plasma HDL-C concentrations were significantly elevated in the hLCAT+ animals of each LDLr status. However, LDL-C levels were significantly reduced only in hLCAT+/LDLr+/+ and hLCAT+/LDLr+/− rabbits but not in hLCAT+/LDLr−/− rabbits (405±14 versus 392±31 mg/dL). Metabolic studies revealed that the fractional catabolic rate (FCR, d−1) of LDL apolipoprotein (apo) B-100 was increased in hLCAT+/LDLr+/+ (26±4 versus 5±0) and hLCAT+/LDLr+/− (4±1 versus ±0) rabbits, whereas the FCR of LDL apoB-100 in both groups of LDLr−/− rabbits was nearly identical (0.16±0.02 versus 0.15±0.02). Consistently, neither aortic lipid concentrations nor the extent of aortic atherosclerosis was significantly different between hLCAT+/LDLr−/− and hLCAT−/LDLr−/− rabbits. Significant correlations were observed between the percent of aortic atherosclerosis and both LDL-C (r=0.985) and LDL apoB-100 FCR (−0.745), as well as between LDL-C and LDL apoB-100 FCR (−0.866). These data are the first to establish that LCAT modulates LDL metabolism via the LDLr pathway, ultimately influencing atherosclerosis susceptibility. Moreover, LCAT’s antiatherogenic effect requires only a single functional LDLr allele, identifying LCAT as an attractive gene therapy candidate for the majority of dyslipoproteinemic patients. (Arterioscler Thromb Vasc Biol. 2000;20:450-458.)

Key Words: familial hypercholesterolemia ■ metabolism ■ gene therapy ■ WHHL rabbits ■ low density lipoproteins

Elevated LDL cholesterol (LDL-C) and reduced HDL cholesterol (HDL-C) concentrations are independent risk factors for coronary heart disease (CHD),1–3 creating the potential for synergistic effects on disease pathophysiology. An example of this synergism is observed in patients with the autosomal codominant disease familial hypercholesterolemia (FH). FH heterozygotes and homozygotes not only have LDL-C levels that are elevated as much as 2- and 6-fold,4 respectively, but also have significant reductions in HDL-C. Thus, an ideal therapeutic intervention for FH patients should decrease LDL-C concentrations while simultaneously increasing those of HDL-C. We have previously reported that, when overexpressed in New Zealand White (NZW) rabbits, human lecithin:cholesterol acyltransferase (hLCAT) has precisely these effects, reducing LDL-C and raising HDL-C,5,6 consequently preventing diet-induced atherosclerosis.7

As the major enzyme responsible for the esterification of free cholesterol (FC) present in plasma lipoproteins, LCAT,5,9 a 63-kDa glycoprotein, plays a critical role in the metabolism of HDL and in reverse cholesterol transport,10 a process that involves the movement of cholesterol from peripheral cells to the liver for catabolism. Although the role of LCAT in HDL
metabolism has long been known, our finding that hLCAT overexpression also reduced LDL-C concentrations in both chow- and cholesterol-fed NZW rabbits was unexpected and has led to the generation of several hypotheses as to the mechanism(s) involved in this regulation. Kinetic studies conducted in the cholesterol-fed animals indicated that LCAT reduced LDL-C concentrations by accelerating LDL clearance from the plasma compartment, rather than by decreasing the synthesis of apolipoprotein (apo) B–containing lipoproteins. In view of the fact that 60% to 80% of LDL is cleared via hepatic LDL receptor (LDLr)–mediated endocytosis in the normal rabbit, we hypothesized that the significantly reduced concentrations of LDL observed in our hLCAT-transgenic NZW rabbits might be due to upregulation of the LDLr pathway. If correct, LCAT would be an attractive treatment modality for heterozygous FH patients, whereas it would likely prove less beneficial for homozygous FH patients who lack normal LDLrs.

The present study was designed with 2 goals in mind: (1) to establish the involvement of the LDLr pathway in the LCAT-mediated reductions of LDL and (2) to determine the influence of hLCAT overexpression on atherosclerosis susceptibility. Moreover, LCAT’s antiatherogenic effect requires only a single functional LDLr allele, identifying LCAT as an attractive gene therapy candidate for the majority of dyslipoproteinemic patients.

