Adenovirus-mediated expression of hepatic lipase in LCAT transgenic mice

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Abstract *In order to evaluate the coordinate role that* hepatic lipase (HL) and lecithin:cholesterol acyltransferase (LCAT) play in modulating HDL particle heterogeneity and function in vivo we utilized recombinant adenovirus to express HL in control and LCAT transgenic mice. Adenovirus-mediated expression of human HL in control (n = 4, LCAT activity = 42 ± 1 nmol/ml per h) and LCAT-tg mice (n = 4, LCAT activity = 3566 ± 93 nmol/ml per h) resulted in post heparin HL activities of 24358 ± 6080 and 27266 ± 7985 nmol/ml per min, respectively. Overexpression of HL led to significant reductions in total cholesterol, phospholipids, and HDL cholesterol in both LCAT-tg (62, 62, and 63%, $P < 0.05$) and control mice (68, 63, and 78%, $P < 0.01$) as well as to the formation of more homogenous HDL. However, compared to control animals, the reductions in the plasma concentrations of HDL-cholesterol and apoA-I were less in LCAT-tg mice (HDL-cholesterol: −62 ± 15% vs. −78 ± 15%, $P = 0.18$; apoA-I: −36 ± 7% vs. −76 ± 8%, $P < 0.0005$). Gel filtration analysis revealed that in LCAT-tg mice the apoE-rich HDL_1 was preferentially reduced by expression of HL in vivo. Compared to control mice the reduction in the apoA-I/A-II HDL in transgenic mice was significantly less indicating that a subset of HDL in LCAT transgenic mice are resistant to the action of HL. These combined data support a role for both HL and LCAT in modulating HDL heterogeneity and function, properties which may ultimately affect the ability of LCAT transgenic mouse HDL to function in the process of reverse cholesterol transport.—**Dugi, K. A., B. L. Vaisman, N. Sakai, C. L. Knapper, S. M. Meyn, H. B. Brewer, Jr., and S. Santamarina-Fojo.** Adenovirus-mediated expression of hepatic lipase in LCAT transgenic mice. *J. Lipid Res.* 1997. **38:** 1822–1832.

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In humans, high density lipoprotein (HDL) comprises several distinct subspecies, which include HDL_1, HDL_2, HDL_3 as well as nascent HDL. These different lipoprotein fractions may have distinct physiologic functions in vivo. Thus, low plasma levels of HDL_2 have been shown to correlate with the development of premature coronary artery disease (CAD) (1, 2) and nascent HDL have been proposed to play an important role in reverse cholesterol transport, one of several major processes by which HDL may modulate the development of CAD (3). Although evaluation of mouse lipoproteins by standard electrophoretic and gel filtration techniques reveals a more homogeneous HDL pattern than in humans, careful analysis of mouse HDL reveals heterogeneity with respect to lipid and apolipoprotein composition (4). A better understanding of the mechanisms influencing the generation of HDL with different composition and function may provide insights into metabolic pathways that modulate the processes of reverse cholesterol transport and atherosclerosis.

Three major plasma proteins appear to play a critical role in modulating HDL size, heterogeneity, and function: cholesteryl ester transfer protein (CETP), lecithin:cholesterol acyl transferase (LCAT), and hepatic lipase (HL). In humans, CETP catalyzes the exchange of triglycerides and cholesteryl esters between HDL and (apo) apolipoprotein B-containing lipoproteins, but CETP activity is absent in mice (5). CETP deficiency leads to the generation of cholesteryl ester-rich and triglyceride-poor mouse HDL that are the main carriers for cholesteryl ester. HL is a glycoprotein consisting of 476 amino acids (6–8). It is synthesized by hepatocytes and bound to glycosaminoglycans of the capillary endothelium (9). The main function of HL is the hydrolysis of phospholipids and triglycerides present in intermediate density lipoproteins (IDL) (10) and HDL (11–13), in particular HDL_2 (14). In vitro, HL has been shown to generate nascent pre-β_1 particles from HDL (15, 16)

Abbreviations: apo, apolipoprotein; CER, cholesterol esterification rate; FC, free cholesterol; kb, kilobases; FPLC, fast protein liquid chromatography; HDL, high density lipoproteins; HL, hepatic lipase; LCAT, lecithin:cholesterol acyltransferase; LCAT-tg, LCAT-transgenic; Lucif, firefly luciferase; PL, phospholipids; rAdV, recombinant adenovirus; TC, total cholesterol; TG, triglycerides; CAD, coronary artery disease.

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suggesting an important role of hepatic lipase in reverse cholesterol transport.

Human LCAT is a 47 kD glycoprotein synthesized in the liver and consists of 416 amino acids (17). LCAT catalyzes the synthesis of cholesteryl esters and lysolecithin from phosphatidylcholine and unesterified cholesterol present in plasma lipoproteins (18). It has been suggested that LCAT promotes the efflux of cholesterol from peripheral tissues to HDL, the first step of reverse cholesterol transport, by maintaining a concentration gradient for free cholesterol (19). In addition, esterification of free cholesterol on nascent pre-β_1 HDL particles by LCAT appears to be essential for the maturation of these particles to HDL_2 and HDL_3 (20, 21). This concept is supported by the accumulation of abnormal small spherical as well as aggregated discoidal particles in patients with LCAT deficiency (22–24).

