Overexpression of human lecithin:cholesterol acyltransferase in cholesterol-fed rabbits: LDL metabolism and HDL metabolism are affected in a gene dose-dependent manner

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Abstract Lecithin:cholesterol acyltransferase (LCAT) is an enzyme well known for its involvement in the intravascular metabolism of high density lipoproteins; however, its role in the regulation of apolipoprotein (apo) B-containing lipoproteins remains elusive. The present study was designed to investigate the metabolic mechanisms responsible for the differential lipoprotein response observed between cholesterol-fed hLCAT transgenic and control rabbits. ^{131}I-labeled HDL apoA-I and ^{125}I-labeled LDL kinetics were assessed in age- and sex-matched groups of rabbits with high (HE), low (LE), or no hLCAT expression after 6 weeks on a 0.3% cholesterol diet. In HE, the mean total cholesterol concentration on this diet, mg/dl (230 ± 50), was not significantly different from that of either LE (313 ± 46) or controls (332 ± 52) due to the elevated level of HDL-C observed in HE (127 ± 19), as compared with both LE (100 ± 33) and controls (31 ± 4). In contrast, the mean nonHDL-C concentration for HE (103 ± 33) was much lower than that for either LE (213 ± 39) or controls (301 ± 55). FPLC analysis of plasma confirmed that HDL was the predominant lipoprotein class in HE on the cholesterol diet, whereas cholesteryl ester-rich, apoB-containing lipoproteins characterized the plasma of LE and, most notably, of controls. In vivo kinetic experiments demonstrated that the differences in HDL levels noted between the three groups were attributable to distinctive rates of apoA-I catabolism, with the mean fractional catabolic rate (FCR, d^{-1}) of apoA-I slowest in HE (0.282 ± 0.03), followed by LE (0.340 ± 0.01) and controls (0.496 ± 0.04). A similar, but opposite, pattern was observed for nonHDL-C levels and LDL metabolism (h^{-1}), such that HE had the lowest nonHDL-C levels with the fastest rate of clearance (0.131 ± 0.027), followed by LE (0.057 ± 0.009) and controls (0.031 ± 0.001). Strong correlations were noted between LCAT activity and both apoA-I ($r = -0.868$, $P < 0.01$) and LDL ($r = 0.670$, $P = 0.06$) FCR, indicating that LCAT activity played a major role in the mediation of lipoprotein metabolism. In summary, these data are the first to show that LCAT overexpression can regulate both LDL and HDL metabolism in cholesterol-fed rabbits and provide a potential explanation for the prevention of diet-induced atherosclerosis observed in our previous study.—**Brousseau, M. E., S. Santamarina-Fojo, B. L. Vaisman, D. Applebaum-Bowden, A. M. Bérard, G. D. Talley, H. B. Brewer, Jr., and J. M. Hoeg.** Overexpression of human lecithin:cholesterol acyltransferase in cholesterol-fed rabbits: LDL metabolism and HDL metabolism are affected in a gene dose-dependent manner. *J. Lipid Res.* 1997. **38:** 2537–2547.

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Lecithin:cholesterol acyltransferase (LCAT) is a pivotal enzyme involved in the intravascular metabolism of high density lipoproteins (HDLs) (1). LCAT is synthesized primarily by hepatocytes and catalyzes the reaction whereby a fatty acid is transferred from the *sn*-2 position of phosphatidylcholine to the 3-hydroxyl group of cholesterol, yielding cholesteryl esters and lysolecithin (1, 2). Cholesteryl esters generated by LCAT may be transported directly to the liver or, alternatively, may be transferred to apolipoprotein (apo) B-containing particles by the action of cholesteryl ester transfer protein (CETP) (3) and, ultimately, removed by hepatic

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; FCR, fractional catabolic rate; FED, fish-eye disease; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; HE, high expressor; HL, hepatic lipase; LAT, lysolecithin acyltransferase; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LE, low expressor; LPL, lipoprotein lipase; PL, phospholipid; PR, production rate; RT, residence time; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein.

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receptor-mediated pathways (4–6). Hence, LCAT has been suggested to play an important role in reverse cholesterol transport by creating a concentration gradient for the efflux of free cholesterol from peripheral cells to HDL particles (7).

In plasma, LCAT preferentially associates with HDL (α-LCAT), but several investigators have also demonstrated that LCAT may act directly on apoB-containing lipoproteins (β-LCAT) (8–11). Liu, Krul, and Subbaiah (12) have shown that low density lipoprotein (LDL) subfractions were capable of activating both lysolecithin acyltransferase (LAT) and LCAT, with proper apoB conformation being critical for activation. Additional evidence for an association between LCAT and LDL is found in certain patients with partial LCAT deficiency, or fish-eye disease (FED). In some FED patients, residual LCAT activity may reside solely on apoB-containing lipoproteins (10, 13), while in others it may reside on both the α- and β-containing lipoproteins (14).

