Overexpressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice

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Abstract **Lipoprotein lipase (LPL) is known to play a crucial role in lipoprotein metabolism by hydrolyzing triglycerides; however its role in atherogenesis has yet to be determined. We have previously shown that low density lipoprotein receptor knockout mice overexpressing LPL are resistant to diet-induced atherosclerosis due to the suppression of remnant lipoproteins. Plasma lipoproteins and atherosclerosis of apolipoprotein (apo) E knockout mice which overexpress the human LPL transgene (LPL/APOEKO) were compared with those of control apoE knockout mice (APOEKO). On a normal chow diet, LPL/APOEKO mice showed marked suppression of the plasma triglyceride levels compared with APOEKO mice (54 vs. 182 mg/dl), but no significant changes in plasma cholesterol and apoB levels. Non-high density lipoproteins (HDL) from LPL/APOEKO mice had lower triglyceride content, a smaller size, and a more positive charge compared with those from APOEKO mice. Cholesterol, apoA-I, and apoA-IV were increased in HDL. Although both groups developed hypercholesterolemia to a comparable degree in response to an atherogenic diet, the LPL/APOEKO mice developed 2-fold smaller fatty streak lesions in the aortic sinus compared to the APOEKO mice. In conclusion, overproduction of LPL is protective against atherosclerosis even in the absence of apoE.**—Yagyu, H., S. Ishibashi, Z. Chen, J. Osuga, M. Okazaki, S. Perrey, T. Kitamine, M. Shimada, K. Ohashi, K. Harada, F. Shionoiri, N. Yahagi, T. Gotoda, Y. Yazaki, and N. Yamada. **Overexpressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice.** ***J. Lipid Res.*** **1999.** 40: **1677–1685.**

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Lipoprotein lipase (LPL) hydrolyzes triglycerides in plasma lipoproteins such as chylomicrons and very low density lipoproteins (VLDL) and causes a wide variety of alterations in lipoprotein metabolism. These effects include: *i*) the conversion of triglyceride-rich lipoproteins to denser lipoproteins such as chylomicron remnants, intermediate density lipoproteins (IDL), and low density lipoproteins (LDL) *ii*) stimulation of the hepatic removal of the lipolyzed lipoproteins in concert with apolipoprotein (apo) E, and *iii*) transfer of surface components of triglyceride-rich lipoproteins to high density lipoproteins (HDL) (See refs: 1–3 for review).

The effects of LPL on atherosclerosis have been controversial. As atheromatous plaques contain substantial amounts of LPL in situ, Zilversmit (4) proposed that local LPL is atherogenic. Furthermore, LPL mediates the lipolytic conversion of triglyceride-rich lipoproteins to atherogenic cholesterol-rich lipoproteins such as LDL and chylomicron remnants (1–3). Supporting this, LPL deficiency in humans, a common genetic cause of chylomicronemia syndrome, results in very low plasma levels of LDL-cholesterol, and is believed to cause resistance to premature atherosclerosis (1). However, Benlian et al. (5) have recently reported that several LPL-deficient patients have developed relatively advanced atherosclerosis. It has also been reported that even individuals who are heterozygous for LPL mutations are predisposed to premature atherosclerosis (6–9). Furthermore, several clinical studies have shown that fibric acid derivatives induce LPL activity, lower plasma triglycerides, and suppress atherosclerosis (10–12). This leads to the question of what the exact role of LPL in atherosclerosis is as a whole.

In order to address this issue, we have previously established a line of transgenic mice that overexpress LPL ubiquitously (13), and cross-bred these animals to the LDL receptor knockout mice to generate mice that lack

Abbreviations: LPL, lipoprotein lipase; apo, apolipoprotein; APOEKO, apoE knockout mice; LPL/APOEKO, apoE knockout mice overexpressing human lipoprotein lipase, VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LPDS, lipoprotein-deficient serum; LRP, LDL low density lipoprotein receptor-related protein; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; HPLC, high performance liquid chromatography.