### Methods

#### Animals and LDLr Status

The hLCAT gene was introduced into LDLr-deficient rabbits by selective breeding. The generation of transgenic rabbits was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, as was the experimental protocol. Rabbits were determined to be transgenic for human LCAT by Southern blot analysis. Six experimental groups were defined by the presence or absence of the hLCAT gene, as well as by LDLr status. For the LDL kinetic studies, each group comprised 3 age-matched males with the exception of the hLCAT+/LDLr+/– group, which consisted of 2 females and 1 male. For the atherosclerosis experiments, 19 LDLr+/– rabbits (14 hLCAT+, 5 hLCAT–) and 16 LDLr–/– rabbits (8 hLCAT+, 8 hLCAT–), ~22 months of age, were studied. The data in Figure 5A were generated from 19 LDLr+/– rabbits (9 hLCAT+, 10 hLCAT–) and have been previously reported.

Heterozygosity or homozygosity for the LDLr defect in the present study was established by polymerase chain reaction (PCR) by using genomic DNA that was isolated from whole blood. The mutation in WHHL rabbits involves a 12-bp in-frame deletion that removes 4 amino acids from the fourth ligand-binding repeat of the LDLr. As shown in Figure 1, a 306-bp portion of exon 4 of the rabbit LDLr gene, including the 12-bp mutant region from 369 to 380 that removes 4 amino acids from the fourth ligand-binding repeat of the LDLr in the normal rabbit,12 led to hypothesized that the significantly reduced concentrations of LDL observed in our hLCAT-transgenic NZW rabbits might be due to upregulation of the LDLr pathway. If correct, LCAT would be an attractive treatment modality for heterozygous FH patients, whereas it would likely prove less beneficial for homozygous FH patients who lack normal LDLrs.

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#### Plasma LCAT Activity, Mass, and Lipids

Samples were collected from the central ear artery of each rabbit at each designated time point and were added to tubes containing tripotassium EDTA. Plasma was isolated by centrifugation at 2500 rpm for 30 minutes at 4°C. Plasma α-LCAT activity was determined in duplicate by using a proteoliposome assay, as previously de-
scribed, and LCAT mass was determined by radioimmunoassay. Total cholesterol (TC) and triglyceride (TG) concentrations (Sigma Chemical Co) were measured with a Hitachi 911 autoanalyzer (Hitachi USA) with the use of enzymic reagents. All lipid determinations were performed on plasma from rabbits that had been fasted overnight. Plasma HDL-C was determined after dextran sulfate–MgCl₂ precipitation of VLDL and LDL.

**Gel Filtration Chromatography**

Two hundred microliters of rabbit plasma was applied to a fast protein liquid chromatography (FPLC) system consisting of 2 Superose 6 columns connected in series (Pharmacia Biotech, Inc). Lipoproteins were eluted at 0.3 mL/min with PBS containing 1 mmol/L EDTA and 0.02% (wt/wt) NaN₃. After the initial 10 mL was eluted, the next 30 mL was collected in 0.5-mL fractions. TC content and LCAT mass distribution were determined for each fraction as described above.

**LDL Composition**

LDL composition was determined subsequent to sequential ultracentrifugation (d=1.019 to 1.063 g/mL). TC and TG were analyzed as above, and FC and phospholipids (PL) (Wako Chemicals) in the LDL fraction were also determined with a Hitachi 911 autoanalyzer by using enzymic reagents. Cholesteryl ester (CE) was calculated by subtracting FC from TC. Total protein (Pro) content was measured with the bicinchoninic acid protein assay (Pierce Chemical Co). LDL apoB-100 concentrations were determined by a competitive ELISA assay utilizing a chicken polyclonal antiserum directed against rabbit LDL apoB-100 (Covance Laboratories, Inc).

