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Adenoviral expression of human lecithin-cholesterol acyltransferase in nonhuman primates leads to an antiatherogenic lipoprotein phenotype by increasing high-density lipoprotein and lowering low-density lipoprotein

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Abstract

Lecithin-cholesterol acyltransferase (LCAT), a key enzyme in high-density lipoprotein (HDL) metabolism, has been proposed to have atheroprotective properties by promoting reverse cholesterol transport. Overexpression of LCAT in various animal models, however, has led to conflicting results on its overall effect on lipoproteins and atherosclerosis. In this study, the effect of overexpression of LCAT in nonhuman primates on lipoprotein metabolism is examined. Human LCAT was expressed with adenovirus in squirrel monkeys (n = 8), resulting on day 4 in a 22-fold increase of LCAT activity (257 ± 23 vs 5618 ± 799 nmol mL^{-1} h^{-1}, $P < .0001$). At its peak, LCAT was found to nearly double the level of HDL cholesterol from baseline (113 ± 7 vs 260 ± 24 mg/dL, $P < .01$). High-density lipoprotein formed after treatment with the adenovirus was larger in size, as assessed by fast protein liquid chromatography (FPLC) analysis. By kinetic studies, it was determined that there was a decrease in apolipoprotein (Apo) A-I resident time (0.373 ± 0.027 vs 0.685 ± 0.045 d^{-1}, $P < .0001$) and almost a doubling in the ApoA-I synthetic rate (22 ± 2 vs 41 ± 3 mg kg^{-1} d^{-1}, $P < .0001$), but no overall change in ApoA-I levels. In addition, increased expression of LCAT was associated with a 37% reduction of ApoB levels (12 ± 1 vs 19 ± 1 mg/dL, $P < .05$) due to increased low-density lipoprotein catabolism (fractional catabolic rate = 1.7 ± 0.1 d^{-1} in controls vs 4.2 ± 0.3 d^{-1} in LCAT-treated group, $P < .05$). In summary, overexpression of LCAT in nonhuman primates leads to an antiatherogenic lipoprotein profile by increasing HDL cholesterol and lowering ApoB, thus making LCAT a potential drug target for reducing atherosclerosis.
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1. Introduction

Lecithin-cholesterol acyltransferase (LCAT), a plasma enzyme produced by the liver, catalyzes the conversion of cholesterol to cholesteryl esters on lipoproteins by the transacylation of fatty acid from the sn-2 position of phosphatidylcholine to the 3-hydroxyl group on the A-ring of cholesterol [1]. Most LCAT activity is found on high-density lipoprotein (HDL), but approximately 30% is also on apolipoprotein (Apo) B–containing lipoproteins [2]. Because of the increased hydrophobicity of cholesteryl ester compared with cholesterol, cholesteryl ester formed by LCAT on the surface of lipoprotein particles partitions into the neutral lipid core of lipoproteins. This has a profound effect on lipoprotein structure, particularly HDL; it converts the nascent discoidal-shaped HDL to the mature spherical-shaped α-migrating form of HDL.

The physiologic consequences of LCAT on atherosclerosis, however, have not been definitively established. A long-standing hypothesis is that LCAT is antiatherogenic because it promotes the reverse cholesterol transport pathway, the pathway by which excess cellular cholesterol is returned to the liver for excretion [3]. This potentially occurs by 2 mechanisms. First, LCAT increases the level of HDL, which in itself may increase the flux of cholesterol from cells by increasing the amount of extracellular acceptors of cholesterol. Second, the esterification of cholesterol by LCAT on HDL would be predicted to limit the spontaneous back exchange of cholesterol from HDL to cells and to instead promote the net delivery of cholesterol to HDL and then to the liver. In support of this model, the level of LCAT activity, in some studies, has been shown to be positively correlated with HDL cholesterol (HDL-C) and appears to be

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inversely related to the risk of coronary heart disease (CHD) [4]. In addition, patients with a genetic deficiency of LCAT have a profound decrease in HDL-C; but paradoxically, these patients do not appear to have a significantly increased risk of CHD [5,6]. This may be due, at least in part, to the fact that LCAT deficiency also leads to a decrease in low-density lipoprotein (LDL) cholesterol [7], possibly as a consequence of the decreased formation of cholesteryl esters on HDL, which is normally transferred from HDL to LDL and other ApoB containing lipoproteins by the cholesteryl ester transfer protein (CETP).

