Overexpression of Lipoprotein Lipase in Transgenic Rabbits Inhibits Diet-induced Hypercholesterolemia and Atherosclerosis*

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Lipoprotein lipase (LPL) is a key enzyme in the hydrolysis of TG-rich lipoproteins. To elucidate the physiological roles of LPL in lipid and lipoprotein metabolism, we generated transgenic rabbits expressing human LPL. In postheparinized plasma of transgenic rabbits, the human LPL protein levels were about 650 ng/ml, and LPL enzymatic activity was found at levels up to 4-fold greater than that in nontransgenic littermates. Increased LPL activity in transgenic rabbits was associated with as much as an 80% decrease in plasma triglycerides and a 50% decrease in high density lipoprotein-cholesterol. Analysis of the lipoprotein density fractions revealed that increased expression of the LPL transgene resulted in a remarkable reduction in the level of very low density lipoproteins as well as in the level of intermediate density lipoproteins. In addition, LDL cholesterol levels in transgenic rabbits were significantly increased. When transgenic rabbits were fed a cholesterol-rich diet, the development of hypercholesterolemia and aortic atherosclerosis was dramatically suppressed in transgenic rabbits. These results demonstrate that systemically increased LPL activity functions in the metabolism of all classes of lipoproteins, thereby playing a crucial role in plasma triglyceride hydrolysis and lipoprotein conversion, and that overexpression of LPL protects against diet-induced hypercholesterolemia and atherosclerosis.

Lipoprotein lipase (LPL)* plays a crucial role in lipid metabolism and transport by catalyzing the hydrolysis of triglyceride-rich (TG-rich) lipoproteins such as chylomicrons and very low density lipoproteins (VLDL). Through the hydrolysis of TG in these particles, LPL converts these lipoproteins to denser lipoproteins such as chylomicron remnants, intermediate density lipoprotein (IDL), and low density lipoproteins (LDL) (1–3). This process generates free fatty acids (FFA), which are taken up and used for metabolic energy or stored as TG after reesterification and also results in the generation of surface remnants, which give rise to high density lipoproteins (HDL). It has been suggested that LPL influences not only plasma TG levels but also plasma HDL levels (4).

LPL is mainly produced by mesenchymal cells such as adipose and muscle cells and then transported to the luminal surface of the vascular endothelium, where it is bound to heparan sulfate proteoglysns (HSPG). Small amounts of LPL are also present in other types of tissues, including the adrenals, brain, lung, and spleen (5). Furthermore, LPL is also expressed by macrophages and smooth muscle cells in atherosclerotic lesions (6, 7), suggesting that LPL modulates vascular functions and may be involved in atherogenesis. Elucidation of the precise roles of LPL in atherosclerosis has been compounded by the fact that LPL has multiple functions in lipoprotein metabolism through its catalytic properties and acts as a ligand for the LDL receptor-related protein (8) or a bridge between lipoproteins and HSPG (9). In humans, it has been found that familial LPL deficiency resulted in premature atherosclerosis associated with chylomicronemia (10). Several lines of evidence suggest that increased LPL activity may be either proatherogenic or antiatherogenic, depending on the site of LPL expression. For example, systemically incremental expression of LPL in either LDL receptor or apoE knock-out (KO) mice showed protection against atherosclerosis (11, 12), whereas in bone marrow-transplanted LDL receptor or apoE KO mice, macrophage-derived LPL enhanced the lesion development (13, 14). To further investigate the mechanisms of the LPL role in lipoprotein metabolism and its multiple functions in atherosclerosis, we attempted to generate transgenic rabbits that express elevated levels of LPL in either a systemic or macrophage-specific fashion.