**In Vivo Metabolic Studies**

Narrow-cut LDL (d=1.030 to 1.050 g/mL) for metabolic studies was isolated from the plasma of fasting LDLr-deficient rabbits by sequential ultracentrifugation. We chose this tracer because it provided us with the best means of isolating a large quantity of LDL apoB that was not significantly contaminated with other apolipoproteins and, furthermore, provided for consistency among experiments. After isolation, the LDL was dialyzed against PBS/EDTA to remove excess KBr. Agarose gel electrophoresis and FPLC analysis confirmed that the isolated LDL was pure and not contaminated with other lipoprotein classes.

The isolated LDL was next radiolabeled with ³¹I (Dupont/NEN) by using a modification of the ICI method previously described. Immediately before injection, the ³¹I-labeled LDL solution was filtered-sterilized (0.22-μm Millex-GV filters, Millipore), and a 25-μCi infusate dose was prepared for each rabbit. Infusates were injected into the marginal ear vein of each rabbit, and blood samples were obtained at 5 and 30 minutes and at 1, 3, 6, 9, 24, 32, 48, 72, and 144 hours after injection. LDL (d=1.019 to 1.063 g/mL) was isolated from each time point of each rabbit by sequential ultracentrifugation. The resultant LDL fractions for each rabbit were then subjected to SDS–polyacrylamide gel electrophoresis on 4% to 20% gradient gels, and LDL apoB-100 bands were excised and subjected to SDS–polyacrylamide gel electrophoresis. Fractional catabolic rate (FCR) was calculated as the reciprocal of the RT. LDL apoB pool sizes were derived from the formula: [plasma volume (dL) × LDL apoB concentration (mg/dL)]/body weight (kg). Plasma volume was estimated at 3.28% of body weight. Production rate (PR) was calculated as the product of FCR and pool size.

**Analysis of Aortic Lesions**

Rabbits were killed by using intravenous sodium pentobarbital. Aortas were harvested and sliced longitudinally. One half was stained with Sudan IV, and the percentage of the surface area stained was determined by planimetry of the digitized image. The remaining half of the aorta, specifically the arch and upper thoracic down to the left subclavian artery ostia, was used for lipid extraction. Each slice of aorta was weighed and extracted in 20 mL of chloroform/methanol (2:1, vol/vol), according to the method of Folch and coworkers. After the organic phase was dried under N₂, lipid content was determined gravimetrically, and the lipids were resolubilized in isopropanol. Pro, lipid, TC, and unesterified cholesterol contents were determined as previously described.

**Statistical Analysis**

Data for hLCAT-transgenic rabbits were assessed for significance relative to those of nontransgenic littermate control rabbits of the same LDLr status by using Student’s nonpaired, 2-tailed t test. Correlation coefficients were determined by the method of Pearson. In all cases, statistical significance was set at P<0.05. Data presented in the text, tables, and figures represent mean±SEM.

**Results**

**Determination of LDLr Status**

Heterozygosity or homozygosity for the 12-bp deletion in exon 4 of the rabbit LDLr gene was established by PCR, as described in Methods. Figure 1 shows the gel-purified PCR products of LDLr+/+, LDLr++/−, and LDLr−/− rabbits with the exception of lane 2, which shows the undigested PCR product of an LDLr+/+ animal before gel purification. The additional high-molecular-weight band was present owing to the lack of gel purification. Subsequent to gel purification and BglI digestion, PCR products from LDLr+/+ rabbits generated 2 bands of 212 and 94 bp (lane 3). In the case of LDLr++/− rabbits, 2 bands at 306 and 294 bp were generated before digestion (lane 4), whereas 3 product bands were observed in LDLr++/− animals after incubation with BglI (lane 5) because of the presence of 1 normal and 1 mutant allele. In contrast, LDLr−/− rabbits generated only 1 band at 294 bp, both before (lane 6) and after (lane 7) incubation with BglI.