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the LDL receptor and overproduce LPL (LPL/LDLRKO) (14). LPL/LDLRKO mice had reduced plasma levels not only of triglycerides but also of total cholesterol, and were very resistant to diet-induced atherosclerosis (14). In this animal model, a subfraction of VLDL was selectively reduced and HDL-cholesterol levels were not changed. Therefore we ascribed the anti-atherogenic effects of the overexpressed LPL to the reduction of remnant lipoproteins. There were not significant alterations in either plasma apoB concentrations or plasma clearance of apoB-containing lipoproteins in LPL/LDLRKO mice. Therefore it is unlikely that the overproduced LPL stimulates the interaction of apoB-containing lipoproteins with hepatic receptors distinct from the LDL receptor, an apparent contradiction to the reported function of LPL in vitro (15–19).

In order to further characterize the effects of the overexpressed LPL on lipoprotein metabolism and atherosclerosis in the setting of apoE deficiency, we have generated APOEKO mice that overexpress LPL (LPL/APOEKO) by cross-breeding to apoE knockout (APOEKO) mice (20, 21), and analyzed their lipoprotein profiles and susceptibility to atherosclerosis as compared to APOEKO mice without the overexpression of LPL.

EXPERIMENTAL PROCEDURES

Mice. The LPL transgenic mice (13) and APOEKO mice (20) were created as described in the indicated references. The LPL transgenic mice express human LPL cDNA under control of a chicken β-actin enhancer/cytomegalovirus (CMV) promoter in a wide variety of tissues such as the heart, skeletal muscle, adipose tissue and, to a lesser extent, in other tissues including the aorta (13). To obtain LPL/APOEKO mice, the LPL transgenic mice were bred to the homozygous APOEKO mice. The resultant offspring, which overexpressed LPL and were obligatorily heterozygous for the mutant apoE locus, were further bred to the homozygous APOEKO mice to obtain homozygous APOEKO mice with or without the LPL transgene. Male offspring of brother–sister matings of each line were used for experiments. PCR was used to assign the genotype. The parental APOEKO mice were F2 hybrids between 129/Sv and C57BL/6, and parental LPL transgenic mice were F2 hybrids between C57BL/6 and DBA/2, which had been back-crossed to C57BL/6 three times. Therefore, 72%, 25%, and 3% of the genetic background of the mice was derived from C57BL/6, 129/Sv, and DBA/2 strains, respectively. Two diets were used: *i*) a normal chow diet (MF diet from Oriental Yeast Co., Tokyo) that contained 5.6% (w/w) fat with 0.09% (w/w) cholesterol was provided, and *ii*) an atherogenic diet: MF diet containing 0.15% (w/w) cholesterol and 15% (w/w) butter. Before killing, mice were anesthetized with pentobarbital. The current experiments were performed in accord with institutional guidelines for animal experiments at University of Tokyo.

Northern blot analysis

Total RNA was isolated from the cells by Trizol (Gibco-BRL) according to the manufacturer's instructions. Ten μg of total RNA was subjected to electrophoresis in 1% agarose gel containing formamide, and transferred to a nylon filter (Hybond N, Amersham Pharmacia). cDNA probes were radiolabeled with [α-^{32}P]deoxy-CTP. After prehybridization for 2 h, blots were hybridized in a Rapidhyb^{R} buffer (Amersham Pharmacia) for 1 h at 65°C with the probes.

Human LPL mass in plasma

After a 16-h fast, heparin (100 units/kg) was injected intravenously as a bolus. After 3 min, post-heparin plasma was taken. Human LPL mass was determined as previously described (22).

Plasma lipids and lipoproteins

Blood was collected from the retro-orbital venous plexus into tubes containing EDTA. VLDL (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml), LDL (d 1.019–1.063 g/ml), HDL (d 1.063–1.21 g/ml), HDL2 (d 1.063–1.125 g/ml), and HDL3 (d 1.125–1.21 g/ml) were isolated from the pooled plasma by sequential ultracentrifugation (23). Total cholesterol and triglyceride concentrations in plasma or lipoprotein fractions were determined enzymatically by kits (Determiner TC555 and Determiner TG555, Kyowa Medex, Tokyo), and their concentrations in original plasma were calculated as mg/dl. Plasma apoB concentrations were measured by single radial immunodiffusion method using a kit designed for measurement of human apoB levels (Apo B plate 'Daiichi'; Daiichi Pure Chemicals, Tokyo, Japan), according to the manufacturer's instruction (24, 25).