Various animal models of both the absence and overexpression of LCAT have been described [4,8-13], but the effect of LCAT on lipoproteins and atherosclerosis varies depending on the animal model. In mice, overexpression of LCAT results in an increase in the level of a large lipid-rich form of HDL and accelerates atherosclerosis. In contrast, increased LCAT expression in rabbits also raises HDL-C but markedly decreases atherosclerosis [14]. Mice, unlike rabbits and humans, lack CETP, which causes excess cholesteryl esters to accumulate on HDL in LCAT transgenic mice [15]. This results in a large dysfunctional form of HDL, with decreased ability to deliver cholesterol to the liver [15]. In contrast, when LCAT was expressed in transgenic mice that also express CETP, LCAT was found line in rabbits [8,14] to decrease atherosclerosis. Interestingly, LCAT K/O mice also have a decreased propensity for atherosclerosis [16].

In this study, we examined the effect of LCAT in nonhuman primates, namely, squirrel monkeys. Because of the conflicting animal and genetic studies, it is important to establish the effect of LCAT in another animal model, which more closely resembles humans in their lipoprotein metabolism. Squirrel monkeys have been commonly used to investigate lipoprotein metabolism, as well as the impact of various drug therapies on plasma lipids [17,18]. Squirrel monkeys, like humans, also contain CETP; but they do not have the very high levels found in rabbits [19] and contain, like humans, ApoA-II, which is not present in rabbits [20]. Furthermore, squirrel monkeys among New World monkeys have the greatest propensity to develop diet-induced atherosclerosis and have also been found to spontaneously develop atherosclerosis [21]. In this study, human LCAT was overexpressed in squirrel monkeys using adenovirus, which was found to raise HDL-C and lower ApoB. Results from this study raise the possibility of LCAT as a target for the development of new drugs for modulating lipoprotein metabolism and reducing atherosclerosis.

2. Materials and methods

2.1. Animals

Sixteen adult male squirrel monkeys (*Saimiri sciureus*), ranging in weight from 850 to 1200 g, were fed with Fiber-Plus Monkey Diet 5049 (PMI Nutrition, St Louis, MO). The animals used in these studies were maintained in accordance with the National Institutes of Health *Guide for the Care of Laboratory Animals*, protocol H-0059/H-0059R1.

2.2. Generation of recombinant adenovirus

A full-length human wild-type LCAT and luciferase complementary DNAs were subcloned into a shuttle vector (pAdl2HL) containing cytomegalovirus promoter/enhancer fragment (*Hin*dII-*Pbs*l,−601 to f52 base pairs) promoter elements, as well as the SV40 polyadenylation signal. Recombinant adenovirus encoding luciferase (rLucif-AdV) and LCAT (rLCAT-AdV) was generated after cotransfection of pAdl2LCAT and pJM17 (Ad5 genome) in 293 cells, as previously described [22], propagated in 293 cells, and purified by cesium chloride density ultracentrifugation. The purified virus was then titered and diluted in 0.2% albumin (Sigma Chemical, St Louis, MO) before infusion into the animals; 2×10^{10} plaque-forming units of either rLCAT-AdV or rLucif-AdV was infused in each monkey by tail venipuncture.

2.3. Analysis of lipids and apolipoproteins

Blood was collected after overnight fasting either by tail venipuncture or from an indwelling catheter into tubes containing 0.1 mol/L EDTA, centrifuged for 15 minutes, and stored at 4°C before analysis. Total cholesterol, triglycerides, phospholipids, free cholesterol, ApoA-I, and ApoB were determined using enzymatic or immunoturbidimetric kits from Sigma-Aldrich (St Louis, MO) and Wako Chemicals (Richmond, VA). High-density lipoprotein cholesterol was measured after dextran sulfate (Ciba-Corning, Medfield, MA) precipitation [23]. Plasma lipoproteins were further analyzed by fast protein liquid chromatography (FPLC) by gel filtration chromatography using 2 Superose 6 HR 10/30 columns connected in series (GE Healthcare Biosciences Corp, Piscataway, NJ). Lipoproteins were eluted at a constant flow rate of 0.3 mL/min with phosphate-buffered saline (PBS) buffer containing 1 mmol/L EDTA and 0.02% sodium azide.

2.4. LCAT activity assay

The LCAT activity was determined by the formation of cholesteryl esters using proteoliposomes containing ApoA-I as substrate [24].

2.5. Isolation of lipoproteins

High-density lipoprotein (d = 1.063-1.21 g/mL) and LDL (d = 1.030-1.050 g/mL) were isolated from monkey plasma by sequential ultracentrifugation, followed by dialysis at 4°C against PBS containing 0.01% (wt/vol) EDTA. Agarose gel electrophoresis and FPLC analysis were used to confirm the purity of each lipoprotein fraction.