Rabbits have been used successfully to express a number of transgenes (15). As an experimental model for the study of lipid metabolism and atherosclerosis, rabbits have several advantages over mice (16). Rabbits have higher levels of apoB-containing lipoproteins than mice, a lipoprotein profile more like that of humans, and a pattern of hepatic apoB100 and intestinal apoB48 synthesis resembling that of humans. Like humans, and unlike mice, rabbits have cholesteryl ester transfer
protein, which has been showed to exert dual (proatheroscle-
rosis as well as antiatherosclerosis) functions in the develop-
ment of atherosclerosis (17). The larger plasma volumes in
rabbits permit metabolic studies of lipoprotein subclasses and
facilitate lipoprotein turnover studies. Furthermore, rabbits
are very susceptible to the development of atherosclerosis, with
the lesions resembling those seen in human atherosclerosis.
For these reasons, we created human LPL transgenic rabbits
and characterized the effects of the constitutively increased
systemic expression of human LPL on plasma lipoproteins in
the transgenic rabbits. We found that essentially all classes of
lipoproteins were affected by the presence of elevated levels of
LPL. Finally, LPL transgenic rabbits showed protection against
cholesterol diet-induced hypercholesterolemia and athero-
resis.

EXPERIMENTAL PROCEDURES

Generation of Human LPL Transgenic Rabbits—Transgenic rabbits
were produced by the method described previously (15). In this study,
specific pathogen-free Japanese white rabbits (Tokyo Laboratory An-
imal Co., Tokyo, Japan) were used. Zygotes were microinjected with
human LPL cDNA transgenic constructs under the control of the
chicken β-actin promoter (18), designated as CBA-hLPL. All animal
experiments were performed with the approval of the Animal Research
Committee of the University of Tsukuba. The presence of the transgene
in founder transgenic rabbits was examined by Southern blotting using
a human LPL cDNA clone (19).

Northern Blot Analysis—Total RNA was isolated from various tis-
sues of the transgenic rabbits (aorta, heart, lung, kidney, adrenal, liver,
spinal, intestine, stomach, muscle, thymus, brain, bone marrow, and
testis) using Trizol reagent (Life Technologies, Inc.). Thioglycolate-
elicted peritoneal macrophages and alveolar macrophages were col-
clected as described (20). Ten μg of RNA was denatured in the presence
of dimethyl sulfoxide, and glyoxal was subjected to electrophoresis in a
1.2% agarose gel transferred to a Nitrin nylon membrane (Schleicher & Schuell Inc.). The membrane was hybridized with the 32P-labeled human LPL cDNA probe.

Analysis of LPL Protein and Enzymatic Activity—Postheparin plasma was prepared from a blood sample taken 10 min after a bolus
injection of heparin at a dose of 30 units/kg, body weight, and prehe-
parin plasma was collected from a blood sample taken before the injec-
tion of heparin. For determination of the amount of LPL protein, an
enzyme-linked immunosorbent assay was performed by using anti-
human LPL monoclonal antibody (21). The enzymatic activity of LPL
was determined using a 14C-labeled triolein emulsion substrate. The
presence of human LPL in postheparin plasma was further studied by
Western blot analysis using monoclonal antibody against human LPL
(5SD).

Plasma Lipid and Lipoprotein Analysis—The plasma lipid and li-
oprotein profiles of LPL transgenic rabbits were compared with those
of age-matched control littersmates at the age of 4–5 months. Blood was
collected in both the fasting and postprandial states to evaluate the
lipoprotein changes caused by LPL expression. Blood samples were
collected from rabbits after 16 h of food deprivation. A second bleeding
was performed after these animals were fed a chow diet for 6 h after
overnight fasting. Plasma TG, total cholesterol (TC), HDL cholesterol
levels were measured using Wako assay kits. The plasma nonesterified fatty acids were determined using an hLPL cDNA probe revealed that hLPL in trans-
genric rabbits was expressed in multiple tissues such as muscle,
aorta, heart, adipose tissue, spleen, kidney, and lung (Fig. 1A).

Western blot analysis revealed that hLPL was present in the
postheparin plasma but not in the preheparin plasma, indicating
that human LPL can be associated with HSPG on the endothelial cell surface. The molecular mass of hLPL produced by
transgenic rabbits was ~60 kDa, the same size as LPL found
in human postheparin plasma (Fig. 1B).