**Plasma LCAT Activity, Lipids, and LDL ApoB-100 Levels**

Table 1 summarizes the mean plasma LCAT activity, lipid, and LDL apoB data for hLCAT-transgenic and nontransgenic rabbits of each LDLr status. As expected, plasma LCAT activity was significantly increased in transgenic animals relative to controls, with at least 3-fold increases in activity observed for each group. Similarly, plasma HDL-C concentrations were significantly elevated in hLCAT-transgenic animals, independent of LDLr status. hLCAT+/+LDLr++/+ rabbits had the highest mean plasma LCAT activity and consequently, the highest levels of HDL-C compared with their nontransgenic littermate controls. The mean plasma HDL-C concentration of the hLCAT+/+LDLr++/+ group was 3 times greater than that of the hLCAT−/−LDLr++/+ group, whereas that of the hLCAT+/+LDLr−/− group was 5 times greater than that of its control group. With regard to LDL-C and apoB concentrations, only in the LDLr++/+ and LDLr++/+ groups, and not in the LDLr−/− group, did hLCAT-transgenic rabbits have significant reductions relative to controls. Unexpectedly, plasma TGs were reduced by 66% in hLCAT+/+LDLr++/+ rabbits and by 56% in hLCAT−/−LDLr−/− rabbits compared with those of their respective control groups. Preliminary studies in these animals have shown that this reduction is in part due to accelerated VLDL apoB-100 catabolism, suggesting that hLCAT overexpression may influence both VLDL and LDLr-mediated
pathways. No differences were noted in plasma TGs between transgenic and nontransgenic LDLr+/- rabbits.

**Distribution of Cholesterol and hLCAT Mass Among Lipoproteins and LDL Composition**

Figure 2 illustrates the distribution of TC in the plasma of representative hLCAT+ and hLCAT− LDLr+/-, LDLr+/-, and LDLr−/- rabbits, as assessed by gel filtration chromatography. The elution profile of the radiolabeled rabbit LDL tracer is also provided for comparative purposes. As discussed above, plasma HDL-C content was greater in the hLCAT+ animals of each LDLr status. The enrichment of LDL with CE is shown in Table 1. The mean HDL-C content increased in all 3 groups of transgenic rabbits relative to their controls, with the Pro, CE, and TG components accounting for 29%, 26%, and 22% of LDL, respectively. Similarly, the LDLs of all 3 groups of transgenic rabbits were found to contain Pro, CE, and TG components accounting for 29%, 26%, and 22% of LDL, respectively. Conversely, apoB-containing lipoproteins were prevalent in the plasma of LDLr−/− rabbits, independent of the degree of LCAT expression. The distribution of LDL among the lipoprotein fractions of transgenic rabbits of each LDLr status is shown in Figure 3. LDLr−/− rabbits were due to enhanced catabolism of LDL apoB-100 relative to nontransgenic controls. The decline in 131I-LDL apoB-100–associated radioactivity was very rapid in hLCAT+/+LDLr+/- rabbits, such that only 1% of the initial dose of 131I-LDL remained 6 hours after injection relative to >10% in that of the control group. In hLCAT+/+LDLr+/- rabbits, <1% of the initial dose of 131I-LDL remained 6 hours after injection, whereas >20% remained associated with the LDL apoB-100 FCR (d−1). 3.2 ± 0.2 for hLCAT+/+LDLr+/- rabbits, which was 80% lower than that of 26.2 ± 4.1 for hLCAT−/LDLr−/− rabbits. Similarly, the LDL apoB-100 FCR of hLCAT−/LDLr−/− rabbits (1.2 ± 0.2) was reduced by 71% relative to that of hLCAT+/LDLr+/- rabbits (4.1 ± 0.5). In contrast, Figure 4C shows that the catabolism of LDL apoB-100 in LDLr homozygotes was independent of LCAT overexpression, with hLCAT+ and hLCAT− animals having nearly identical radioactivity decay curves and, thus, LDL apoB-100 FCRs. Also of note was the fact that LCAT overexpression improved the LDL catabolic defect in LDLr−/− rabbits.