In addition to LCAT, a number of different enzymes, including hepatic and lipoprotein lipases and CETP, are critical to the proper functioning of the lipoprotein metabolic cascade, and the activity of each may be influenced by dietary cholesterol. Lipoprotein and hepatic lipase activities have both been shown to increase with cholesterol feeding in rabbits (15, 16), as has plasma CETP activity (16–18). The regulation of CETP in the rabbit occurs at the mRNA level, with increased hepatic CETP mRNA abundance noted after cholesterol-feeding (18). Interestingly, an inverse association has been demonstrated between plasma CETP concentration and liver LDL receptor mRNA abundance in transgenic mice (19). In view of the fact that approximately 50% of very low density lipoprotein (VLDL) and 60–80% of LDL are cleared by hepatocytes via LDL receptor-mediated endocytosis in the normal rabbit (20, 21), it is not unreasonable to speculate that LCAT overexpression could mediate the metabolism of LDL via a similar mechanism.

Recently, we have reported that the overexpression of human LCAT (hLCAT) in transgenic rabbits prevents diet-induced atherosclerosis, with significant reductions determined by both quantitative planimetry and quantitative immunohistochemistry (22). In addition to having elevated HDL cholesterol levels, the concentrations of proatherogenic apoB-containing lipoproteins were substantially lower in cholesterol-fed hLCAT transgenic rabbits relative to littermate controls, leading us to hypothesize that hLCAT overexpression might modulate nonHDL, as well as HDL, particle metabolism. Thus, the present study was designed to investigate the metabolic mechanisms responsible for the differences observed in both HDL and LDL cholesterol levels between cholesterol-fed hLCAT transgenic and control rabbits, and, ultimately, to provide a potential explanation for the prevention of diet-induced atherosclerosis. The results of this study confirm our hypothesis that hLCAT overexpression modulates nonHDL-C, as well as HDL-C, concentrations in cholesterol-fed animals by affecting lipoprotein particle clearance in a gene dose-dependent manner.

METHODS

Animals and diet

The production of the human LCAT transgenic rabbits used in these experiments has been described elsewhere (23). Briefly, fertilized eggs from New Zealand White rabbits were microinjected with a 6.2 kb genomic fragment consisting of the entire LCAT gene, including 0.85 kb and 1.134 kb of the 5′- and 3′-flanking regions, respectively. Integration of the human LCAT gene in newborn rabbits was determined by Southern blot analysis. Three experimental groups, each comprised of three age-matched males, were defined on the basis of baseline total and HDL cholesterol levels as well as by LCAT activity, and were, thus, designated as high expressors (HE), low expressors (LE), and controls. It has previously been demonstrated that the number of integrated copies of the LCAT transgene correlates highly with plasma human LCAT concentrations, as well as with total and HDL cholesterol levels in transgenic animals (24).

Animals were maintained on a 120 gm daily ration of 0.3% cholesterol diet (Product number 4109000, Ziegler Brothers, Inc., Gardeners, PA) for the duration of the studies (6 weeks), and weekly blood samples were obtained for plasma lipid determinations. These data indicated that plasma lipid values increased through week 4, after which point values plateaued. Body weights remained stable throughout the study period with mean values (kilograms) of 4.0 ± 0.1, 3.5 ± 0.2, and 3.5 ± 0.3 for HE, LE, and controls, respectively. Metabolic studies were conducted during week 6, at which point samples were also obtained from representative animals for FPLC analyses. The research protocol used in these studies was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute.

Plasma lipids and apolipoproteins

To facilitate blood sampling for the metabolic studies, a central venous catheter was placed via the right external jugular vein. Samples were collected from the

central vein catheter at each designated time point and were added to tubes containing tripotassium EDTA. Plasma was isolated by centrifugation at 2500 rpm for 30 min at 4°C. Total cholesterol (TC) and triglycerides (TG) (Sigma, St. Louis, MO) and free cholesterol (FC) and phospholipids (PL) (Wako Chemicals USA, Inc., Richmond, VA) in both whole plasma and FPLC fractions were measured with a Hitachi 911 Autoanalyzer (Hitachi USA, Indianapolis, IN) using enzymic reagents. Plasma HDL cholesterol was determined after dextran sulfate-Mg^{2+} precipitation of very low density and low density lipoproteins (25). Cholesteryl ester (CE) values were calculated by subtracting free cholesterol from total cholesterol concentrations. ApoA-I and apoB concentrations were determined by competitive ELISA assays, utilizing a monoclonal antiserum directed against purified rabbit apoA-I and a chicken polyclonal antiserum directed against rabbit LDL, respectively.

Human LCAT, hepatic and lipoprotein lipase, and CETP activities

α-LCAT activity was determined, in duplicate, as previously described (26). Total post-heparin plasma lipolytic activity was quantitated as previously reported (27) using glycerol tri-[^{14}C]oleate (Amersham, Arlington Heights, IL) as the substrate. Hepatic and lipoprotein lipase activities were determined in duplicate by selectively blocking lipoprotein lipase with 1 mmol/L NaCl. Plasma CETP activity was assessed using the method of Albers et al. (28).

Gel filtration chromatography

Two hundred microliters of rabbit plasma was applied to a fast protein liquid chromatography (FPLC) system consisting of two Superose 6 columns connected in series (Pharmacia Biotech, Inc., Piscataway, NJ). Lipoproteins were eluted at 0.3 ml/min with phosphate-buffered saline, containing 1 mM EDTA and 0.02% (w/w) sodium azide (29). After the initial 10 ml was eluted, the next 30 ml was collected in 0.5-ml fractions. Total, free, and esterified cholesterol concentrations, as well as phospholipids, were determined for each rabbit. Total protein content was assessed with the BCA protein assay (Pierce, Rockford, IL).