Effect of Increased LPL Expression on Plasma Lipid Lev-
elnts—As summarized in Table III, CBA-hLPL transgenic rabbits shown a marked reduction of plasma lipids; there were 70 and
80% reductions of TG in the fasting and postprandial states compared
with the levels in control rabbits. Total cholesterol contents were slightly reduced in transgenic rabbits due to 56% (fasting) and 59% (postprandial) reductions of HDL-C and the
disappearance of VLDDL and LDL cholesterol (also see Fig. 3). In addition, the plasma nonesterified fatty acids were signifi-
cantly decreased in fasting transgenic rabbits compared with
nontransgenic littersmates (Table III).

Lipoprotein profiles on agarose gels stained with Fat Red 7B showed that preβ VLDL lipoproteins were consistently absent in
both fasting and postprandial transgenic rabbits (Fig. 2A). We also compared the apoB levels of transgenic rabbits with
those of nontransgenic rabbits in the fasting and postprandial conditions. Transgenic rabbits had lower levels of apoB100 in

| Table I | Number of animals used to generate transgenic rabbits |
|---|---|
| Donor rabbits (total) | 297 |
| Zygotes recovered (total) | 4045 |
| Zygotes implanted | 2664 |
| Recipient rabbits | 116 |
| Pregnancy rate (%) | 48 (41%) |
| Bunnies born (total) | 166 |
| Transgenic positive founders (surviving) | 7 (3) |
Transgenic Rabbits Expressing Human Lipoprotein Lipase

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**TABLE II**

| Human LPL protein contents and enzymatic activity in transgenic (Trg) rabbit postheparin plasma |
|---------------------------------|-------------------------|-------------------------|
| Sex   | Transgene copy no. | hLPL protein mass (ng/ml) | Enzymatic activity (μmol FFA/ml/min) |
|-------|-------------------|---------------------------|-----------------------------------|
| CBA-hLPL Trg (F0)<sup>a</sup> | | | |
| L01   | Male 3           | 395                       | 0.455                             |
| L04   | Male 2           | 157                       | 0.234                             |
| L17   | Male 10          | 652                       | 0.897                             |
| Control rabbits (n = 8) | NA<sup>d</sup> | | 0.244 ± 0.08                      |

<sup>a</sup> F0, transgenic founder rabbits.  
<sup>b</sup> For control rabbits, four females and four males were analyzed.  
<sup>c</sup> Not applicable.  
<sup>d</sup> Rabbit endogenous LPL protein was not detected by the antibody to human LPL in this assay.

**A. Northern Blot Analysis**

![Northern blot analysis of hLPL tissue distribution](image)

**B. Western Blot Analysis**

![Western blot assay of hLPL in postheparin plasma](image)

**Fig. 1.** Northern blot analysis of hLPL tissue distribution (A) and Western blot assay of hLPL in postheparin plasma (B). Human LPL tissue distribution in CAB-hLPL transgenic was examined by Northern blot analysis as described under “Experimental Procedures.” Preheparin and postheparin plasma from CAB-hLPL transgenic rabbit and a control littermate were resolved by 10% SDS-polyacrylamide gel electrophoresis. The separated proteins were then electrophoretically transferred to a sheet of nitrocellulose membrane and incubated with monoclonal antibody against human LPL (5D2). Human LPL in postheparin plasma of the transgenic rabbit was ~60 kDa. Bovine milk LPL (lot number 45H8025; Sigma) was loaded as a positive control.

the fasting state than nontransgenic rabbits. Six hours after animals were fed a chow diet, apoB100 levels in control rabbits were elevated, and a small amount of apoB48 was also detected. However, in transgenic rabbits, apoB100 levels were consistently lower than those of controls in both the fasting and fed states, and apoB48 was not detected (Fig. 2B).