We have described the generation of C57BL/6 mice transgenic for human LCAT (LCAT-tg) (25). Compared to control animals, homozygous LCAT-tg mice have a 2.5-fold increase in the plasma HDL cholesterol concentrations. FPLC profiles revealed a shift of the homogeneous HDL particles to heterogeneous HDL consisting of apoA-I/apoA-II lipoproteins and apoE-rich HDL_1. However, despite the increased plasma concentrations of HDL cholesterol and apoA-I, LCAT-tg mice have increased diet-induced atherosclerosis (26). The mechanism(s) leading to the dissociation between increased HDL and reduced atherosclerosis in these animals are unclear but one potential explanation may be the generation of an abnormal HDL in LCAT-tg mice that is unable to function in the process of reverse cholesterol transport.

In the present study we utilized recombinant adenovirus to express high plasma concentrations of HL, a key enzyme involved in HDL metabolism, in both control and LCAT-tg mice to investigate the proposed coordinate role of HL and LCAT in modulating plasma HDL concentrations, heterogeneity, and function. Specifically, we evaluated whether adenovirus-mediated expression of HL reversed LCAT-induced changes in the plasma concentrations and heterogeneity of mouse HDL as well as provided insights into potential mechanisms leading to the enhanced susceptibility of LCAT-tg mice compared to control mice for developing diet-induced atherosclerosis. Our studies demonstrate that HL overexpression in LCAT-tg mice reduces plasma concentrations of total and HDL cholesterol as well as decreases the heterogeneity of LCAT-tg mouse HDL, thus providing evidence for the important coordinate role that these two enzymes play in modulating HDL plasma concentrations and heterogeneity. However, compared to control HDL, a subset of HDL particles in LCAT-tg mice appear more resistant to either the enzymatic or non-enzymatic actions of HL in vivo indicating that the functional properties of transgenic mouse HDL are altered, thus providing a potential mechanism for the enhanced atherosclerosis observed in LCAT-tg mice.

METHODS

Animals

The LCAT transgenic C57BL/6 mice utilized in these studies have been previously described (25). Control C57BL/6 mice were obtained from Charles River (Wilmington, MA). For both lines, male animals 5–6 months of age and weighing 25–35 g were utilized. The mice were bred and housed at the National Institutes of Health under protocols approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute. Animals had free access to food (NIH 07 chow diet, 4.5% fat, 0% cholesterol; Zeigler Brothers Co., Gardners, PA) and water. The research protocol was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute of the National Institutes of Health.

Recombinant adenovirus

The recombinant adenoviruses HL-rAdV and Lucif-rAdV, containing the human HL cDNA (6–8) or the firefly luciferase (27, 28), respectively, were generated as described by McGrory, Bautista, and Graham (29). First, adenoviral shuttle plasmid vectors were constructed using polylinkers by the addition of the CMV promoter/enhancer fragment [HindII-Pbsl, −601 bp to +52 bp] (30), the late viral protein gene 16s/19s splice donor and acceptor SV40 signal fragment [XhoI-PstI, 470 bp to 545 bp and 1391 bp to 1480 bp] (31, 32) and the SV40 polyadenylation signal fragment [BamHi-HpaI, 2519 bp to 2666 bp] (31) to the XbaI site (bp # 3329) of the plasmid pXCX2 (gift from Dr. Frank Graham, McMaster University, Hamilton, Ontario, Canada) based on pXC1 (33). Human HL cDNA was amplified using the polymerase chain reaction and primers: 5′-CTT CAG AAA TTA TCT AGA AAG CCT GGA CCC-3′ and 5′-ATT CAT TTA TTC GTT AAC CTG GGT CTT CAT-3′ and the HL cDNA fragment was inserted immediately 3′ of the splice acceptor signal. The shuttle vectors were then cotransfected with pJM17 (29) into human embryonal kidney 293 cells (34) (ATCC, Rockville, MD) by the calcium phosphate coprecipitation method (35). Viral plaques were isolated as described (36). Recombinant adenoviruses were identified by the polymerase chain reaction (37) and subjected to two rounds of plaque purification prior to large scale amplification in 293 cells. Infected cells were

harvested 2–5 days after infection and subjected to 5 cycles of freeze/thaw lysis. Crude lysates were extracted with Freon (Halocarbon 113, Matheson Gas Products, Bridgeport, NJ), banded twice in CsCl, supplemented with 0.2% mouse albumin (Sigma Chemical Co., St. Louis, MO), and dialyzed extensively against physiologic saline supplemented with 10 mM HEPES (pH 7.4), 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$. Recombinant adenovirus was stored at −70°C and titered by plaque assay as described (38). Plaque forming units (7×10^8) of HL-rAdV or Lucif-rAdV were injected into the saphenous vein of anesthetized mice on day 0 of the study. Anesthesia was achieved by intraperitoneal injection of 0.011 ml per g body weight of 2.5% Avertin prepared by dissolving 10 g 2,2,2-tribromoethanol (Aldrich, Milwaukee WI) in 10 ml tertiary amyl alcohol (Aldrich).

Blood sampling

For all blood sampling, the mice were fasted for 4 h and anesthetized with methoxyflurane (39). Mice were bled from the retroorbital plexus using capillary tubes coated with heparin (Scientific Products, McGaw Park, IL). Coagulation was prevented with EDTA (final concentration of 4 mM). Samples were kept on ice until centrifugation at 2500 *g* for 20 min at 4°C. Plasma was removed, aliquoted, flash frozen, and stored at −70°C.