Agarose gel electrophoresis of plasma was performed using Universal Gel/8 electrophoresis system (Ciba Corning Diagnostic Corp) according to the manufacturer's instruction.

Immunoblot of apoA-I was performed using an ECL kit and an anti-rat apoA-I antibody as described previously (25).

For apolipoprotein analyses, each lipoprotein fraction was dialyzed against a solution containing 0.15 m NaCl, 1 mm EDTA, and 1 mm PMSF at pH 7.4 and delipidated with ethanol/ether (26). Apolipoproteins in each lipoprotein fraction equivalent to 10 μl of plasma were loaded onto each well of 5–15% gradient SDS/polyacrylamide gel and proteins were visualized by staining with Coomassie Brilliant Blue.

High performance liquid chromatography (HPLC) analyses

HPLC analyses of plasma lipoproteins were performed as described (27, 28). In brief, 100-μl samples of the diluted plasma were applied onto four columns of TSK gel Lipopropak XL (TOSOH, Tokyo) connected in tandem. This system provides high resolution especially in the range of large lipoproteins. The elution was performed at a flow rate of 0.6 ml/min using an TSK eluent LP-2 (TOSOH, Tokyo) and 0.3 ml/min for an enzyme solution (Determiner L TC, Kyowa Medex Co., Tokyo). The detection of cholesterol in a post-column effluent was carried out by A550 following the enzymatic reaction in an on-line system.

Pathology

Atheromatous plaques in the aortas were visualized by staining with Sudan IV as described (29). The luminal side of the stained aortas was photographed. Image capture and analysis was performed using Adobe Photoshop™ 3 image analysis software, as essentially described. The extent of atherosclerosis was determined using selection threshold ranges in the three basic colors and was expressed as the percent of surface area of the entire aorta covered by lesions; this was designated as the en face surface lesion area (30). The cross-sectional lesion area was evaluated according to a modified method of Paigen et al. (31). In brief, the hearts were perfused with saline containing 4% (w/v) formalin and fixed for more than 48 h in the same solution. The basal half of the hearts was embedded in Tissue-Tek OCT compound (Miles, Inc.), and the serial sections were captured using Cryostat (6 μm thick) as described (13). Four sections, each separated by 60 μm, were used to evaluate the lesions: two at the end of the aortic sinus and two at the junctional site of sinus and ascending. The sections were stained with Oil Red O and counterstained with hematoxylin.

Cells

One ml of thioglycolate broth was injected into the peritoneal cavities of mice aged 3 months. After 4 days, the peritoneal cavities were lavaged with 10 ml/mouse of ice-cold saline. The cells were washed three times with PBS and resuspended in DMEM and 2×10^6 cells were plated in one well of 12-well plates (Corning). After incubation at 37°C for 2 h, the non-adherent cells were removed by washing three times with pre-warmed PBS. The adherent cells were incubated with DMEM containing 5 mg/ml of LPDS for 24 h at 37°C in the atmosphere of 5% CO_2 and 95% air unless otherwise stated.

Cholesteryl ester formation assay

VLDL and d < 1.063 g/ml lipoproteins were isolated from the plasma by ultracentrifugation at densities of 1.006 g/ml and 1.063 g/ml, respectively. β-VLDL, apoE- and cholesterol-enriched lipoproteins were isolated from LDLRKO mice fed an atherogenic diet. The lipoproteins were re-floated at the same density and dialyzed against a solution containing 0.15 M NaCl, 1 mM EDTA, and 1 mM PMSF at pH 7.4. After dialysis, the lipoproteins were sterilized by filtration through Milex 0.45 μm and their protein concentrations were measured by method of Lowry et al. (32). Cholesteryl ester formation from [1-^{14}C] oleate was essentially determined as described (33).