**Analysis of Lipoproteins and Apolipoproteins—** Plasma lipoproteins from F1 male transgenic and nontransgenic rabbits were separated by sequential density gradient ultracentrifugation, resolved further by 1% agarose gel electrophoresis, and stained with Fat red 7B. The major lipoprotein classes in each fraction were as follows: d < 1.006 g/ml, pre-β-migrating VLDL; d = 1.006–1.02 g/ml, IDL; d = 1.02–1.04 g/ml, large LDL; d = 1.04–1.06 g/ml, β-migrating small LDL and α-migrating HDL1; d = 1.06–1.08 and 1.08–1.10 g/ml, HDL1 and HDL2; and d = 1.10–1.21 g/ml, HDL3. Quantitative analysis of TC and TG in each fraction consistently showed three prominent changes in the relative amounts of different lipoproteins. First, transgenic rabbits had remarkably low levels of VLDL and IDL. As shown in Fig. 3, cholesterol contents in the VLDL and IDL fractions of transgenic rabbit lipoproteins were not detectable, and TG contents were 10 and 20%, respectively, of those of nontransgenic rabbits. Second, transgenic rabbits had a significant increase of LDL (d = 1.04–1.06 g/ml) cholesterol level, and third, the HDL content, especially the content of HDL<sub>2</sub> (d = 1.06–1.10 g/ml), was notably reduced in transgenic rabbits compared with nontransgenic littersmates. We also assessed the distribution of TG and TC within plasma lipoprotein fractions by HPLC to confirm the results obtained from the ultracentrifugation studies. On a standard chow diet, there were striking reductions of VLDL and HDL levels accompanied by dramatic increases in LDL-C in the transgenic rabbits compared with age- and sex-matched nontransgenic rabbits (Fig. 4A).

The distribution of apolipoproteins among the various density lipoprotein fractions was examined by the reaction of specific antibodies with Western blots of lipoproteins that had been resolved by agarose gel electrophoresis (Fig. 5). The observed decreases in VLDL and IDL contents in transgenic rabbits were confirmed by notable reductions in the amount of apoB and apoE, whereas the increase of small density LDL (d = 1.06–1.10 g/ml) content was accompanied by increased apoB and apoE. It seems that apoB/apoE-rich β-migrating particles in transgenic rabbits were shifted from the lower density range (d = 1.006–1.04 g/ml) to the high density range (d = 1.06–1.08 g/ml), presumably due to enhanced lipolysis. In addition, the apoAI content was decreased in HDL<sub>1–2</sub> fractions in transgenic rabbits compared with controls.

**Cholesterol-rich Diet-induced Hypercholesterolemia—** When fed a cholesterol-rich diet for 16 weeks, transgenic rabbits showed a consistent suppression of the diet-induced hypercholesterolemia throughout the experiment (Fig. 6A). Triglyceride levels were not significantly affected by cholesterol-rich diet consumption in either transgenic or nontransgenic rabbits, while transgenic rabbits had a consistently lower level of plasma TG than nontransgenic rabbits (Fig. 6A). In cholesterol-fed animals, the hypercholesterolemia is characterized by an increase in remnant lipoproteins (β-VLDL (d < 1.006 g/ml), IDL (d = 1.006–1.06 g/ml)), and the appearance of cholesterol-rich lipoprotein referred to as HDLc (d = 1.02–1.06 g/ml), which had α mobility but lacked the apoB (28). As shown in Fig. 6B, these remnant lipoproteins (d < 1.006 and d = 1.02–1.06 g/ml) were dramatically diminished in transgenic rabbits. The reduction of remnant lipoproteins was associated with decreased levels of apoB and apoE in transgenic rabbits (Fig. 7). The HDL contents in transgenic rabbits were reduced, as were the levels of apoAI (Fig. 7). HPLC analysis showed that plasma remnant lipoproteins in the transgenic rabbits were dramatically reduced (Fig. 4B), as evaluated in an ultracentrifugation assay.

**Electron Microscopic Evaluation of Lipoprotein Particles—** The average diameters of the apoB-containing lipoproteins in each density fraction and the size distribution are illustrated in Fig. 8, A and B. There was little difference in lipoproteins within d = 1.006–1.02 and d = 1.02–1.04 g/ml between transgenic and control rabbits. The mean sizes of lipoproteins within d < 1.006 and d = 1.04–1.6 g/ml in transgenic rabbits were relatively larger than those in control rabbits due to the appearance of a small number of large particles in transgenic rabbits. The large particles in the d < 1.006 g/ml fraction were postulated to be chylomicrons (>90 nm in diameter), and the
small particles were thought to be VLDL and remnant lipoproteins (<90 nm), whereas in the δ = 1.04–1.6 g/ml fraction, the large lipoproteins (>14 nm) were thought to be LDL, and the small lipoproteins (<14 nm) were thought to be cholesterol-rich HDLc as evaluated by their electrophoretic mobility and apolipoprotein content. Since the absolute number of the particles in each fraction was reduced in transgenic rabbits compared with control rabbits, a relative increase of these large particles in each fraction was reduced in transgenic rabbits compared with control rabbits. This suggests that hLPL was bound to the plasma lipoproteins. Density gradient fractions from chow-fed rabbits were collected by ultracentrifugation, and cholesterol and TG contents were quantitated as described under “Experimental Procedures.” The combined recovery for each animal averaged ~80% of the total amount in plasma.