2.6. In vivo metabolic studies

The ^{131}I-ApoA-I HDL was prepared by a modification of the iodine monochloride method [25]. Lyophilized purified human apo A-I was dissolved in a 6-mol/L guanidine-HCl, 1-mol/L glycine (pH 8.5) buffer containing 1 mCi of $Na^{131}I$;

iodinated after adding 0. 33 mmol/L iodine monochloride; and then dialyzed. The ^{131}I-labeled ApoA-I was then reassociated with HDL from either untreated squirrel monkey plasma or rAdV-LCAT modified plasma for 30 minutes at 37°C and extensively dialyzed at 4°C against PBS containing 0.01% (wt/vol) EDTA. All radiolabeled HDL preparations were analyzed by FPLC and native agarose gel to confirm the purity and integrity.

Low-density lipoprotein isolated by ultracentrifugation from squirrel monkeys was dialyzed against 1 mol/L glycine (pH 10.0) before iodination using a modification of the iodine monochloride method [25]. Briefly, 1 mCi of ^{125}I was added to LDL rapidly followed by 0.33 mmol/L iodine monochloride with no vortexing. After dialysis, the final LDL preparations were then analyzed by FPLC and agarose gel electrophoresis to assess the integrity and purity of the labeled LDL.

All radiolabeled lipoproteins were filter sterilized through a 0.22-μm filter before injection into the femoral artery. Sequential blood samples of less than 50 μL were obtained for the kinetic analysis at the indicated times from an indwelling catheter or by tail vein venipuncture. Multiexponential functions were fit to the plasma decay curves with the use of WINSAAM program. Residence times were obtained from the areas under the plasma decay curves for the first 72 hours, and the fractional catabolic rate (FCR) was calculated as the reciprocal of the residence time.

2.7. Statistical analysis

Unless otherwise indicated, all values are expressed as mean ± SEM. Comparisons between groups of mice were made using 2-tailed Student *t* test for independent samples.

3. Results

3.1. Plasma LCAT activity levels

Four days after injection of adenovirus containing human LCAT transgene (rLCAT-AdV) into squirrel monkeys, a marked increase in LCAT activity was observed (Table 1). Compared with a control group treated with an adenovirus encoding luciferase (rLucif-AdV), there was approximately a 22-fold increase in LCAT activity by day 4, which returned to baseline by day 8.

3.2. Effect of LCAT on plasma lipids and lipoproteins

The increase of LCAT activity in the rLCAT-AdV–treated group was associated with a significant increase in total plasma cholesterol and HDL-C, as well as a reduction in ApoB (Table 1). No significant changes in lipids and lipoproteins were observed in the control group treated with rLucif-AdV, indicating that adenoviral infection in itself did not significantly alter lipoprotein metabolism by nonspecific liver damage. Compared with baseline results on day 0, LCAT expression significantly increased total cholesterol from 178 ± 11 to 325 ± 20 mg/dL by day 4 ($P < .001$). Approximately 90% of the increase in total cholesterol was due to an increase in cholesteryl esters, which nearly doubled by day 4 ($P < .001$). Almost all of the increase in total cholesterol was also associated with HDL, which increased 2.3-fold on day 4 ($P < .001$). No significant change was observed in the level of non–HDL-C. No change was also observed in the plasma concentration of ApoA-I; but interestingly, the level of plasma ApoB decreased by approximately 37% on day 4 ($P = .001$). As shown in Fig. 1, the effect of the adenoviral expression of LCAT on plasma cholesterol reached a plateau on day 4 and started to decrease by days 7 to 8.

Increased LCAT activity was also associated with a change in the size of HDL as assessed by FPLC analysis (Fig. 2). A significant shift of HDL toward larger-sized particles was observed (peak elution shifted from 30 mL elution volume to 27 mL). Consistent with the LCAT-induced increase of HDL-C observed after dextran sulfate precipitation (Table 1), there was also an overall increase in cholesterol from the FPLC fractions for HDL from the rLCAT-AdV–treated monkeys. A relatively small LDL peak was observed from both the control and experimental groups; but the rLCAT-AdV–treated monkeys appeared to have slightly lower cholesterol content, although no difference was observed for non–HDL-C levels (Table 1).

3.3. Effect of LCAT on apolipoprotein kinetics

To determine the mechanism for the effect of LCAT on lipid and lipoprotein changes, a kinetic analysis of

Table 1
Plasma levels of LCAT, lipids, and lipoproteins.