**TABLE 1. Plasma LCAT Activity, Lipids, and LDL ApoB in hLCAT-Transgenic and Nontransgenic Rabbits of Each LDLr Status**

<table>
<thead>
<tr>
<th>Group</th>
<th>LCAT Activity, nmol · mL⁻¹ · h⁻¹</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TG</th>
<th>LDL ApoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLr+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hLCAT+</td>
<td>1144 ± 129*</td>
<td>121 ± 12*</td>
<td>2 ± 0*</td>
<td>29 ± 1</td>
<td>1 ± 0*</td>
</tr>
<tr>
<td>hLCAT−</td>
<td>125 ± 6</td>
<td>28 ± 4</td>
<td>4 ± 1</td>
<td>27 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>LDLr+/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hLCAT+</td>
<td>297 ± 57*</td>
<td>62 ± 8*</td>
<td>10 ± 2*</td>
<td>17 ± 1*</td>
<td>14 ± 4*</td>
</tr>
<tr>
<td>hLCAT−</td>
<td>55 ± 11</td>
<td>21 ± 1</td>
<td>47 ± 3</td>
<td>50 ± 6</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>LDLr−/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hLCAT+</td>
<td>219 ± 50*</td>
<td>27 ± 2*</td>
<td>405 ± 14</td>
<td>69 ± 16*</td>
<td>221 ± 25</td>
</tr>
<tr>
<td>hLCAT−</td>
<td>62 ± 4</td>
<td>5 ± 1</td>
<td>392 ± 31</td>
<td>157 ± 18</td>
<td>226 ± 23</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 3 animals of each hLCAT status per group. All lipid values are in mg/dL.

*P<0.05 compared with nontransgenic control group of the same LDLr status.
heterozygotes to a value that was not significantly different from that of nontransgenic LDLr\(^1/1\) rabbits. No significant differences were observed in LDL apoB PR between the hLCAT\(^1\) and hLCAT\(^2\) rabbits of each LDLr group.

**Analysis of Aortic Lesions**

To further evaluate the role of the LDLr pathway in LCAT’s ability to reduce LDL-C concentrations and ultimately prevent atherosclerosis, we assessed spontaneous atherosclerosis in hLCAT-transgenic and control LDLr\(^+/+\) and LDLr\(^{-/-}\) rabbits. Aortic lipid concentrations as well as the percent surface area covered by plaque are provided in Table 4. No significant differences were noted in either total lipid, FC, or esterified cholesterol content when transgenic and control LDLr\(^1/2\) rabbits were compared, nor was spontaneous atherosclerosis evident in the aortas of these animals. This was likely due to the fact that, although the mean LDL-C concentration of hLCAT\(^2/LDLr\(^1/2\) rabbits was significantly greater than that of hLCAT\(^+\)/LDLr\(^1/2\) rabbits (47 versus 10 mg/dL), this concentration of LDL was not sufficient to promote spontaneous atherosclerosis in the former group. In contrast, significant atherosclerosis was present in both hLCAT\(^-\) and hLCAT\(^+\)/LDLr homozygotes, with both groups having similar aortic cholesterol content and 84\(\pm\)3% of the aortic surface covered by plaque. This is illustrated in

Figure 2. Distribution of TC in the plasma of hLCAT\(^+\) and hLCAT\(^-\)/LDLr\(^+/+\) (A), LDLr\(^+/-\) (B), and LDLr\(^{-/-}\) (C) rabbits, as assessed by gel filtration chromatography. The elution profile of the radiolabeled rabbit LDL tracer is also provided for comparative purposes. Plasma TC concentrations (mg/dL) for the hLCAT\(^+\) and hLCAT\(^-\) groups were 125\(\pm\)12 and 35\(\pm\)2 for LDLr\(^+/+\) rabbits, 76\(\pm\)14 and 84\(\pm\)11 for LDLr\(^+/-\) rabbits, and 600\(\pm\)31 and 554\(\pm\)20 for LDLr\(^{-/-}\) rabbits. In each case, HDL-C content was greater in the hLCAT\(^+\) group. However, only in the LDLr\(^+/+\) and LDLr\(^+/-\) groups, and not in the LDLr\(^{-/-}\) group, were LDL-C concentrations reduced relative to those of their nontransgenic controls.