**DISCUSSION**

LPL is the rate-limiting enzyme for hydrolysis of lipoprotein TG and has been hypothesized to exert either pro- or antiatherogenic effects, depending on its localization (reviewed in Ref. 15 and 16). In order to test this hypothesis, it is essential to establish appropriate animal models such as transgenic animals that overexpress the LPL gene in a tissue-specific fashion. For this purpose, we generated transgenic rabbits that have increased LPL expression either under the control of a systemic expression promoter as shown here or a macrophage-specific expression promoter.

The rabbit was specifically selected for this undertaking because of its usefulness in investigating the relationship of lipoprotein and cholesterol metabolism to the development of atherosclerosis (see reviews in Refs. 15 and 16), and the cDNA sequence of human LPL shows about 80% homology to that of rabbit LPL. Successful generation of transgenic rabbit lines required extensive effort and was made more difficult by a high rate of variability in the number of fertilized zygotes recovered from each donor rabbit, the small number of pups, and the relatively high rate of neonatal mortality, including cannibalism, as shown in Table I. A transgenic line (L17) was established and used for the current study. In this study, we characterized the plasma lipid levels and lipoprotein levels in CBA-hLPL transgenic rabbits fed both normal chow and cholesterol-rich diets. In CBA-hLPL transgenic rabbits, human LPL was expressed in multiple tissues (Fig. 3B), resulting in 4-fold higher LPL activity in postheparin plasma (but not in preheparin plasma) than that in nontransgenic rabbits. This suggests that hLPL was bound to the vascular surface via HSPG.

On a standard chow diet, increased LPL activity in CBA-hLPL transgenic rabbits led to a remarkable reduction of the

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**TABLE III**

**Plasma lipids in CBA-hLPL transgenic and nontransgenic littermate rabbits**

| Triglycerides | Total cholesterol | HDL-C | NEFA* |
|--------------|------------------|-------|-------|
| mg/dl        | mg/dl            | mg/dl | μg/liter |
| Fasted       | Fed              | Fasted | Fed    | Fasted | Fed          |
| Control (n = 15) | 42 ± 6.6 | 51.4 ± 8.8 | 32.5 ± 9.2 | 33.6 ± 9.2 | 16 ± 7.8 | 18 ± 7.1 | 5.46 ± 0.5 | 1.65 ± 1.0 |
| Transgenic (n = 10) | 12.2 ± 5.6b | 10.2 ± 6.6b | 27 ± 13.3 | 28 ± 15.5 | 7 ± 3.5b | 7.3 ± 3.4b | 3.25 ± 1.0b | 0.73 ± 0.5 |

*Nonesterified fatty acids.

b p < 0.05 versus control.

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**Fig. 3. Quantitation of cholesterol and triglyceride contents in plasma lipoproteins.** Density gradient fractions from chow-fed rabbits were collected by ultracentrifugation, and cholesterol and TG contents were quantitated as described under “Experimental Procedures.” The combined recovery for each animal averaged ~80% of the total amount in plasma.

**A. Agarose gel electrophoresis**

**B. SDS-PAGE**

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**Fig. 2. Lipoprotein profiles (A) and plasma apoB levels (B).** Plasma (2 μl) from rabbits that had fasted for 16 h (A, left panel) or that had been fed a chow diet for 6 h after fasting (A, right panel) was electrophoresed on a 1% agarose gel and stained for neutral fat with Fat red 7B. For comparison of plasma apoB levels (fasting versus fed) between CBA-hLPL transgenic and nontransgenic rabbits, density fractions of δ < 1.006 g/ml were separated by ultracentrifugation and resolved by 3.5% SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue.