Low density lipoprotein isolation

Narrow-cut LDL (d 1.030–1.050 g/ml) for metabolic studies was isolated from the plasma of fasting LDL receptor-deficient rabbits by sequential ultracentrifugation. We chose this tracer for it provided us with the best means of isolating a large quantity of LDL apoB that was not significantly contaminated with other apolipoproteins and, further, provided for consistency among experiments. Moreover, previous studies have not shown an effect of donor LDL on recipient LDL apoB kinetics (30, 31). After isolation, the LDL was dialyzed against PBS/EDTA to remove excess potassium bromide. Agarose gel electrophoresis and FPLC analysis confirmed that the isolated LDL was pure and was not contaminated with other lipoprotein classes.

In vivo metabolic studies

Purified rabbit apoA-I (32) and rabbit LDL were radiolabeled with ^{131}I and ^{125}I (Dupont/NEN, Boston, MA), respectively, using a modification of the iodine monochloride method previously described (33, 34). ^{131}I-labeled apoA-I was reassociated with autologous plasma from each animal for 30 min at 39°C, the normal body temperature of a rabbit. Unbound iodine was removed by extensive dialysis against 0.1% (v/v) PBS, 0.01% (w/v) EDTA. Four milliliters of the dialyzed autologous plasma from each rabbit was subjected to density gradient ultracentrifugation for 22 h at 39,000 rpm, 10°C, according to the method of Terpstra and Pels (35), for the isolation of ^{131}I-labeled HDL apoA-I. Each isolated radiolabeled HDL fraction was further dialyzed against PBS/EDTA to remove excess potassium bromide.

Immediately prior to injection, 25 μCi of ^{125}I-labeled LDL was added to the ^{131}I-labeled, autologous HDL (25 μCi) of each rabbit, and each preparation was filter-sterilized (0.22 μm Millex-GV filters, Millipore, Bedford, MA). Infusates were injected into the marginal ear vein of each rabbit. Blood samples were collected at 5 min and at 1, 3, 6, 9, 12, 24, 48, 72, 96, 120, 144, and 168 h post-injection. Plasma was isolated by centrifugation at 2500 rpm, 4°C for 30 min. Five hundred microliters of each sample was analyzed for radioactivity on a Packard Cobra gamma counter (Packard Instrument Co., Downers Grove, IL).

An additional set of studies was performed in which LDL apoB-100 kinetics were assessed in HE and control rabbits, after 6 weeks on the same 0.3% cholesterol diet as that used in the gene-dosage experiments. In these studies, blood samples were obtained through 30 h post-injection, and LDL (d 1.019–1.063 g/ml) was isolated from each time point of each rabbit by sequential ultracentrifugation. Subsequently, 200 μl of each LDL fraction was subjected to SDS-PAGE using 4–22.5% gradient gels (36), and LDL apoB-100 bands were excised and analyzed for their radioactive content.

Residence time (RT) was determined from the area under the plasma radioactivity decay curve, using a multiexponential computer curve-fitting program (SAAM31) (37). Fractional catabolic rate (FCR) was calculated as the reciprocal of the RT. ApoA-I and apoB pool sizes were derived from the formula: [plasma volume (dl) × apoprotein concentration (mg/dl)]/body weight (kg). Plasma volume was estimated as 3.28% of

body weight (38). Production rate (PR) was calculated as the product of FCR and pool size.

Statistical analysis

Data were assessed for significance using the Bonferroni multiple comparison procedure. As three comparisons were made, the observed level of significance was set at $P < 0.02$. Correlation coefficients were determined by the method of Pearson, and their statistical significance was set at $P < 0.05$. Multiple regression analysis was performed using SPSS for Windows (release 5.0, SPSS Inc., Chicago, IL). Data presented in the text, tables, and figures represent mean ± SEM.

RESULTS

Plasma LCAT activity, lipids, and lipoprotein composition

Table 1 summarizes the mean plasma LCAT activity and lipids of control and hLCAT transgenic rabbits on the 0.3% cholesterol diet. HE had twice the level of plasma LCAT activity than did LE ($P < 0.07$), and, as expected, the difference between HE and controls was even greater ($P < 0.02$). The more than 2-fold elevation in plasma LCAT activity noted in LE relative to controls just failed to reach statistical significance ($P < 0.06$). We have previously shown that the degree of LCAT expression is directly associated with the concentrations of plasma total, free, and esterified cholesterol (23); however, as can be seen in Table 1, this was not the case for the present study. This was undoubtedly due to the differential distribution of cholesterol that occurred on the 0.3% cholesterol diet, as is discussed below.

Plasma total, free, and esterified cholesterol concentrations were lower in HE relative to both LE and controls, although none of these differences were statistically significant. The lack of difference in total cholesterol levels between the groups was largely due to the elevated level of HDL-C noted in HE, with a mean level that was 1.3-fold and 4-fold greater than those of LE and controls, respectively. Conversely, plasma nonHDL-C concentrations were reduced in HE when compared with the values for either LE (−52%) or controls (−66%). The preceding changes resulted in a TC:HDL-C ratio for HE that was significantly lower than that observed for controls.