Lipid, lipoprotein, and apolipoprotein quantification

Lipids in 10 μl of mouse plasma were quantitated by the COBAS MIRA Plus automated chemistry analyzer (Roche Diagnostic Systems Inc., Branchburg, NJ) and enzymic assays using commercially available kits for total cholesterol (Sigma Diagnostics, St. Louis, MO) as well as free cholesterol and phospholipids (Wako Chemicals USA Inc., Richmond, VA). Cholesteryl ester concentrations were calculated as the difference of total and free cholesterol. HDL cholesterol was determined as the cholesterol remaining in the plasma after precipitation of apolipoprotein B-containing lipoproteins with heparin and calcium as previously described (40). Apolipoproteins A-I and B were quantitated by sandwich ELISA utilizing polyclonal antibodies raised in rabbits and purified mouse apoA-I and mouse LDL as protein standards. Apolipoprotein E levels were semiquantitatively determined by Western blotting (see below) and scanning densitometry using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale CA). The standard curve was generated by different dilutions of purified mouse apo E.

Determination of LCAT, lipase, and luciferase activities

α-LCAT activity was assayed using artificial proteoliposome substrate as previously described (41). Post heparin plasma samples were obtained 5 min after tail vein injection of 500 U/kg of heparin. LPL and HL activities were assayed using radiolabeled triolein substrate as described (42). Selective HL activity was quantitated in 1 M NaCl and LPL activity was determined as the difference of total and HL activities. Mouse plasma was diluted up to 100-fold to maintain linearity of the assay.

For luciferase assay, tissues were obtained on day 4 after injection with Lucif-rAdV. One hundred mg of tissue was homogenized in 0.5 ml of extraction buffer (0.1 M potassium phosphate buffer, pH 7.4, and 1 mM DTT). After three freeze/thaw cycles, the homogenates were centrifuged for 20 min at 10,000 *g* and 4°C. Thirty μl of the supernatant phase was then incubated with luciferin in the presence of 330 μl of reaction mixture (16.4 mM $MgCl_2$ and 5.4 mM ATP) and the resulting relative light units were determined with the Monolight luminometer, Analytic Luminescence Laboratory, San Diego, CA (43).

Fast protein liquid chromatography

Plasma lipoproteins were separated by gel filtration utilizing two Superose 6 HR 10/30 columns (Pharmacia-LKB Biotechnology Inc., Piscataway, NJ) connected in series as described (5). Lipoproteins from 50 μl of plasma were eluted at 0.3 ml/min with PBS buffer containing 1 mM EDTA and 0.02% sodium azide. Lipids in the recovered fractions were assayed as described above. For Western blotting of apolipoproteins, the FPLC fractions were concentrated 15-fold using Ultrafree-MC filters with 10,000 NMWL cellulose membranes (Millipore, Bedford MA). FPLC profiles were performed either on plasma from individual mice or pooled from several mice as stated in the figure legends.

Gel electrophoresis and Western blots

Two-dimensional gels for the detection of HDL particles with pre-β_1 mobility were done as previously described (44). Mouse apoA-I was visualized using a polyclonal anti-mouse apoA-I antibody from rabbit. Ten μl of concentrated FPLC fractions or 1 μl of whole plasma was subjected to SDS polyacrylamide gel electrophoresis through 4–20% gradient or 10% gels in Tris/glycine buffer (gels and buffer from Novex, San Diego, CA) and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Immobilon-P membranes were coupled to polyclonal antibodies to mouse apoA-I or apoE raised in rabbits and stained with horseradish peroxidase using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA).

Statistical analysis

Data are presented as mean ± standard deviation. Comparisons among groups of animals were performed

TABLE 1. Plasma LCAT and HL activities before and after injection of recombinant hepatic lipase adenovirus

Mice	rAdV Injected	LCAT Activity		Hepatic Lipase Activity	
		Day 0	Day 5	Day 0	Day 5
		nmol/ml/h		*nmol/ml/min*	
LCAT-tg (n = 4)	Hepatic lipase	3566 ± 93	3499 ± 145	525 ± 23[b]	27266 ± 7985[c]
Control (n = 4)	Hepatic lipase	42 ± 1	ND[a]	324 ± 54	24358 ± 6080[c]
LCAT-tg (n = 3)	Luciferase	3591 ± 96	3634 ± 70	437 ± 50	374 ± 70

[a]ND, not determined.
[b]$P < 0.01$ compared to control mice.
[c]$P < 0.001$ compared to day 0.

with unpaired Student's *t*-test for independent variables and according to Levene's test for equality of variances. Analyses of mice before and after injection of recombinant adenovirus were done with the paired *t*-test. All calculations were performed with SPSS for Windows, release 5.0, SPSS Inc., Chicago, IL.

RESULTS

A total of four LCAT-tg and four control mice were injected with recombinant adenovirus expressing human HL and three LCAT-tg as well as five control mice were injected with recombinant adenovirus coding for firefly luciferase. **Table 1** summarizes the LCAT and postheparin HL activities before (day 0) and on day 5 after virus injection. HL-rAdV injection did not alter the level of LCAT activity in mouse plasma indicating intact liver protein synthesizing capacity. Five days after injection of HL-rAdV, the baseline postheparin HL activities in LCAT-tg and control mice were increased to a similar level ($P < 0.001$) indicating successful expression of a functional HL. No changes in LCAT or postheparin HL activities were evident after the injection of Lucif-rAdV.