Briefly, after incubation with a medium containing 5 mg/ml of LPDS for 24 h, the cells were incubated with a medium containing 12.5 μg/ml of lipoproteins, the [1-^{14}C]oleate-albumin complex, and 5 mg/ml of LPDS at 37°C for 24 h. Plates were washed twice with PBS containing 2 mg/ml BSA and once with PBS. Cholesteryl ester was extracted with hexane–isopropanol 3:2 to which [^{3}H]cholesteryl-oleate and unlabeled oleic acid were added as the internal standard and carrier, respectively. The organic phase was evaporated to dryness under flowing N_2. Cholesterol [1-^{14}C]-oleate was separated on silica-coated TLC plates (#5583, Merck, Germany) using a solvent system composed of heptane–ethyl ether–acetic acid 90:30:1 (v/v/v), their position was identified using a BAS2000 phosphoimager (Fuji Film, Tokyo). The spots corresponding to cholesteryl esters were scraped and their radioactivities were quantified by liquid scintillation. Cellular proteins were dissolved in 0.1 N NaOH and determined by the Lowry method (32).

Statistics

Student's *t*-test was used to compare mean values between two sets of mice.

RESULTS

Expression of the LPL transgene

In order to compare the expression levels of the human LPL transgene in various organs, Northern blot analysis was performed (**Fig. 1**). The human LPL transgene was expressed in skeletal muscle, heart, adipose tissue and, to a lesser extent, in the aorta as previously reported (13).

Plasma lipoprotein profile of mice fed normal chow

Plasma lipid concentrations and LPL mass were compared between APOEKO and LPL/APOEKO mice fed a normal chow diet (**Table 1**). LPL/APOEKO mice had high concentrations of human LPL, while APOEKO mice did not. As expected, LPL/APOEKO mice had 69% lower plasma triglyceride levels than APOEKO mice. Although a relatively large variation exists in the plasma total cholesterol levels in both APOEKO and LPL/APOEKO mice, no significant difference was observed between the two groups. LPL/APOEKO mice had higher plasma HDL cholesterol levels than APOEKO mice, though they were not statistically significant.

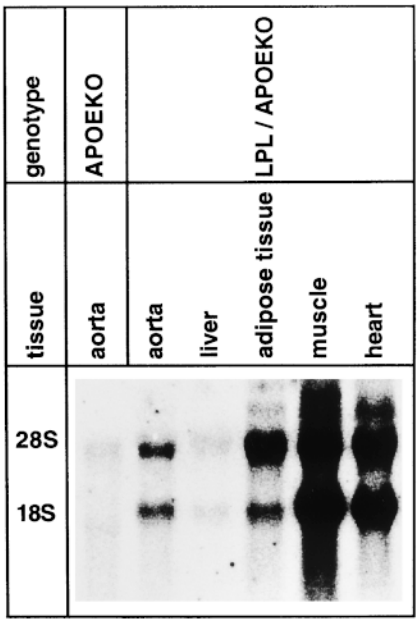

Fig. 1. Expression of human LPL transgene in various tissues of LPL/APOEKO mice. Total RNA was isolated from the indicated organs and subjected to Northern blot analysis.

In order to determine the size distribution of lipoproteins which accumulated in LPL/APOEKO mice, we performed HPLC analyses on the total plasma. The non-HDL cholesterol peak of LPL/APOEKO mice was smaller than that of APOEKO mice (retention time: 46.9 vs. 45.6 min)

TABLE 1. Human LPL mass and lipoproteins in the plasma of LPL/APOEKO and APOEKO mice

	APOEKO	LPL/APOEKO
Normal chow		
n	17	10
Post-heparin LPL (ng/ml)	<20	1288 ± 328
Pre-heparin LPL (ng/ml)	<20	174 ± 40
Total cholesterol (mg/dl)	636 ± 253	654 ± 170
Triglycerides (mg/dl)	182 ± 90	54 ± 16[a]
HDL-cholesterol (mg/dl)	22 ± 12	29 ± 26
ApoB (mg/dl)	131 ± 49	142 ± 28
Atherogenic diet		
n	17	10
Total cholesterol (mg/dl)	1164 ± 409	1129 ± 330
Triglycerides (mg/dl)	206 ± 125	51 ± 9[a]
HDL-cholesterol (mg/dl)	15 ± 8	13 ± 7
ApoB (mg/dl)	198 ± 63	224 ± 63

Blood was collected from the mice before and 8 weeks after consumption of an atherogenic diet. Plasma cholesterol and triglyceride levels were determined enzymatically. HDL cholesterol levels were determined from the HPLC profiles and total cholesterol levels. The mice were 8–12 weeks of age at the start of the feeding. Values are mean ± SD.