	LCAT act	TC	TG	PL	FC	CE	ApoA-I	HDL	ApoB	Non-HDL
	(nmol mL^{-1} h^{-1})					(mg/dL)				
LCAT d0 (n = 11)	257 ± 23	178 ± 11	67 ± 12	245 ± 21	42 ± 3	136 ± 8	134 ± 8	113 ± 7	19 ± 1	65 ± 7
LCAT d4 (n = 11)	5618 ± 799*	325 ± 20*	35 ± 6*	196 ± 9†	57 ± 5*	268 ± 18*	136 ± 6	260 ± 24*	12 ± 1†	65 ± 13
Luc d0 (n = 9)	251 ± 13	166 ± 10	41 ± 4	199 ± 10	35 ± 2	130 ± 8	135 ± 6	103 ± 9	20 ± 2	63 ± 11
Luc d4 (n = 9)	257 ± 12	171 ± 10	43 ± 3	210 ± 11	39 ± 3	132 ± 8	132 ± 4	113 ± 13	20 ± 2	57 ± 8

LCAT act indicates LCAT activity; TC, total cholesterol; TG, triglycerides; PL, phospholipids; FC, free cholesterol; CE, cholesteryl ester; d0, before the study; d4, at the peak expression day, at a steady-state level. *Non–HDL-C* was defined as TC − HDL.

* Increased where *P* is less than .00001 compared with day 0.

† Decreased where *P* is less than .05 compared with day 0.

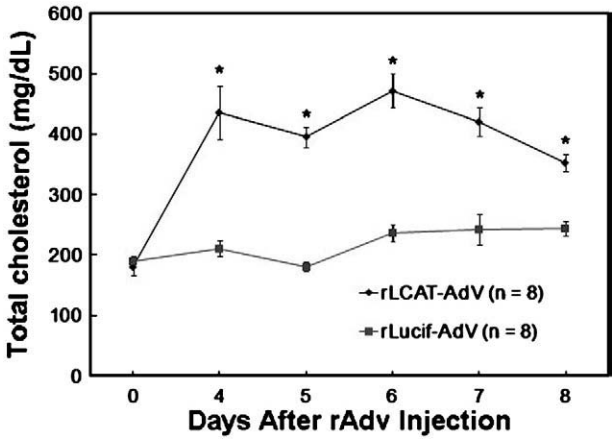

Fig. 1. Effect of LCAT on plasma total cholesterol. rLCAT-AdV (♦) or rLucif-AdV (■) was injected into squirrel monkeys, and plasma total cholesterol was measured from baseline to 8 days postinjection. Results are expressed as mean ± SEM (n = 8 per group) (*$P < .01$ compared with day 0).

radioiodinated ApoA-I associated with HDL and radioiodinated ApoB on LDL was performed (Fig. 3). We first established that potential liver damage from the adenoviral infection did not significantly alter apolipoprotein kinetic parameters. As can be seen in Fig. 3A, treatment of squirrel monkeys with luciferase by rLucif-AdV did not change the catabolism of radioiodinated ApoA-I on HDL compared with a control group not treated with adenovirus over the 8 days of the study (FCR = 0.373 ± 0.027 pools per day). Afterward, the effect of LCAT expression on ApoA-I catabolism was assessed (Fig. 3B). This was done on day 4 after treatment with rLCAT-AdV because the effect of LCAT on lipoproteins appeared to peak at that time and to be relatively stable for at least the following 3 days, as shown by the total plasma cholesterol levels (Fig. 1). The LCAT-treated group was divided into 2 arms: 1 arm received HDL isolated from control monkeys that had not received any adenovirus, whereas the other arm received HDL isolated from monkeys previously treated 4 days earlier with rLCAT-AdV. Before injection, the 2 different HDL preparations were associated with radioiodinated ApoA-I. The objective of this experiment was to test if ApoA-I from either the native control HDL or HDL that had been modified by LCAT would have a different catabolic or production rate (PR). Compared with rLucif-AdV–treated monkeys that had received native control HDL (FCR = 0.373± 0.027 pools per day), monkeys treated with rLCAT-AdV showed a similar increased rate of catabolism for both the native control HDL (FCR = 0.685 ± 0.045 pools per day) and the LCAT-modified HDL (FCR = 0.731 ± 0.077 pools per day). Analysis of the estimated PR (Table 2) that was calculated 72 hours after injection of the labeled HDL indicated that there was also a significant increase (1.9-fold) in the ApoA-I PR for both groups. The rLucif-AdV–treated monkeys that had received the control native HDL had an increased PR of 41 ± 3 mg kg^{-1} d^{-1}, which was similar to the group receiving LCAT-modified HDL (PR = 39 ± 5 mg kg^{-1} d^{-1}) and much higher compared with the rLucif-AdV control group (PR = 22 ± 2 mg kg^{-1} d^{-1}, $P < .05$). The overall plasma level of ApoA-I, however, did not change after LCAT treatment (Table 1); so the increased catabolism of ApoA-I from the LCAT treatment was fully compensated by an increase in the PR of ApoA-I.