Figure 1 illustrates the changes in the plasma cholesterol concentrations observed in both LCAT-tg and control mice during the course of the study. The injection of Lucif-rAdV (dotted lines, open circles) into LCAT-tg (top panel) or control mice (bottom panel) did not significantly alter baseline cholesterol levels. Adenovirus-mediated expression of HL, however, resulted in significant lowering of plasma cholesterol concentrations in both control and LCAT-tg mice. The maximum cholesterol lowering effect of HL-rAdV was evident on day 4 after injection with cholesterol levels returning to baseline between days 8 and 12 after rAdV infusion.

The changes in the plasma lipid, lipoprotein, and apolipoprotein concentrations of both groups of animals before (day 0) and after (day 4) injection of HL-rAdV are summarized in **Table 2** and **Fig. 2.** In LCAT-tg mice, baseline plasma concentrations of cholesterol, phospholipids, triglycerides, and HDL were reduced by 62 ± 14% ($P < 0.005$), 62 ± 12% ($P < 0.005$), 32 ± 17% ($P < 0.05$), and 62 ± 15% ($P < 0.05$), respectively. Similarly, injection of HL-rAdV in control mice resulted in significant lowering of plasma cholesterol (76 ± 3 vs. 24 ± 8 mg/dl, −69 ± 10%, $P < 0.0005$), phospholipid (176 ± 14 vs. 65 ± 18 mg/dl, −63 ± 10%, $P < 0.005$

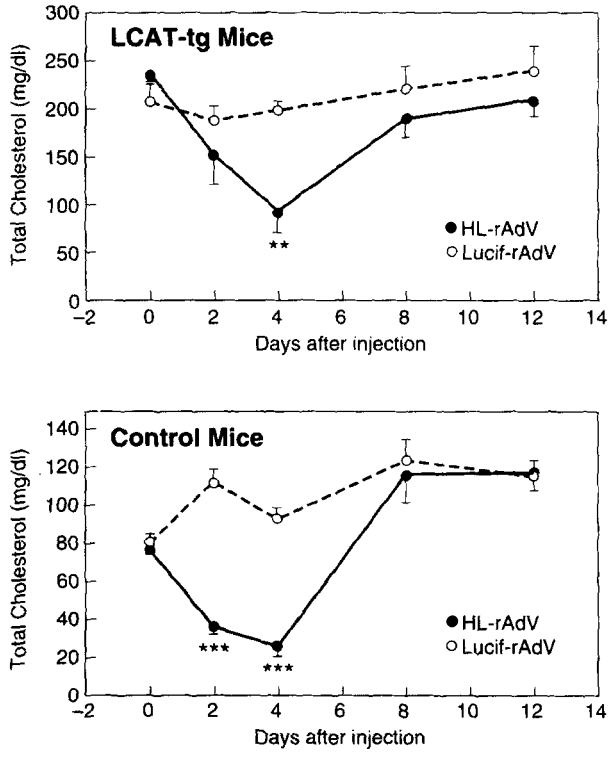

Fig. 1. Changes in the plasma concentrations of total cholesterol before and several days after the injection of HL-rAdV or Lucif-rAdV are illustrated for LCAT-Tg mice injected with HL-rAdV (n = 4, solid line) or Lucif-rAdV (n = 3, dotted line) and for control C57BL/6 mice injected with HL-rAdV (n = 4, solid line) or Lucif-rAdV (n = 5, dotted line). Data are expressed as mean ± SEM. $^{**}P < 0.05$, $^{***}P < 0.00005$.

TABLE 2. Lipid, HDL cholesterol, and apoAp-I levels in LCAT-tg and control mice before and on day 4 after injection of HL-rAdV or adenovirus

Mice	Virus	Day	TC	TG	PL	HDL-C	Apo E	ApoA-I
					mg/dl			
LCAT-tg	HL-rAdV	0	235 ± 11	37 ± 11	229 ± 15	199 ± 31	10.2 ± 1.3	55 ± 37
(n = 4)	HL-rAdV	4	89 ± 33[b]	25 ± 9[a]	87 ± 24[b]	74 ± 24[a]	6.3 ± 2.2	33 ± 18
Control	HL-rAdV	0	76 ± 3	55 ± 13	176 ± 14	77 ± 9	3.4 ± 0.3	40 ± 17
(n = 4)	HL-rAdV	4	24 ± 9[c,d]	55 ± 24	65 ± 18[b]	17 ± 12[c,d]	3.5 ± 0.3	9 ± 1[c,e]
LCAT-Tg	Lucif-rAdV	0	207 ± 30	35 ± 8	186 ± 27	207 ± 33	nd	62 ± 21
(n = 3)	Lucif-rAdV	4	199 ± 12	46 ± 7	179 ± 12	186 ± 24	nd	53 ± 6
Control	Lucif-rAdV	0	80 ± 9	97 ± 26	139 ± 26	95 ± 8	nd	71 ± 25
(n = 5)	Lucif-rAdV	4	93 ± 10	120 ± 29	177 ± 15	104 ± 9	nd	103 ± 38

[a] $P < 0.05$ compared to baseline values.
[b] $P < 0.01$ compared to baseline values.
[c] $P < 0.001$ compared to baseline values.
[d] $P < 0.01$ control vs. LCAT-tg mice after injection of HL-rAdV.
[e] $P < 0.05$ control vs. LCAT-tg mice after injection of HL-rAdv.