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2 J. Fan, unpublished data.

3 H. Deng, H. Unoki, and J. Fan, unpublished data.
levels of plasma TG, especially in VLDL and IDL, suggesting that LPL activity determines the rate of hydrolysis of lipoprotein-triglycerides. This was found to be true in both the fasting and postprandial states in transgenic rabbits. Reduced VLDL and IDL levels indicate that these particles were rapidly cleared from the plasma, possibly through the receptor-mediated uptake by the liver (29). It has been suggested that after hydrolysis of VLDL triglycerides by LPL, exposure of unreactive apoE, and conformational changes in apoB-100 may enhance binding of VLDL to the LDL receptor (30, 31). Merkel et al. (32) showed that hepatic expression of LPL increases VLDL mass clearance in LPL-KO mice. It is currently unknown whether hLPL functions in transgenic rabbits exclusively through its catalytic activity or also through its noncatalytic function or both (33). In accordance with the reduction in plasma TG in transgenic rabbits, low TG levels and the absence of TC in plasma VLDL and IDL were associated with reduced apoB and apoE contents, as demonstrated by sequential density ultracentrifugation. The increase in LDL content, especially that of the smaller and denser LDL (d = 1.04–1.06 g/ml) was associated with increased apoB and apoE in these particles, suggesting that LPL enhances the conversion of VLDL to small density LDL (18). In this respect, LPL resembles hepatic lipase, which is also involved in the conversion of IDL to LDL (19). Despite increased TG hydrolysis by LPL, plasma FFA levels in transgenic rabbits were lower than those in nontransgenic rabbits, suggesting that LPL may enhance the uptake of FFA in the peripheral tissues such as adipose tissue and muscles. In LPL transgenic mice driven by a muscle-specific promoter, the mouse creatine kinase promoter, Levak-Frank et al. (34) demonstrated that LPL augmented the FFA uptake in the muscles. However, these transgenic mice with high expression of LPL suffered from body weight loss and premature death, presumably caused by myopathy, in contrast to our transgenic rabbits.

Initially, we hypothesized that overexpression of LPL in transgenic rabbits might enhance the production of HDL particles; however, we found that HDL levels were actually re-

Fig. 4. HPLC assay of lipoprotein profiles of transgenic (red line) and control (blue line) rabbits fed a chow diet (A) or cholesterol diet (B). Tri
glycerides and cholesterol contents were analyzed as described under “Experimen
tal Procedures.”

Fig. 5. Apoprotein analysis. Plasma lipoproteins from a chow-fed control (upper panel) and a CBA-hLPL transgenic (lower panel) rabbit were separated by sequential density ultracentrifugation using the density ranges shown above the gels. An equal volume of each fraction was resolved by electrophoresis in a 1% agarose gel. Lipoproteins were visualized using Fat red 7B staining, and apolipoproteins were identified by immunoblotting with specific antibodies against apoB, apoE, and apoAI. α and β indicate electrophoretic mobility.
duced in transgenic rabbits. One possible explanation for this phenomenon is that LPL may hydrolyze TG-rich HDL directly or enhance selective uptake of HDL cholesterol ester by altering HDL particle components (35, 36), which eventually leads to increased HDL catabolism. Increased LPL activity in transgenic rabbits may also enhance the receptor-mediated uptake of VLDL particles, thereby reducing the availability of VLDL-derived surface components for HDL biosynthesis. It is also likely that smaller HDL in transgenic rabbits transferred their cholesterol to apoB-containing particles rapidly through high activity of cholesteryl ester transfer protein in rabbits. This mechanism would account for the observed reduction of total HDL cholesterol. In support of this possibility, incubation of cultured hepatic cells with LPL-treated HDL resulted in enhanced uptake of cholesteryl esters from HDL (36). This effect is dependent on cell surface heparan sulfate proteoglycans but independent of lipolysis and of endocytosis mediated by the LRP or LDL receptor (36).