Figure 3. Distribution of hLCAT mass among the lipoprotein fractions of transgenic LDLr\(^+/+\) (A), LDLr\(^+/-\) (B), and LDLr\(^{-/-}\) (C) rabbits is provided in this figure. hLCAT was principally associated with HDL in LDLr\(^+/+\) rabbits, whereas its distribution was more heterogeneous in transgenic LDLr\(^+/-\) rabbits, with hLCAT mass detected in IDL-LDL, HDL\(_1\), and HDL particles. Interestingly, in transgenic LDLr\(^{-/-}\) rabbits, the majority of hLCAT was associated with LDL rather than HDL. Plasma hLCAT mass concentrations (\(\mu\)g/mL) were 27\(\pm\)2, 5\(\pm\)0, and 5\(\pm\)0 for LDLr\(^+/+\), LDLr\(^+/-\), and LDLr\(^{-/-}\) transgenic rabbits, respectively.
Figure 5, wherein the probability map for aortic lesion development in LDLr−/− rabbits shows substantial staining, indicative of severe atherosclerosis, independent of the degree of hLCAT expression. A comparison of the extent of aortic lesions in cholesterol-fed hLCAT-transgenic and control LDLr+/+ rabbits was included in Figure 5A to illustrate the protective effect of LCAT in the presence of functional LDL receptors. Only 5% of the aortic surface was covered by plaque in hLCAT+/+ LDLr+/+ rabbits versus 35% in the hLCAT−/LDLr+/+ group. The data in Figure 5 suggest that LCAT only protects against atherosclerosis when normal LDL receptors are present. Consistent with this, significant correlations were observed between the percent of aortic atherosclerosis and LDL-C concentration (r = 0.985, P < 0.001) and LDL FCR (r = −0.745, P < 0.035). Moreover, both aortic FC (r = −0.705) and esterified cholesterol concentrations were significantly (P < 0.05) associated with LDL FCR.

Relationships Between LCAT Activity, LDL-C, and LDL Metabolic Parameters

The results of linear regression analyses are provided in Table 5. LDL-C concentrations were highly correlated with LDL apoB concentrations. LDL apoB concentrations were inversely associated with plasma LCAT activity.

Moreover, plasma LCAT activity was significantly correlated with LDL apoB-100, but not with LDL apoB PR. This finding, taken together with the fact that LDL apoB concentrations were correlated with FCR only, indi-
TABLE 4. Aortic Lipid Content in hLCAT-Transgenic and Nontransgenic LDLr+/− and LDLr−/− Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Total Lipid</th>
<th>FC, μg/mg</th>
<th>CE, μg/mg</th>
<th>Percent Surface Area*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLr+/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hLCAT+ (n=14)</td>
<td>0.06±0.01</td>
<td>2±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>hLCAT− (n=5)</td>
<td>0.06±0.02</td>
<td>2±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>LDLr−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hLCAT+ (n=8)</td>
<td>0.13±0.02</td>
<td>43±4</td>
<td>29±3</td>
<td>84±3</td>
</tr>
<tr>
<td>hLCAT− (n=8)</td>
<td>0.16±0.01</td>
<td>40±2</td>
<td>33±3</td>
<td>84±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Percent of the aortic surface covered by plaque.

cates that LCAT-mediated alterations in LDL apoB-100 FCR were responsible for the observed reductions of LDL in our transgenic rabbits with functional LDL receptors.

Figure 5. Comparison of aortic atherosclerosis in hLCAT+ and hLCAT− LDLr+/+ (A), LDLr+/− (B), and LDLr−/− rabbits, as determined by quantitative planimetry. Compilation of images is summarized for transgenic and control rabbits of each LDLr status. Beneath panel A, a graded collection of the probability of distribution is shown for cholesterol-fed LDLr+/+ rabbits, whereas that for spontaneous atherosclerosis in LDLr+/− and LDLr−/− rabbits is provided beneath panel C. The data from cholesterol-fed LDLr rabbits (A) illustrate the protective effect of LCAT in the presence of functional LDL receptors. *P<0.01. In contrast, the data in panel C suggest that LCAT does not protect against spontaneous atherogenesis in rabbits lacking functional LDLr’s.