[a] $P < 0.01$, APOEKO vs. LPL/APOEKO.

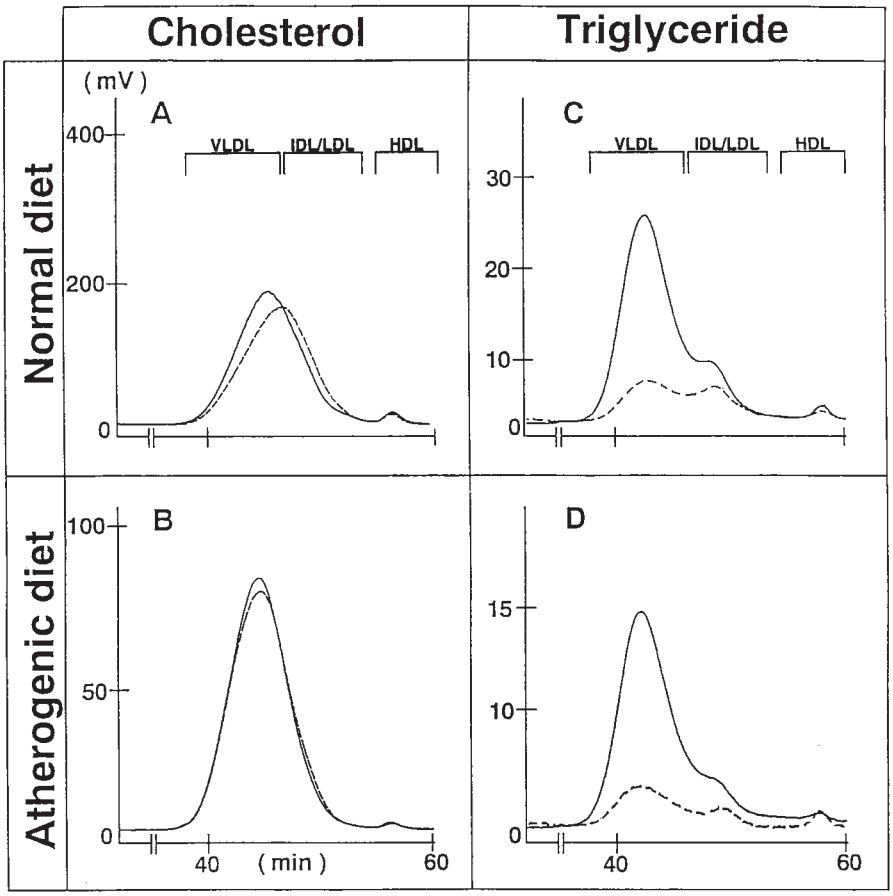

Fig. 2. HPLC lipoprotein profiles of APOEKO (solid line) and LPL/APOEKO (dashed line) mice fed a normal chow (A, C) and atherogenic diet (B, D). Cholesterol (A, B) and triglycerides (C, D) contents were measured enzymatically. The original volume of the plasma applied to the columns was as follows: A, 5 μl; B, 1 μl; C, 20 μl; D, 4 μl.

(**Fig. 2A**), while the size distribution of HDL cholesterol was not different between the two groups. For non-HDL triglycerides, there were two peaks in both types of mice: one peak in the VLDL range and another peak in the IDL/LDL range (Fig. 2C). A significant size reduction was observed in both peaks in LPL/APOEKO mice compared to APOEKO mice. In contrast to the cholesterol profile, the differences in the retention time for each peak were less remarkable between the two groups (47.7 vs. 48.2 mm for the peaks with smaller molecular size and 42.0 vs. 42.7 min for the peaks with larger molecular size).