For all parameters, the differences noted between LE and controls were always intermediate to those observed between HE and controls. The most substantial difference between the former two groups was in HDL-C levels, with a greater than 3-fold elevation observed in LE. This comparison failed to reach statistical significance ($P < 0.1$) most likely due to the variability observed in the HDL-C levels of LE on this diet. Neither plasma phospholipids nor triglycerides were significantly different when any of the three groups were compared (data not shown).

In **Fig. 1,** FPLC lipoprotein profiles from representative HE (A), LE (B), and control (C) rabbits are given. In agreement with the plasma lipid determinations, the FPLC profile of HE was characterized by the presence of a large CE- and PL-enriched HDL, the levels of which were elevated relative to LE and controls. In contrast, both VLDL and IDL + LDL cholesterol concentrations were significantly lower in HE when compared with LE, and, most dramatically, with controls. Thus, on the 0.3% cholesterol diet, HDL was the predominant lipoprotein class in HE, whereas the presence of CE-rich apoB-containing lipoproteins characterized the plasma of controls. Compared with HE, an accumulation of VLDL and IDL + LDL cholesterol was also noted in LE, but to a far lesser extent than that observed in controls. VLDL, IDL, and LDL percent composition analyses revealed that the apoB-containing lipoproteins of hLCAT transgenic and control rabbits were not dramatically different, with control VLDL and IDL having relatively more cholesteryl ester and less triglyceride when compared with those of HE (data not shown). Transgenic and control LDL were also of similar composition, suggesting a decrease in particle number, rather

TABLE 1. Plasma LCAT activity and lipids of control and hLCAT transgenic rabbits on a 0.3% cholesterol diet

Level of Expression	LCAT Activity	TC	FC	CE	HDL-C	nonHDL-C	TC:HDL-C
	nmol·ml·h⁻¹			*mg/dl*			
High	889 ± 180[b]	230 ± 50	65 ± 13	165 ± 38	127 ± 19[a]	103 ± 33[b]	1.8 ± 0.2[b]
Low	412 ± 94	313 ± 46	78 ± 11	235 ± 37	100 ± 33	213 ± 39	3.1 ± 0.8
Control	176 ± 14[b]	332 ± 52	86 ± 8	246 ± 44	31 ± 4[a]	301 ± 55[b]	10.7 ± 2.5[b]

Values are mean ± SEM. Mean values with a common superscript are significantly different from one another. LCAT, lecithin:cholesterol acyltransferase; TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; HDL-C, high density lipoprotein cholesterol.

[a] $P < 0.008$.

[b] $P < 0.02$.