Because we observed a decrease in plasma ApoB levels (Table 1), we also investigated the effects of increased LCAT activity on LDL kinetics (Fig. 3C). Compared with the rLucif-AdV treatment group (FCR = 1.7 ± 0.1 pools per day), monkeys treated with rLCAT-AdV showed a significant increase in the catabolism of ApoB (FCR = 4.2 ± 0.3 pools per day, $P < .01$). The ApoB PR was also slightly increased (1.4-fold) compared with the control group, which apparently was negated by the much larger relative increase in LDL catabolism, resulting in lower ApoB plasma levels (Table 1).

To gain further insight into the effect of LCAT on the catabolism of HDL and LDL, samples collected at various time points after injection with rLCAT-AdV or Lucif-AdV were analyzed by FPLC (Fig. 4). For the Lucif-AdV–treated group, there was no apparent change in the size distribution of the HDL or LDL tracer over time (data not shown). In other words, there was a gradual decrease in the total radioactive counts of the radiolabeled lipoproteins with no shift in the lipoprotein size distribution with time. The same was also true for radiolabeled LDL given to the rLCAT-AdV–treated group (Fig. 4A). In contrast, in the case of radiolabeled HDL given to the rLCAT-AdV–treated monkeys, most of the ApoA-I tracer on HDL shifted after 30 minutes to a larger-sized HDL particle, as assessed by FPLC analysis (Fig. 4B). Thereafter, the tracer remained on

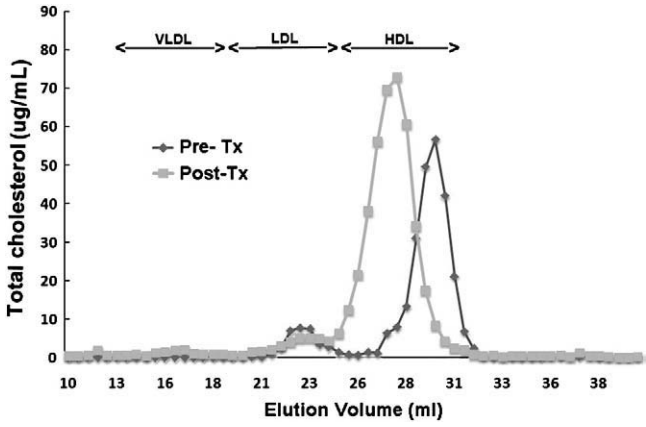

Fig. 2. Elution profiles of lipoproteins on gel permeation chromatography. Plasma pool from squirrel monkeys (n = 4) that received rLCAT-AdV were fractionated by using 2 Superose 6 HR 10/30 columns connected in series. Each fraction was analyzed for the total cholesterol content (micrograms per milliliter). The dark line (♦) represents the pretreatment value, whereas the lighter line (▒) represents the result from plasma collected on day 4 after injection of adenovirus. Arrows indicate elution position for major lipoproteins. Results are expressed as mean ± SEM.

this larger HDL particle and then gradually decreased over time, which is consistent with a direct catabolism of the larger form of HDL produced by the increased expression of LCAT.