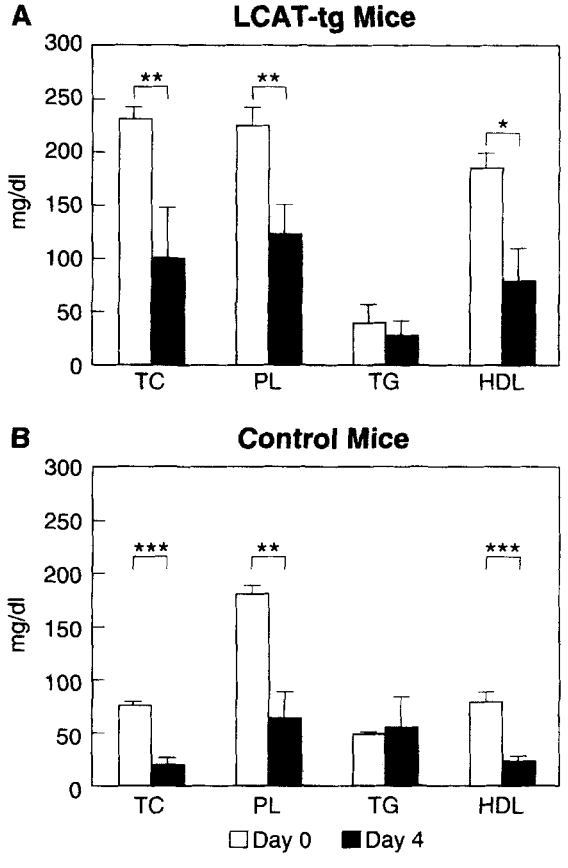

Fig. 2. Plasma lipid and HDL concentrations before and on day 4 after injection of HL-rAdV into LCAT-tg (n = 4; panel A) or control mice (n = 4; panel B). The plasma concentrations of total cholesterol (TC), phospholipids (PL), triglycerides (TG), and HDL cholesterol (HDL) are shown. Values are expressed as mean ± SD. Open bars represent values before and closed bars on day 4 after injection of HL-rAdV. No significant changes in plasma lipoproteins or HDL were seen after the injection of Lucif-rAdV into LCAT-tg (n = 3) or control (n = 5) mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

and HDL cholesterol (77 ± 9 vs. 17 ± 12 mg/dl, −78 ± 15%, $P < 0.001$) but not triglycerides (55 ± 13 vs. 55 ± 24 mg/dl). Due to higher baseline values, the absolute reduction of plasma lipid and lipoprotein levels was higher in LCAT-tg than control mice. However, the percent reduction in HDL cholesterol concentrations after HL expression was less in LCAT-tg than in control mice (−78 ± 15% vs. −62 ± 14%). Consistent with these data, the baseline plasma concentrations of apoE and apoA-I were not significantly decreased in LCAT-tg mice, although determination of plasma apoE levels revealed a trend towards reduction after HL expression. In contrast, apoA-I was markedly reduced in control mice injected with HL-rAdV (40 ± 17 vs. 9 ± 1 mg/dl, −76 ± 8%, $P < 0.05$). The difference in apoA-I reduction between LCAT-tg (−36 ± 7%) and control mice (−76 ± 8%) after HL-rAdV injection was highly significant ($P < 0.0005$) suggesting that at least a subset of apoA-I-containing lipoproteins in LCAT-tg mice were resistant to the action of hepatic lipase. No significant decrease in plasma cholesterol, phospholipid, triglyceride, and HDL cholesterol concentrations was seen in LCAT-tg (n = 3) or control (n = 5) mice injected with the control adenovirus Lucif-rAdV (Table 2).

To further evaluate the ability of HL to reduce the amount of apoE-rich and apoA-I. HDL in transgenic mice we performed FPLC analysis of plasma samples obtained before or on day 4 after injection of HL-rAdV. **Figure 3,** panel A, illustrates the baseline FPLC profiles of LCAT-tg and control mice. Control mice (dotted line) have distinct peaks for LDL and HDL with the HDL peak predominating. In contrast, LCAT-tg mice (solid line) have an HDL that is shifted in size towards that of larger particles but is similar in apolipoprotein composition to the apoA-I HDL present in control mice (25). In addition, LCAT-tg mice have a larger apoE-rich HDL_1 particle (25). Panel B illustrates the FPLC