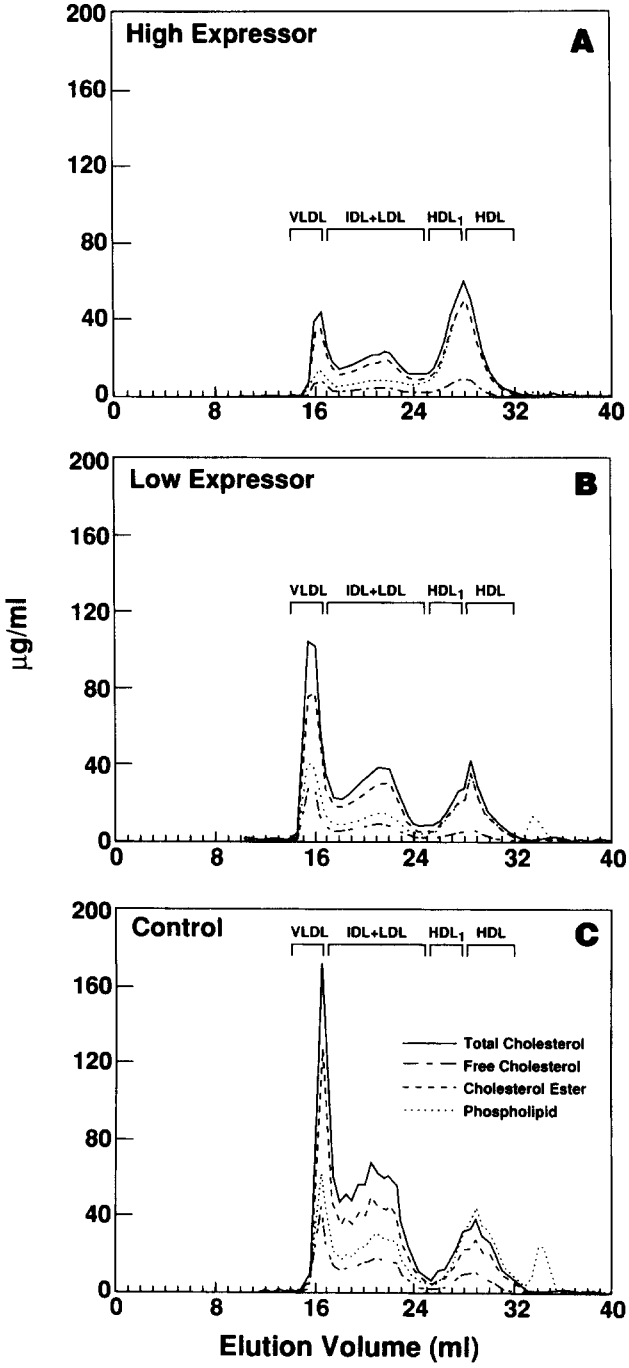

Fig. 1. Compositional analysis of gel filtration chromatography fractions. Panels A, B, and C illustrate the concentrations of total cholesterol, free cholesterol, cholesteryl ester, and phospholipid in the FPLC fractions of representative HE, LE, and control rabbits on the 0.3% cholesterol diet. Cholesteryl ester-enriched HDL was the predominant lipoprotein class in the plasma of HE, whereas cholesteryl ester-enriched apolipoprotein B-containing lipoproteins characterized the plasma of LE and, most notably, of controls.

than change in cholesterol content, was responsible for the reductions in LDL in hLCAT transgenics.

FPLC analysis also revealed an incremental shift in apparent HDL particle size that correlated directly with the level of hLCAT expression such that on the 0.3% cholesterol diet HE had the largest HDL particles and controls the smallest. The isotopic distribution of radiolabeled apoA-I and LDL confirmed this size shift and demonstrated that the radioactivity associated with LDL was not redistributed in vivo, lessening the likelihood that significant labeling of exchangeable apoproteins, other than apoB, were labeled in vitro.

Metabolic parameters of HDL apoA-I

The kinetic parameters of HDL apoA-I in control and hLCAT transgenic rabbits on the 0.3% cholesterol diet are given in **Table 2.** The mean plasma apoA-I pool size was greater in HE, as compared with both LE and controls, with only the difference between HE and controls reaching statistical significance. Similar to the HDL data, the difference in apoA-I pool size noted between LE and controls failed to achieve statistical significance ($P < 0.08$) despite a 2-fold elevation in the former due to the large SEM of LE. As illustrated in **Fig. 2,** these differences in circulating apoA-I levels on the 0.3% cholesterol diet were largely attributable to distinctive rates of HDL apoA-I catabolism among the three groups, such that the decline in plasma radioactivity over time of ^{131}I-labeled HDL apoA-I was fastest in controls, followed by LE, and, last, by HE. These data translated into a mean apoA-I FCR, d^{-1}, for HE (0.282 ± 0.030) which was 17% lower than that for LE (0.340 ± 0.010) and 43% lower than that for controls (0.496 ± 0.040). The 32% reduction in apoA-I FCR noted for LE relative to controls just failed to achieve statistical significance with the Bonferroni adjustment ($P < 0.03$). The preceding FCRs corresponded with apoA-I residence times (days) of 3.6 for HE, 2.9 for LE,

TABLE 2. Metabolic parameters of HDL apoA-I in control and hLCAT transgenic rabbits on a 0.3% cholesterol diet

Level of Expression	ApoA-I Pool Size	ApoA-I PR	ApoA-I FCR
	mg/kg	*mg·kg·d^{-1}*	*d^{-1}*
High	246 ± 12^a	16 ± 2	0.282 ± 0.030^b
Low	218 ± 48	21 ± 3	0.340 ± 0.010^c
Control	101 ± 18^a	14 ± 1	$0.496 \pm 0.040^{b,c}$

Values are mean ± SEM. Mean values with a common superscript are significantly different from one another. HDL, high density lipoproteins; apo, apolipoprotein; LCAT, lecithin:cholesterol acyltransferase; PR, production rate; FCR, fractional catabolic rate.

$^aP < 0.003$.
$^bP < 0.01$.
$^cP < 0.02$.

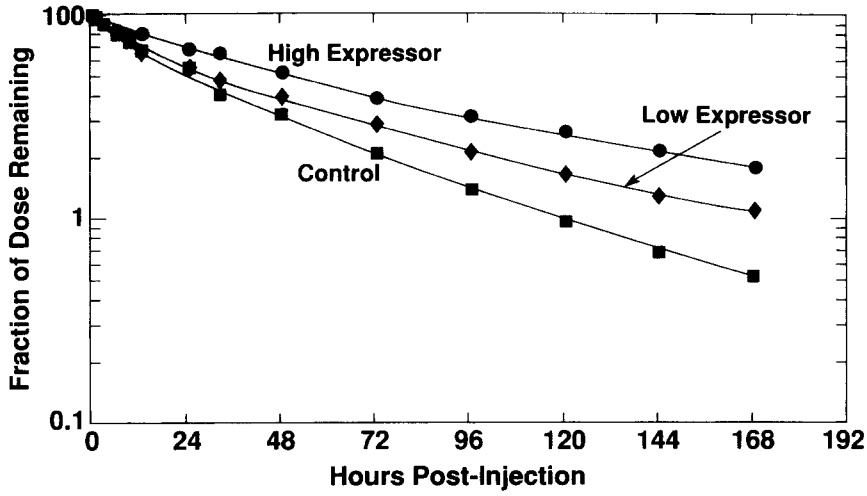

Fig. 2. In vivo metabolism of ^{131}I-labeled HDL apoA-I in control and hLCAT transgenic rabbits on the 0.3% cholesterol diet. Purified rabbit apoA-I was radiolabeled and HDL was isolated as described in Methods. The radioactivity decay curves for HE (●), LE (◆), and controls (■) are illustrated using a two-log scale as the ordinate. The plasma clearance of apoA-I was slowest in HE, followed by LE, and, last, by controls, indicating a gene dose-dependent reduction in apoA-I FCR on the cholesterol diet.