TABLE 5. Correlation Coefficient Analysis

<table>
<thead>
<tr>
<th></th>
<th>LDL ApoB</th>
<th>LDL ApoB-100 FCR</th>
<th>LDL ApoB-100 PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>0.969*</td>
<td>−0.866*</td>
<td>0.247</td>
</tr>
<tr>
<td>LCAT activity</td>
<td>−0.570†</td>
<td>0.973*</td>
<td>−0.333</td>
</tr>
</tbody>
</table>

*P<0.01, †P<0.05.

Discussion

We have previously reported that the overexpression of hLCAT in NZW rabbits prevents diet-induced atherosclerosis. In addition to having elevated HDL-C levels, the concentrations of proatherogenic apoB-containing lipoproteins were substantially lower in cholesterol-fed, hLCAT-transgenic rabbits relative to nontransgenic littermate controls because of the accelerated catabolism of LDL. Although the observed effects on HDL were expected, due to LCAT’s well-established role in HDL metabolism, those on LDL were not. The present study was undertaken to evaluate the role of the LDLr pathway in the LCAT-induced reductions of LDL, as well as to assess LCAT as a potential candidate gene for prevention of atherosclerosis in FH patients.

To investigate the role of the LDLr pathway in the LCAT-mediated reductions of plasma LDL concentrations, we employed an in vivo system, namely the WHHL rabbit, an animal model of FH. hLCAT-transgenic WHHL rabbits, either heterozygous or homozygous for a 4–amino acid deletion in the cysteine-rich ligand-binding domain of the LDLr protein, were generated by selective breeding. hLCAT-transgenic and nontransgenic LDLr+/+ (NZW) rabbits were also studied, allowing for assessment of the interrelationships between the presence or absence of LCAT and LDLr status. We observed that the overexpression of hLCAT resulted in significant reductions of plasma LDL-C and apoB concentrations only in animals with functional LDL receptors. Hence, in both hLCAT+/LDLr+/+ and hLCAT+/LDLr+/− rabbits, HDL rather than LDL was the predominant lipoprotein class present in plasma, whereas apoB-containing lipoproteins were prevalent in the plasma of LDLr homozygotes, independent of the degree of LCAT expression. Metabolic experiments with radiolabeled rabbit LDL revealed that the reductions in LDL observed in the hLCAT+/LDLr+/+ and hLCAT+/LDLr+/− rabbits were due to enhanced catabolism of LDL apoB-100 relative to nontransgenic littermate controls, with no significant differences noted in LDL PRs. Conversely, hLCAT+ and hLCAT− LDLr homozygotes had nearly identical rates of LDL apoB-100 catabolism. Additionally, LCAT overexpression significantly improved the catabolic defect in LDLr heterozygotes such that the LDL apoB-100 FCR of this group was not dramatically different from that of the hLCAT−/LDLr+/+ group. Taken together, the preceding data demonstrate the pivotal role of the LDLr pathway in the LCAT-induced reductions of LDL concentrations.

The precise mechanism(s) responsible for the accelerated catabolism of LDL apoB-100 in hLCAT-transgenic rabbits with functional LDL receptors remains to be defined. However, 1 potential mechanism may be eliminated on the basis of the results of the present study, namely, the role of lipoprotein composition in determining metabolic fate. It is
well documented that both the lipid and apolipoprotein content of LDL can influence LDL catabolism in rabbits.\textsuperscript{25,26} In general, LDL clearance from the plasma compartment is more rapid when LDLs are TG enriched and/or increased in apoE content. When the LDL of hLCAT-transgenic and nontransgenic rabbits of each LDLr status were compared in our study, no significant differences were found in either lipid or apoE content, decreasing the likelihood that LCAT accelerated LDL catabolism by altering the composition of the LDL particles themselves and, ultimately, their interaction with receptor surfaces. Also of note, our LDL composition data in nontransgenic NZW and homozygous WHHL rabbits were not substantially different from those previously reported by Havel and colleagues.\textsuperscript{27} Because it is difficult to completely resolve LDL and HDL, particles by FPLC, we cannot entirely rule out the possibility that LDL-associated hLCAT may have facilitated LDL clearance in both LDLr\textasciitilde and LDLr\textasciitilde rabbits. However, we hypothesize that rather than altering LDL composition, LCAT overexpression, the highest levels of which were in the liver, may have directly influenced LDLr activity by altering intracellular membrane composition, thus increasing membrane fluidity. In support of this hypothesis, increased membrane fluidity due to enrichment with polyunsaturated fatty acids has been shown to accelerate catabolism of LDL via receptor-dependent pathways in nonhuman primates.\textsuperscript{28} LCAT may have induced such changes in hepatic membranes by altering the PL content, as it has been shown to alter HDL PL content in an analogous manner.\textsuperscript{29,30} Alternatively, LCAT may have influenced the LDLr pathway by differentially modulating the synthesis of cholesterologenic enzymes or by affecting intracellular regulatory pools of cholesterol, in turn mediating the transcription of genes encoding the LDLr as well as enzymes involved in cholesterol biosynthesis.\textsuperscript{31} Downregulation of the gene encoding the LDLr is precisely the mechanism by which CE transfer protein, another enzyme with a pivotal role in lipoprotein metabolism, has been shown to modulate the concentrations of apoB-containing lipoproteins in transgenic mice.\textsuperscript{32}