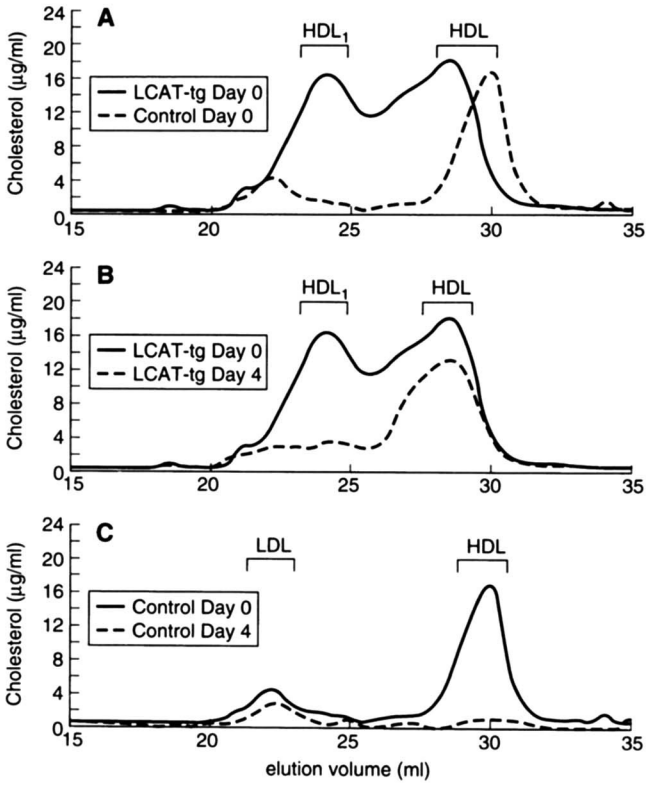

Fig. 3. FPLC profiles of plasma lipoprotein for LCAT-tg (solid line) and control (dotted line) mice before and on day 4 after injection of HL-rAdV. Fifty µl of pooled mouse plasma was run over two Superose 6 columns in series and the cholesterol concentration (µg/ml) in the eluted FPLC fractions was determined. Panel A illustrates the lipoprotein profile in both control and LCAT-tg mice prior to adenovirus infusion. Panels B and C illustrate the FPLC profile of LCAT-tg and control mice, respectively, before (day 0) and after (day 4) infusion of recombinant adenovirus.

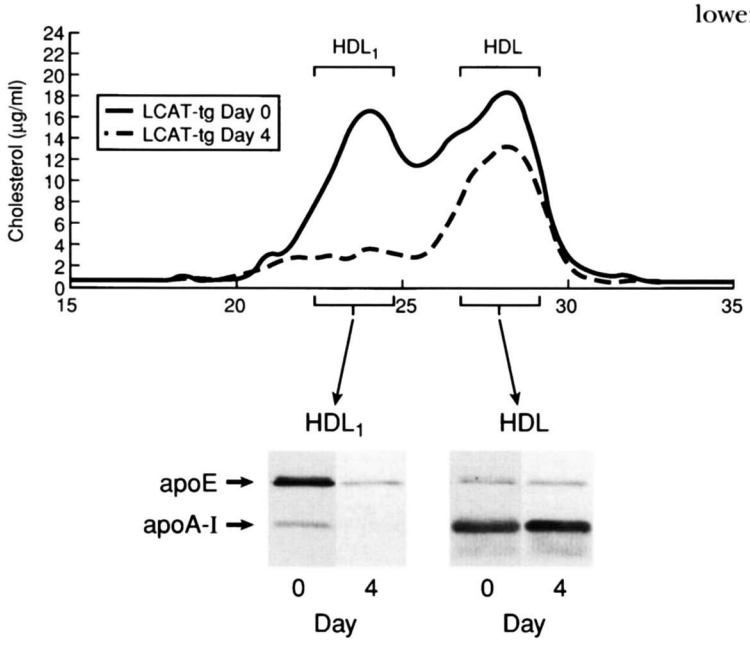

Fig. 4. Apolipoprotein composition of the different FPLC lipoprotein peaks before and on day 4 after injection of HL-rAdV in LCAT-tg mice. The top panel illustrates the FPLC profile of LCAT-tg mice before (solid line) and on day 4 after (dotted line) injection of HL-rAdV. The FPLC fractions pooled for analysis of apolipoprotein composition are indicated. The bottom panel illustrates the Western blot analysis of apoE and apoA-I before and on day 4 after injection of HL-rAdV.

profile of LCAT-tg mice before (solid line) and on day 4 after (dotted line) injection of HL-rAdV. After infusion of HL-rAdV, the size of the apoA-I HDL peak remained virtually unchanged indicating that hepatic lipase was unable to modify the apoA-I-containing HDL particle in LCAT-tg mice. In contrast, the apoE-rich HDL_1 cholesterol peak disappeared almost entirely, indicating that the apoE-rich HDL_1 is susceptible to the action of hepatic lipase in vivo. Figure 3, panel C illustrates the FPLC profile of control mice before (solid line) and on day 4 after (dotted line) injection of HL-rAdV. The peak for apoA-I HDL in control mice virtually disappeared after virus injection. Thus, the same level of hepatic lipase expression resulted in virtually complete disappearance of the apoA-I HDL in control mice without altering the analogous particle in LCAT-tg animals.

Analysis of the changes in the apolipoprotein content in the two HDL particles present in LCAT-tg mice after expression of HL was performed by SDS-PAGE electrophoresis of pooled fractions corresponding to the two distinct HDL FPLC peaks followed by immunostaining. **Figure 4** illustrates that at baseline (day 0) the larger HDL particle, HDL_1, is primarily apoE-rich, while the second particle, like normal mouse HDL, contains primarily apoA-I. Day 4 after injection of HL-rAdV, the apoE content in the fractions corresponding to HDL_1 was greatly diminished. The apoA-I concentration of the apoA-I HDL peak, however, was only marginally reduced. These results are consistent with the differential changes in the cholesterol concentrations of the apoE-rich HDL_1 and apoA-I HDL peaks before and after infusion of HL-rAdV as shown in Fig. 3., panel B.