and 2.0 for controls. Although the apoA-I PR was elevated in LE relative to both HE and controls, no significant differences were noted among the groups. Further evidence that fractional catabolic rate was the predominant mechanism by which HDL apoA-I concentrations were modulated in these animals was provided by linear regression analyses that revealed that plasma HDL-C levels were highly correlated with apoA-I FCR ($r = -0.912$, $P < 0.001$), but not with apoA-I PR ($r = 0.449$, $P = 0.2$). Interestingly, apoA-I FCR was also positively associated with nonHDL-C ($r = 0.780$, $P < 0.01$).

Metabolic parameters of LDL

The metabolic parameters of LDL in control and hLCAT transgenic rabbits on the 0.3% cholesterol diet are provided in **Table 3.** The mean apoB pool size of HE was reduced when compared with both LE and controls, but only the latter comparison achieved significance. ApoB levels were highly correlated with nonHDL-C levels ($r = 0.808$, $P < 0.01$). As depicted in **Fig. 3,** the differences noted among the three groups with respect to apoB pool size on the 0.3% cholesterol diet were primarily due to distinctive rates of LDL catabolism. This finding was similar, but opposite, to the association observed between HDL-C levels and apoA-I FCR, such that HE had the lowest nonHDL-C levels with the fastest rate of clearance, h^{-1}, (0.131 ± 0.027), followed by LE (0.057 ± 0.009), and, last, by controls (0.031 ± 0.001). These data corresponded with LDL residence times (hours) of 7.7 for HE, 17.7 for LE, and 32.5 for controls. Interestingly, the mean apoB production rate of HE was greater than those of both LE and controls. However, apoB PR was not associated with nonHDL-C levels ($r = -0.123$, $P = 0.8$) in this study. Rather, linear regression analysis revealed that nonHDL-C levels were regulated by FCR in these animals ($r = -0.750$, $P < 0.02$).

Additional studies were performed in four HE and three control rabbits to specifically assess the turnover rate of LDL apoB-100. These studies used the same tracer as that used in the gene-dosage experiments; however, in this case, LDL (d 1.019–1.063 g/ml) was reisolated from the plasma time points of each animal by sequential ultracentrifugation and LDL apoB-100 bands were analyzed for their radioactive content, sub-

TABLE 3. Metabolic parameters of LDL in control and hLCAT transgenic rabbits on a 0.3% cholesterol diet

Level of Expression	ApoB Pool Size	ApoB PR	ApoB FCR
	mg/kg	*mg·kg·h*$^{-1}$	*h*$^{-1}$
High	11 ± 2[a]	0.310 ± 0.034	0.131 ± 0.027[b,c]
Low	17 ± 9	0.270 ± 0.104	0.057 ± 0.009[b]
Control	23 ± 1[a]	0.210 ± 0.028	0.031 ± 0.001[c]

Values are mean ± SEM. Mean values with a common superscript are significantly different from one another. LDL, low density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; apo, apolipoprotein; PR, production rate; FCR, fractional catabolic rate.

[a] $P < 0.01$.
[b] $P < 0.02$.
[c] $P < 0.02$.

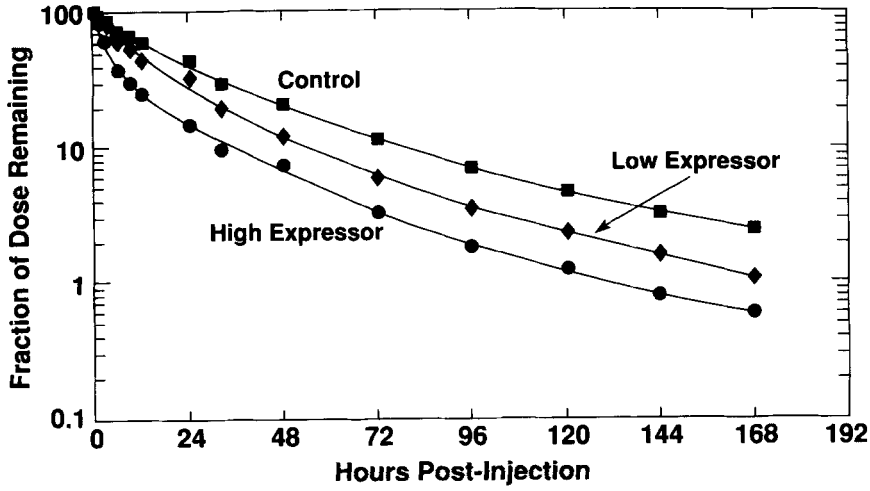

Fig. 3. In vivo metabolism of ^{125}I-labeled LDL in control and hLCAT transgenic rabbits on the 0.3% cholesterol diet. Narrow-cut LDL (d 1.030–1.050 g/ml) was isolated from donor LDL receptor-deficient rabbits and radiolabeled, as described in Methods. The radioactivity decay curves for HE (●), LE (◆), and controls (■) are illustrated using a three-log scale as the ordinate. The plasma clearance of LDL was fastest in HE, intermediate in LE, and slowest in controls, demonstrating that hLCAT overexpression accelerates LDL catabolism in a gene dose-dependent manner in cholesterol-fed rabbits.