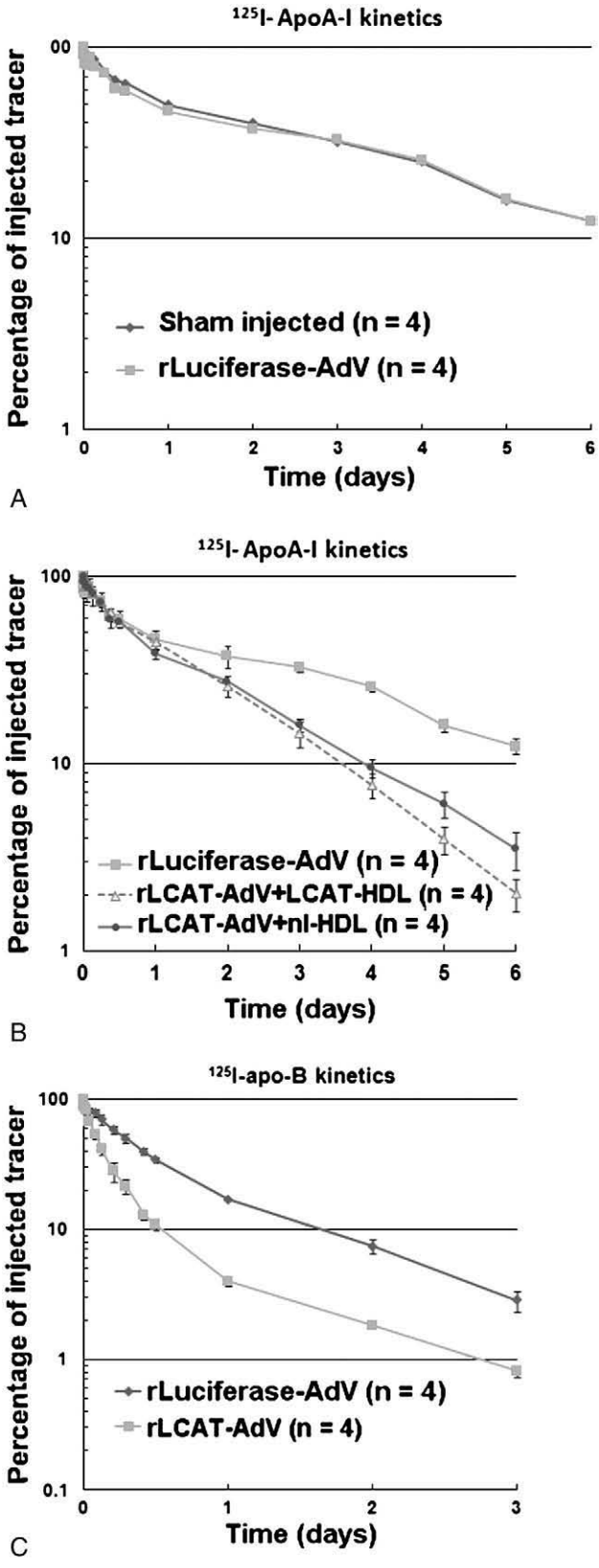

Table 2
Kinetic parameters of HDL and LDL metabolism in nonhuman primates

AdV	ApoA-I (mg/d)	FCR (d^{-1})	PR (mg kg^{-1} d^{-1})
rLCAT-AdV (n = 4)	134 ± 8	0.685 ± 0.045*	41 ± 3*
rLucif-AdV (n = 4)	132 ± 4	0.373 ± 0.027	22 ± 2
AdV	ApoB (mg/d)	FCR (d^{-1})	PR (mg kg^{-1} d^{-1})
rLCAT-AdV (n = 4)	12 ± 2*	4.2 ± 0.3*	22 ± 3
rLucif-AdV (n = 4)	20 ± 2	1.7 ± 0.1	16 ± 2

rLucif-rAdV, control group; rLCAT-rAdV, LCAT adenovirus–injected monkeys.
* *P* less than .05 compared with control.

4. Discussion

There is growing evidence that increasing the level of HDL will reduce the risk for developing CHD [26,27]. The main mechanism that has been proposed for the atheroprotective effect of HDL is its ability to increase the reverse cholesterol transport pathway [28], although HDL has also been reported to have other beneficial effects on atherosclerosis [29,30]. Unfortunately, there are a limited number of drugs for raising HDL; and not all approaches that increase HDL may be antiatherogenic [31]. Interfering with the hepatic uptake of cholesterol from HDL, for example, will markedly raise HDL-C in SR-BI K/O mice but leads to increased atherosclerosis [32]. Similarly, there is concern that drug strategies based on the inhibition of CETP, which also raises HDL-C, may not be atheroprotective because of decreased delivery of cholesterol to the liver by HDL [33]. In contrast, LCAT is believed to raise HDL by enhancing its formation rather than decreasing its catabolism and thus would be predicted to increase overall reverse cholesterol transport. Evidence against a possible atheroprotective role of LCAT is the fact that patients with a genetic defect in LCAT do not appear to have a marked increased risk of CHD [34]; and in mice, increased expression of LCAT leads to increased atherosclerosis, but this may be due to their lack of CETP [15]. One advantage of the present study is that LCAT was expressed in squirrel monkeys, which because they have CETP are likely to be a better animal model than mice for human atherosclerosis [21]. Another strength of this study is that not only the level of HDL was examined but also the effect

Fig. 3. Kinetic analysis of ApoA-I and ApoB in nonhuman primates. A, The ApoA-I kinetic decay curve of control monkeys (◆) vs rLucif-AdV–injected monkeys (▒). B, The kinetic decay curve of ApoA-I in monkeys (n = 4 per group) injected with either rLucif-AdV (▒) or with LCAT-AdV (Δ and ●). Within the group treated with rLCAT-AdV, 4 monkeys received LCAT-modified HDL (Δ); and 4 monkeys received control native HDL (●). C, ApoB kinetics in monkeys (n = 4) treated with either rLCAT-AdV (▒) or with rLucif-AdV (n = 4) (◆). Results are expressed as mean ± SEM.