In order to evaluate the effect of expressing HL at lower levels in LCAT-tg mice we studied the plasma lip-

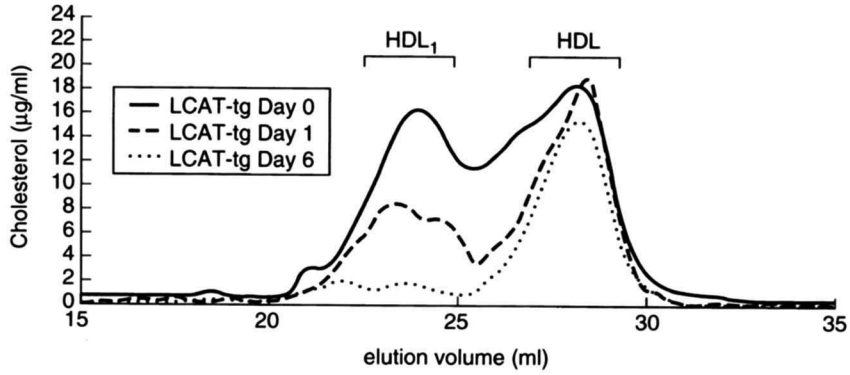

Fig. 5. FPLC profiles from LCAT-tg mice with reduced expression of HL. Fifty µl of pooled mouse plasma was run over two Superose 6 columns in series and the cholesterol concentration (µg/ml) in the eluted FPLC fractions was determined. FPLC profiles were determined before (solid line), on day 1 after (dashed line), and day 6 after (dotted line) injection of HL-rAdV.

ids and FPLC profiles of two mice injected with a lower dose of HL-rAdV. HL activity on day 4 after injection of HL-rAdV increased to a mean of 6332 nmol/ml per min in these mice. This lower level of HL activity led to only a slight decrease of total cholesterol, phospholipids and HDL cholesterol (mean reduction of 12%, 16%, and 9%, respectively). Compared to baseline, apoA-I HDL is unchanged on both days **(Fig. 5).** ApoE-rich HDL_1, in contrast, is clearly reduced on day one and almost completely abolished on day 6 after injection. Therefore, the susceptibility of apoE-rich HDL_1 to the action of HL in LCAT-tg mice is also apparent in mice with expression of HL at levels that are not associated with a profound alteration of plasma lipids.

Figure 6 illustrates the electrophoretic pattern of lipoproteins in normal as well as LCAT-tg mice before and on day 4 after injection of HL-rAdV after analysis by two-dimensional gel electrophoresis. β-Migrating HDL particles are more heterogeneous in LCAT-tg (panel A) than control mice (panel B). On day 4 after injection of HL-rAdV, the HDL in LCAT-tg mice appeared more homogenous than on day 0, indicating that HL overexpression leads to the generation of more homogeneous HDL. In addition (panel B), pre-β_1 nascent HDL particles that are present in control mice are virtually undetectable in LCAT-tg mice (panel A). After injection of HL-rAdV, no increase of pre-β_1 HDL particles was seen in either group of animals.

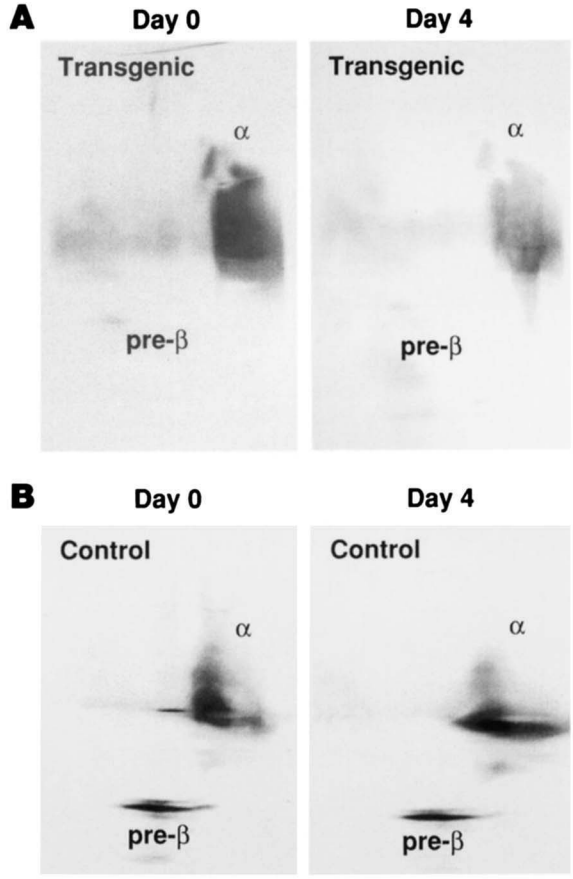

Fig. 6. Two-dimensional gel electrophoresis of whole plasma before or on day 4 after in LCAT-tg and control mice. Ten-µl plasma samples obtained before and on day 4 after injection of HL-rAdV were separated by agarose gel electrophoresis followed by 2–36% PAGE. After Western blotting, membranes were developed with anti-mouse apoA-I antibodies. LCAT-tg mice are shown in panel A, control mice in panel B. The positions of α- and pre-β-migrating lipoproteins are indicated.

DISCUSSION

In the present study we utilize recombinant adenovirus to express high plasma concentrations of HL, a key enzyme involved in HDL metabolism, in both control

and LCAT-tg mice to investigate the proposed coordinate role of HL and LCAT in modulating plasma HDL concentrations, heterogeneity, and function. Specifically, we evaluate whether adenovirus-mediated expression of HL reverses LCAT-induced changes in the plasma concentrations and heterogeneity of mouse HDL as well as provides insights into potential mechanisms leading to the enhanced susceptibility of LCAT-tg mice compared to control mice for developing diet-induced atherosclerosis (26).