sequent to SDS-PAGE. As shown in **Fig. 4,** the results of these studies clearly paralleled those in which plasma LDL radioactivity was determined, establishing that hLCAT transgenic rabbits had significantly accelerated catabolism of LDL apoB-100 relative to controls. Moreover, when these data are compared with those in Fig. 3, it is apparent that the values for fraction of dose remaining for each group at the 30-h time point are almost identical, with values of approximately 10% and 30% for hLCAT transgenic and control rabbits, respectively, thus, providing strong evidence that FCR was the predominant mechanism by which LDL concentrations were regulated in these animals.

Relationships between plasma LCAT activity and apoA-I and LDL metabolic parameters

Linear regression analyses revealed that plasma LCAT activity was positively associated with HDL-C (r =

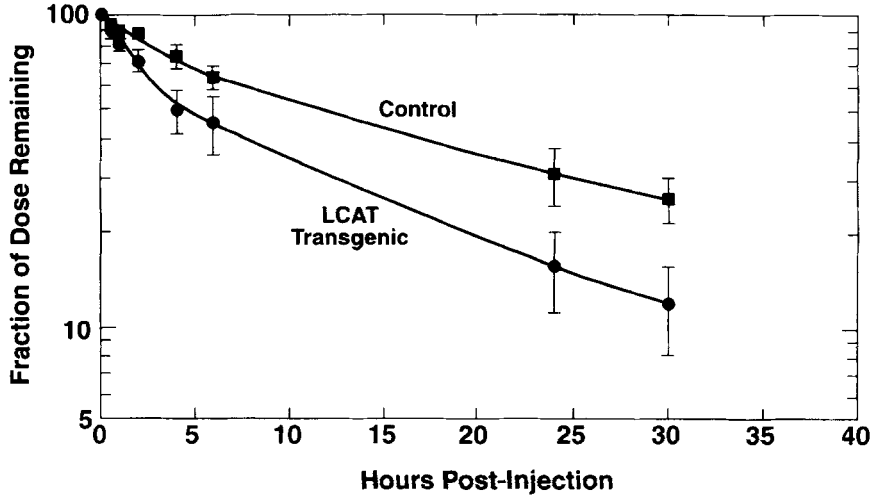

Fig. 4. In vivo metabolism of ^{125}I-labeled LDL apoB-100 in control and hLCAT transgenic rabbits on the 0.3% cholesterol diet. LDL was re-isolated from the plasma of each rabbit and subjected to SDS-PAGE for the isolation of LDL apoB-100, as described in Methods. The radioactivity decay curves for hLCAT transgenic (●) and control (■) rabbits clearly indicate that LDL apoB-100 catabolism was significantly faster in transgenics relative to controls, corroborating the plasma LDL data shown in Fig. 3.

0.864, $P < 0.01$), whereas an inverse relationship was noted between LCAT activity and apoA-I FCR ($r = -0.868$, $P < 0.01$). ApoA-I PR was not significantly correlated with LCAT activity ($r = -0.017$). In contrast to the HDL-C data, an inverse, rather than direct, relationship was observed between plasma nonHDL-C concentrations and LCAT activity ($r = -0.695$, $P = 0.06$). LDL FCR was also related to LCAT activity, such that high levels of activity were associated with an accelerated LDL FCR ($r = 0.670$, $P = 0.06$). LDL PR was not significantly associated with LCAT activity ($r = 0.443$).

Multiple stepwise regression analysis was also performed in order to further define those variables that were most closely linked to the changes in HDL-C and nonHDL-C levels, as well as to the lipoprotein metabolic parameters. Using HDL-C as the dependent variable and LCAT, CETP, HL, and LPL activities as the independent variables, both forward and backward analyses revealed an r^2 of 0.736 for LCAT activity, with this being the only variable required in the model to explain the variability in HDL-C concentrations. A similar result was obtained when nonHDL-C was used as the dependent variable. In this case, LCAT activity accounted for 63% ($r^2 = 0.630$) of the variability in nonHDL-C concentrations. Not surprisingly, both forward and backward analyses indicated that LCAT activity was the primary determinant of apoA-I FCR as well ($r^2 = 0.781$), while LCAT activity accounted for 51% of the variability in LDL FCR. As neither apoA-I nor LDL production rates were significantly correlated with any of the metabolic parameters, these data were not analyzed.