Elevated LDL-C and reduced HDL-C concentrations are independent risk factors for CHD.\textsuperscript{1–3} We and others have previously reported that the loss of LDLr function in homozygous FH patients not only increases LDL-C concentrations but decreases those of HDL-C as well,\textsuperscript{4,33} the latter due in part to a 30% reduction in LCAT activity relative to age- and sex-matched controls.\textsuperscript{33} Thus, a goal of the present study was to evaluate LCAT as a potential gene therapy candidate for FH patients, who are at increased risk for CHD,\textsuperscript{4} owing to their unfavorable lipoprotein profile. Neither the LCAT activity (nmol \cdot mL\textsuperscript{-1} \cdot h\textsuperscript{-1}) values for homozygous FH patients (77\pm4) nor those of human controls (110\pm5) were very different from those reported for our nontransgenic LDLr\textasciitilde (62\pm4) and LDLr\textasciitilde (125\pm6) groups, respectively, allowing us to cautiously extrapolate the clinical relevance of our findings.

In the present study, we assessed spontaneous atherosclerosis in heterozygous and homozygous LDLr-deficient, hLCAT-transgenic, and control rabbits. LCAT did not protect against atherosogenesis in homozygous FH rabbits nor, as mentioned earlier, did it alter LDL metabolism. This lack of protection in hLCAT-transgenic LDLr homozygotes, despite a 5-fold elevation in HDL-C levels, was likely due to the overwhelming presence of apoB-containing lipoproteins in the plasma. The LDL-C–to–HDL-C ratio in hLCAT\textasciitilde LDLr\textasciitilde rabbits remained high at 15. This would suggest that simply raising HDL-C levels in FH homozygotes without simultaneously reducing those of LDL-C is not sufficient to prevent atherosogenesis. Unfortunately, our conclusions for the LDLr heterozygotes were limited by the lack of spontaneous atherosclerosis in the control group. However, LCAT overexpression clearly improved the LDL catabolic defect in these animals. This, taken together with the fact that hLCAT increased HDL-C in LDLr\textasciitilde rabbits,\textsuperscript{34} identifies LCAT as an attractive target gene for treatment of heterozygous FH patients, as well as for the majority of dyslipoproteinemic patients who have at least 1 functional LDLr allele.

In conclusion, our data are the first to establish that the overexpression of an enzyme with a well known role in HDL metabolism, LCAT, also modulates LDL metabolism via the LDLr pathway, ultimately influencing atherosclerosis susceptibility. We propose that a significant interaction exists between the LCAT and LDLr genes, located on chromosomes 16 and 19, respectively, which may contribute to the variable phenotypic expression observed in FH patients.\textsuperscript{35} The results of this study emphasize the important role of epigenetic pathways in our comprehension of complex genetic interrelationships and, most notably, in the formulation of novel therapies.

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References


LCAT Modulates Atherogenic Plasma Lipoproteins and the Extent of Atherosclerosis Only in the Presence of Normal LDL Receptors in Transgenic Rabbits
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