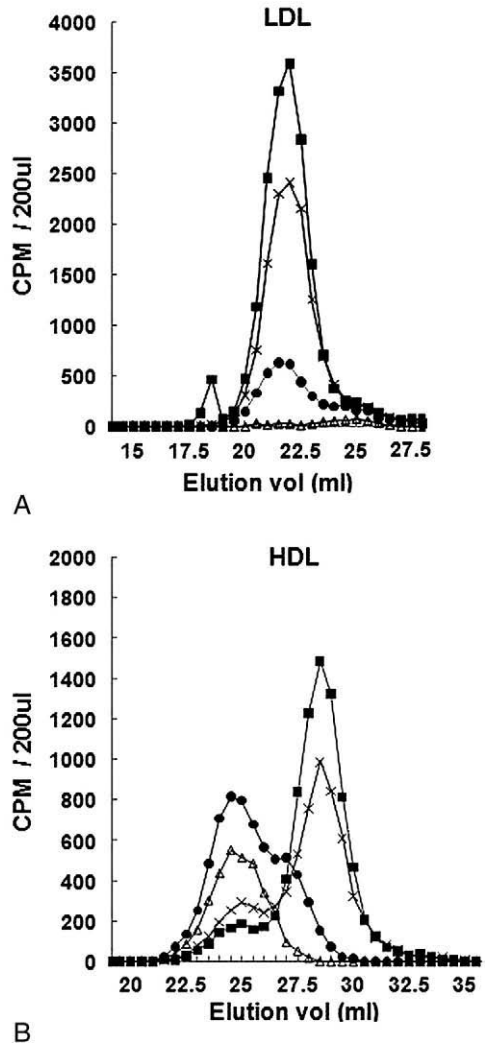

Fig. 4. Elution profiles of radiolabeled lipoproteins on gel permeation chromatography. A plasma pool from 4 monkeys that received either radiolabeled LDL (A) or HDL (B) 4 days after treatment with rLCAT-AdV was fractionated by using 2 Superose 6 HR 10/30 columns connected in series, and radioactive counts were determined. Samples were analyzed at 2 minutes (■), 30 minutes (X), 7 hours (●), and 24 hours (Δ) after injection of the tracer. Results are expressed as mean ± SEM.

of LCAT on the catabolism, and PR of ApoA-I and ApoB was assessed.

Consistent with what has been observed in all other animals models studied to date [35-37], increased LCAT expression in monkeys led to a significant increase in HDL-C; the level of HDL-C more than doubled from 113 to 260 mg/dL (Table 1). ApoA-I, the main protein component of HDL, however, did not significantly change, which would indicate that increased LCAT activity promotes the formation of a lipid-enriched form of HDL. This is consistent with the observed increase in the size of HDL by FPLC (Figs. 2 and 4). As would be expected, a large increase in cholesteryl esters was also observed, which was found to be mostly associated with HDL (Table 1). Similar changes in HDL from humans have been shown to be atheroprotective [38,39]. For example, large lipid-rich HDL, such as the HDL_2 subfraction, has been shown in numerous epidemiologic studies to correlate better with CHD risk reduction than total HDL-C [38,39]. The effect of LCAT on lipoproteins still partially persisted by day 8 even after LCAT returned to baseline most likely because of the lag in time needed for the catabolism of HDL and the clearance of cholesteryl esters formed by LCAT.

Based on previous studies in other animal models [40,41], the effect of LCAT on ApoB concentration appears to better correlate with atherosclerosis than changes in the level of HDL-C. In mice fed a proatherogenic diet, overexpression of LCAT led to an increase in ApoB and atherosclerosis [15]. When LCAT was overexpressed in CETP transgenic mice, there was reduced levels of ApoB and reduced atherosclerosis [8]. Overexpression of LCAT in rabbits, which have CETP, also resulted in reduced ApoB and reduced atherosclerosis [14,40]. Overexpression of LCAT in rabbits that lack LDL receptor, however, did not alter ApoB levels and was not atheroprotective [42], indicating that the LDL receptor is necessary for LCAT to lower ApoB levels, most likely through its ability to remove LDL that has been enriched in cholesteryl esters by LCAT and CETP. In this study, LCAT was also found to significantly lower ApoB levels in squirrel monkeys, but did not significantly change non–HDL-C levels (Table 1). Because squirrel monkeys normally contain relatively low levels of ApoB compared with humans, the effect of the marked increase of LCAT on decreasing ApoB may, however, not be generalized to humans. Lecithin-cholesterol acyltransferase overexpression was also found to reduce total triglycerides by almost 50% (Table 1), which may be the consequence of the exchange of cholesteryl esters for triglycerides on LDL by CETP.