DISCUSSION

We have recently reported that the overexpression of human LCAT in transgenic rabbits prevents diet-induced atherosclerosis (22). In addition to having elevated HDL cholesterol levels, the concentrations of proatherogenic apoB-containing lipoproteins were substantially lower in cholesterol-fed hLCAT transgenic rabbits relative to littermate controls, leading us to hypothesize that hLCAT overexpression might modulate nonHDL, as well as HDL, particle metabolism. The purpose of the present study was to investigate the metabolic mechanisms responsible for the differences observed in both HDL and LDL cholesterol levels between cholesterol-fed hLCAT transgenic and control rabbits, and, ultimately, to provide a potential explanation for the prevention of diet-induced atherosclerosis. Our results further establish the importance of LCAT in the modulation of HDL-C levels, but, more notably, also demonstrate that hLCAT overexpression can mediate the metabolism of LDL in cholesterol-fed rabbits.

In order to determine the mechanism(s) responsible for the elevated HDL-C concentrations noted in high and low expressors relative to controls on the 0.3% cholesterol diet, we assessed ^{131}I-labeled HDL apoA-I kinetics using autologous HDL. These experiments revealed that the catabolism of HDL apoA-I was fastest in controls and slowest in high expressors, with low expressors having an intermediate rate of decay. This finding is consistent with our previous work which reported that the hyperalphalipoproteinemia induced by human LCAT overexpression in chow-fed rabbits was due to a gene dose-dependent reduction in HDL catabolism (39), and indicates that the presence of excess LCAT, rather than of dietary cholesterol, is the primary regulator of HDL-C concentrations in these animals. This concept was supported by both linear and multiple regression analyses which determined that apoA-I FCR was highly regulated by LCAT activity.

The most interesting, and novel, finding of this study concerned the significant relationship found between hLCAT overexpression and the modulation of plasma nonHDL-C concentrations, the latter of which was, in part, associated with changes in LDL catabolic rates. High expressors had the lowest nonHDL-C concentrations with the fastest rate of LDL clearance, followed by low expressors and controls, indicating that hLCAT overexpression affected LDL, as well as HDL, metabolism in a gene dose-dependent manner. Although the interpretation of these results is somewhat limited due to the use of a nonautologous LDL tracer, hLCAT transgenic and control LDL were of similar composition, lessening the likelihood that our kinetic observations were due to differential metabolism of donor and recipient LDL between the groups. Moreover, the data shown in Figs. 3 and 4 clearly indicate that, when injected with the same LDL tracer, hLCAT transgenic rabbits have accelerated catabolism of LDL apoB-100 relative to controls. Both linear and multiple regression analyses also provided strong evidence that LCAT activity played a major role in the determination of nonHDL-C levels, with LCAT activity accounting for 63% of the variability in nonHDL-C levels and for 51% of the variability in LDL FCR in this study. Considering the proatherogenic nature of LDL apoB, these data suggest that the enhanced removal of LDL from the plasma compartment of cholesterol-fed hLCAT transgenic rabbits may partially account for the protection against diet-induced atherosclerosis observed in our previous work.

While several studies have demonstrated that significant amounts of LCAT protein may be associated with LDL in native plasma (8, 40, 41), little is known regarding the role that this association might have in the me-

diation of plasma LDL levels. The modulation of LDL metabolism by LCAT is likely to be a complicated process, perhaps involving both receptor-dependent and -independent pathways. In support of the former, an inverse association has been observed between CETP, another critical enzyme in cholesterol homeostasis, and liver LDL receptor mRNA abundance in transgenic mice (19), leading one to speculate that LCAT could affect LDL receptor number as well. Alternatively, LCAT may not act by influencing LDL receptor synthesis, but rather could potentially affect LDL receptor activity by altering intracellular membrane composition or the cholesteryl ester composition of the particles themselves. The latter could affect both the affinity of apolipoproteins for lipoprotein particles and the interaction of lipoproteins with receptor surfaces, resulting in alterations of lipoprotein metabolism such as those observed in our study. While LDL receptors are the major mediators of LDL clearance from plasma, they have also been implicated as mediators of VLDL clearance via apoE (42). Because VLDL-C, as well as LDL-C, concentrations were clearly lower in high expressors relative to controls, the possibility that hLCAT overexpression might influence VLDL catabolism by either LDL and/or VLDL receptor pathways cannot be excluded.

Another possible explanation for our results involves the demonstration that apoB-100 has been shown to be an activator of the LCAT reaction (12). Thus, one could predict a scenario where increased substrate, apoB-containing lipoproteins, could initiate a chain of events in the metabolic cascade in the presence of excess LCAT in cholesterol-fed transgenic rabbits. Consistent with this concept, Barter (43) has shown that about 10% of the esterified cholesterol in human plasma may be formed by the direct action of LCAT on LDL particles. The cholesteryl esters generated by this reaction could then, ultimately, be removed by hepatic receptor-mediated pathways, resulting in reduced LDL concentrations such as those seen in our hLCAT transgenic rabbits.

In summary, our data indicate that hLCAT overexpression modulates nonHDL-C, as well as HDL-C, concentrations in cholesterol-fed transgenic rabbits by affecting lipoprotein particle clearance in a gene dose-dependent manner, with high expressors having the slowest clearance of HDL and the fastest clearance of LDL. These data are the first to demonstrate that hLCAT overexpression can mediate LDL metabolism, and suggest that LCAT may protect against diet-induced atherosclerosis by modulating both HDL and nonHDL particle metabolism.

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