Adenovirus-mediated expression of HL led to significant reductions in the plasma concentrations of total cholesterol, phospholipids, HDL-cholesterol, and apoA-I in both control and LCAT-tg mice. However, the HL-mediated decrease in HDL cholesterol as well as apoA-I was more significant in control mice when compared to LCAT-tg mice. Furthermore, despite similar levels of HL expression, the reduction of the apoA-I HDL in LCAT-tg mice was significantly less than in control or HL knockout mice (45). Thus, a subset of HDL in LCAT-tg mice appear to be resistant to the in vivo action of a major enzyme necessary for normal HDL metabolism.

Several mechanisms may account for the reduced action of HL on LCAT-tg HDL. The classical role of HL in HDL metabolism has been that of hydrolyzing triglycerides and phospholipids, a process which appears to be influenced by HDL size (14) and apolipoprotein composition (46). Compared to control mice, HDL in homozygous LCAT-tg mice is less dense and cholesterol ester-enriched. While apoA-I and apoA-II levels are increased by only about 30% over control mice, total cholesterol levels are almost 3-fold higher in LCAT-tg mice (25). Therefore, in LCAT-tg mice, HDL is larger and has a diminished protein/cholesterol ratio (25). HDL particle size (47) and cholesterol content (47, 48) have been demonstrated to influence HDL apolipoprotein conformation. Recent studies also suggest that the lipid composition of the HDL surface and core influences its hydrolysis by hepatic lipase (49, 50). Thus, the altered composition found in LCAT-tg mouse HDL (25) may interfere with the classical function of HL in HDL triglyceride and phospholipid hydrolysis.

Alternatively, the ability to participate in HL/receptor-mediated uptake, a process which may be independent of the lipolytic function of the enzyme, may be compromised in LCAT-tg mouse HDL. Recent studies involving CHO cells (51) as well as the expression of catalytically inactive HL in HL-knockout mice (52) have suggested a role of HL in HDL metabolism independent of its lipolytic function that is similar to the proposed role of LPL in receptor-mediated uptake of apoB-containing lipoproteins (53). The changes in LCAT-tg mouse HDL particle size, composition and/or apolipoprotein conformation may preclude this potentially important function of HL in HDL metabolism. Alternatively, the abundance of LCAT protein that is found to be mainly associated with the apoA-I particles (25) could interfere with the association of HL with LCAT-tg mouse HDL. The disruption of HDL metabolism in LCAT-tg mice on a regular chow diet by either reduced HL-mediated HDL lipolysis and/or HL/receptor-mediated lipoprotein uptake could ultimately disrupt the process of reverse cholesterol transport, one of several important mechanisms by which HDL may modulate the development of atherosclerosis. Under these circumstances the pro-atherogenic lipoprotein profile of LCAT-tg mice on a high-fat diet could be further exacerbated.

Our in vivo findings of a more pronounced reduction of apoE-containing HDL, in comparison to apoA-I HDL by HL are consistent with in vitro studies by Thuren, Sisson, and Waite (54) which demonstrated that HL hydrolysis of phospholipids and triglycerides was enhanced by apoE and that apoE-rich HDL particles are better substrates for HL than apoE-poor HDL (55). The authors suggested that the activation of HL by apoE may be due to direct protein protein interaction (55). Thus, the apoE-containing particles may not only be better substrates for the hydrolytic action of HL but also for the receptor-mediated nonlipolytic function of HL in the clearance of lipoproteins. The partial correction of the heterogenous HDL profile present in LCAT-tg mice (25) emphasizes the important coordinate role of HL and LCAT in altering HDL particle size and composition.

Further characterization of HDL by two-dimensional gel electrophoresis demonstrated the presence of pre-β_1 HDL particles in control but not LCAT-tg mice. This finding is consistent with the studies of Francone et al. (56) who demonstrated that the additional presence of the human LCAT gene led to diminished concentrations of pre-β_1 HDL in mice transgenic for human apoA-I alone or apoA-I plus apoA-II. Previous in vitro studies have demonstrated that HL can only generate pre-β_1 HDL from triglyceride-rich HDL_2 (15) or when combined with VLDL and CETP (16, 57). The inability of HL to generate nascent HDL particles in LCAT-tg mice may be due to the altered lipid and apolipoprotein composition present in LCAT-tg HDL (26), in addition to the absence of CETP (5) and the resulting triglyceride-poor HDL. The important role of CETP in the generation of pre-β_1 HDL is also apparent from mice transgenic for apoA-I alone or both apoA-I and CETP (58). The additional presence of CETP in the apoAI/CETP transgenic mice resulted in the generation of dense HDL as well as to a significant increase in nascent, pre-β_1 HDL. The markedly reduced levels of pre-β_1

HDL seen in two-dimensional electrophoresis of our LCAT-tg mouse plasma suggests either abnormal generation or increased catabolism of these nascent HDL particles.

Our combined studies demonstrate that LCAT overexpression alone alters the composition and heterogeneity of HDL and that HL can partially normalize this altered lipoprotein profile in LCAT-tg mice. HL preferentially reduces the amount of apoE-rich HDL_1 in LCAT-tg mice resulting in the formation of a homogenous profile consisting primarily of apoA-I HDL. However, overexpression of LCAT also leads to the absence of pre-β_1 HDL as well as the formation of apoA-I HDL which is resistant to the action of HL. Thus, a subset of HDL in LCAT-tg mice appear to be resistant to the in vivo action of a major enzyme necessary for normal HDL metabolism and reverse cholesterol transport. Our studies establish the important coordinate role that LCAT and HL have in modulating HDL particle heterogeneity and function and suggest altered HDL function as a potential mechanism leading to enhanced atherosclerosis in LCAT transgenic mice.

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