Another potential consideration in predicting the effect of LCAT overexpression on atherosclerosis is the mechanism behind any change in lipoprotein pool size [43]. The pool of HDL and LDL can be altered by a change in the rate of production or catabolism, or both. Because adenoviral constructs only transiently express proteins, the kinetic parameters of ApoA-I and ApoB may be altered if the lipoprotein changes induced by LCAT are not in steady state. To minimize this potential problem, the kinetic analysis on ApoA-I and ApoB was done from day 4 to day 7 when the effect of LCAT on total cholesterol appeared to peak and remained relatively stable (Fig. 1). During this period, LCAT overexpression was associated with an increase in both the production and catabolism of ApoA-I, which balanced each other out (Table 2), resulting in no change in the plasma concentration of ApoA-I, although HDL-C increased. The mechanism for the change in the catabolism of ApoA-I by LCAT is not known but is likely related to the HDL lipid compositional differences (Table 1) caused by LCAT [23]. The effect of LCAT overexpression on the production of ApoA-I is likely an indirect effect, such as an alteration in the hepatic expression of genes like ApoA-I and ABCA1 that are involved in the initial secretion of nascent HDL. In addition,

the increased esterification of cholesterol by LCAT could also potentially facilitate the extracellular formation and stabilization of HDL.

Several studies support the notion that increased production of ApoA-I or increased formation of HDL-C, as was observed in this study, should be antiatherogenic. Transgenic mice and rabbits that overexpress the gene for ApoA-I are protected against atherosclerosis [44,45]. Similarly, increased hepatic expression of ABCA1, which increases the formation of HDL by promoting the initial lipidation of ApoA-I, is atheroprotective [46-48]. Infusion of HDL or ApoA-I mimetic peptides, which would simulate increased production of ApoA-I, is also atheroprotective [49,50].

The effect of increasing the catabolism of HDL or ApoA-I on atherosclerosis is not as clear and appears to depend on other factors. Increased catabolism of HDL, leading to a decrease in HDL pool size as occurs in patients with hypertriglyceridemia [51], is associated with increased CHD risk. Delaying HDL catabolism, however, such as when SR-B1 gene is deleted in the liver, leads to a marked increase in HDL-C and increased atherosclerosis because it interferes with one of the last steps in the reverse cholesterol transport pathway, the hepatic uptake of cholesterol [32]. Mice overexpressing LCAT in the absence of CETP, which have increased atherosclerosis, were found to have delayed catabolism of ApoA-I because the altered lipid composition of HDL interferes with its hepatic uptake [15]. In this study, LCAT increased HDL-C, although it also increased ApoA-I catabolism, presumably because the CETP-mediated transfer of cholesteryl esters from HDL to ApoB–containing lipoproteins prevented the formation of a dysfunctional lipid-rich form of HDL that was found in mice [15]. Additional studies will have to be performed to fully understand the impact of ApoA-I kinetic parameters on atherosclerosis; but overall, these findings are consistent with a model whereby increased catabolism of ApoA-I may not necessarily promote atherosclerosis, so long as the overall HDL-C pool size is not significantly decreased. In this situation, it would be predicted that the net flux of cholesterol from peripheral cells to HDL and then to the liver by the reverse cholesterol transport pathway may in fact be increased.

Lecithin-cholesterol acyltransferase was also found in this study to lower ApoB levels by markedly increasing its catabolism (Table 2), which would be expected to be atheroprotective. Statins, currently our most effective drug for treating atherosclerosis, decrease LDL largely by increasing its catabolism [52,53]. The mechanism for how LCAT modulates ApoB metabolism is not known, but one possible mechanism may be related to its ability to alter the lipid composition of LDL. Besides the CETP-mediated transfer of cholesteryl esters produced by LCAT from HDL to LDL, a significant fraction of cholesteryl esters can also be directly made by LCAT on LDL [54].

In summary, increased expression of human LCAT in squirrel monkeys by adenovirus was associated with increased HDL-C and decreased ApoB levels. These results along with the potential beneficial changes in kinetic parameters for ApoA-I and ApoB suggest that, like in rabbits, increased expression of LCAT in an animal model that also expresses CETP is most likely antiatherogenic. The results from this study, therefore, suggest that drug strategies to increase LCAT activity may be useful for reducing atherosclerosis because of its combined beneficial effect on HDL and LDL metabolism.

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