In rabbits on a cholesterol-rich diet, overexpression of hLPL dramatically inhibited the elevation of plasma cholesterol levels by reducing β-VLDL, IDL, and HDLc, the major atherogenic lipoproteins in cholesterol-fed animals. Rabbits are highly susceptible to diet-induced hyperlipidemia and are especially prone to remnant lipoprotein accumulation. This unique sensitivity to dietary cholesterol has been considered to be caused by extremely low hepatic lipase expression, which is an important determinant in remnant lipoprotein metabolism (37). The finding that increased expression of LPL in transgenic rabbits inhibited the accumulation of plasma remnant lipoproteins may suggest that excess LPL can compensate for hepatic lipase deficiency in rabbits, consequently accelerating the clearance of remnant lipoproteins. This result is in agreement with the observation by Zsigmond et al. (38), who reported that injection of an adenovirus-LPL gene into ApoE KO and LDL receptor KO mice leads to a reduction of VLDL/chylomicron remnant cholesterol and TG.

The current study, together with consistent results in transgenic mice (18), indicates that LPL protects against diet-induced hypercholesterolemia and that the lipoprotein profiles (reduced remnant lipoproteins) of transgenic rabbits reflect antiatherogenesis. We found that transgenic rabbits had virtually no gross lesions, as defined by Sudanophilic areas, whereas nontransgenic rabbits had lesions in up to 20% of the aortic arch. However, this result may not be surprising, because 1) transgenic rabbits had consistently lower cholesterol levels than nontransgenic rabbits, and 2) such a “low level

FIG. 6. Suppression of the cholesterol diet-induced hypercholesterolemia in CBA-hLPL transgenic rabbits. Plasma TC and TGs were determined as described under “Experimental Procedures.” A, values are expressed as mean ± S.D. versus control. 2 μl of plasma from each animal was electrophoresed on a 1% agarose gel and stained for neutral lipids with Fat red 7B (B, left). Density gradient fractions from cholesterol-diet fed rabbits were collected by ultracentrifugation and cholesterol contents were quantitated as described under “Experimental Procedures” (B, right). Representative data from each group are shown.
(<200 mg/dl) of plasma cholesterol is not atherogenic even in normal rabbits based on our previous studies. Therefore, it remains unknown whether LPL protects against diet-induced atherosclerosis independently of its lipid-lowering effects (possibly through the enhancement of remnant clearance in the liver). In future studies, we need to examine this hypothesis by feeding transgenic rabbits a diet containing high amounts of cholesterol to compare the lesions in transgenic and nontransgenie rabbits.

**FIG. 7.** Plasma apolipoprotein analysis of cholesterol-fed rabbits. Plasma lipoproteins from a control (upper panel) and a CBA-hLPL transgenic (lower panel) rabbit fed a cholesterol-rich diet for 16 weeks were separated by sequential density ultracentrifugation using the density ranges shown above the gels. An equal volume of each fraction was resolved by electrophoresis in a 1% agarose gel. Lipoproteins were visualized using Fat red 7B staining, and apolipoproteins were identified by immunoblotting with specific antibodies against apoB, apoE, and apoAI. α and β indicate electrophoretic mobility.

**FIG. 8.** Electron microscopic analysis of lipoproteins. Lipoproteins in individual fractions were examined by negative stain electron microscopy and representative micrographs were shown in A. Each sample was diluted as indicated. Distribution of the sizes of lipoproteins was illustrated in B. The average particle diameter and the total particles counted were listed below the name of each lipoprotein class.
Transgenic Rabbits Expressing Human Lipoprotein Lipase

In conclusion, we have successfully generated transgenic rabbits expressing human LPL. Increased expression of LPL in transgenic rabbits profoundly influenced all classes of lipoproteins, thereby mediating plasma cholesterol homeostasis. The findings presented here confirm the central role of LPL in the hydrolysis of TG-rich lipoproteins and extend our understanding of the regulatory function of LPL in HDL metabolism, LDL conversion, and remnant lipoprotein clearance. Importantly, human LPL transgenic rabbits are highly resistant to diet-induced hypercholesterolemia and atherosclerosis. These results imply that stimulation of LPL expression may be used as a potentially adjunctive therapy for treatment of postprandial hyperlipidemia (47).

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