Agarose gel electrophoresis of the plasma from APOEKO mice showed a broad band whose migratory position was between β and pre-β (**Fig. 3, lane 2**), while LPL/APOEKO mouse plasma had a sharper band with β mobility (Fig. 3, lane 3).

In support of the results of HPLC analyses, lipoprotein analyses by sequential ultracentrifugation showed that more than 90% of total cholesterol was distributed in non-HDL fractions in both types of mice (**Table 2**). VLDL cholesterol was decreased and LDL cholesterol was increased in LPL/APOEKO mice.

Immunoblot analysis revealed that there was an approximately 2-fold increase in the plasma apoA-I levels in LPL/APOEKO mice compared with those in APOEKO mice (data not shown). SDS/PAGE analyses were performed to estimate the distribution of apolipoproteins in lipoprotein fractions (**Fig. 4A** and **B**). ApoB-48 was present in VLDL

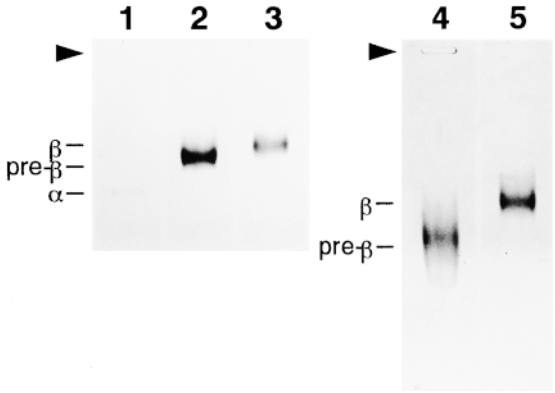

Fig. 3. Agarose gel electrophoresis of lipoproteins from wild-type (lane 1), APOEKO (lane 2) and LPL/APOEKO (lane 3) mice fed a normal chow, and APOEKO (lane 4) and LPL/APOEKO (lane 5) mice fed an atherogenic diet for 8 weeks. Two μl of plasma was applied to Universal Gel/8. Migratory positions for α, pre-β, and β mobility and origin are indicated.

TABLE 2. Distribution of cholesterol between lipoprotein fractions from APOEKO and LPL/APOEKO mice

	VLDL	IDL	LDL	HDL
Normal chow				
APOEKO	523	134	122	10
LPL/APOEKO	479	193	155	32
Atherogenic diet				
APOEKO	614	298	226	25
LPL/APOEKO	520	335	249	25

Lipoproteins were isolated from pooled plasma by sequential ultracentrifugation. Cholesterol content in each lipoprotein fraction was determined enzymatically and expressed as mg/dl of the original plasma. Overall recovery of cholesterol during ultracentrifugation was calculated to be 82–89%.

through LDL, but not in HDL. ApoA-IV and apoA-I were present in all lipoprotein classes. ApoB-48 appears to be decreased in VLDL and increased in LDL fractions in LPL/APOEKO mice compared to the APOEKO mice (compare Fig. 4A with Fig. 4B). LPL/APOEKO mice exhibited a 3-fold increase in apoA-I content in HDL compared to APOEKO mice. Similarly, LPL/APOEKO mice had increased apoA-IV contents in HDL compared to APOEKO mice. The same result was obtained in a separate experiment using different animals (data not shown).

Effects of atherogenic diet on the lipoprotein profile

Both APOEKO and LPL/APOEKO mice were fed an atherogenic diet for 8 weeks, and the changes in lipoprotein profiles and the extent of atherosclerosis were compared between the two types of mice. After 8 weeks of feeding, plasma cholesterol levels increased 1.74-fold in APOEKO and 1.8-fold in LPL/APOEKO mice, with no significant difference between the two groups (Table 1). On the other hand, LPL/APOEKO mice had lower plasma triglyceride levels than APOEKO mice (51 vs. 206 mg/dl, $P < 0.01$). Upon HPLC lipoprotein analyses, the non-HDL cholesterol peaks from the mice fed an atherogenic diet were larger than those from mice fed a normal chow diet (Fig. 2B). However, no differences were observed in either the retention time or the amount of non-HDL cholesterol between the two groups. Notably, non-HDL tri-

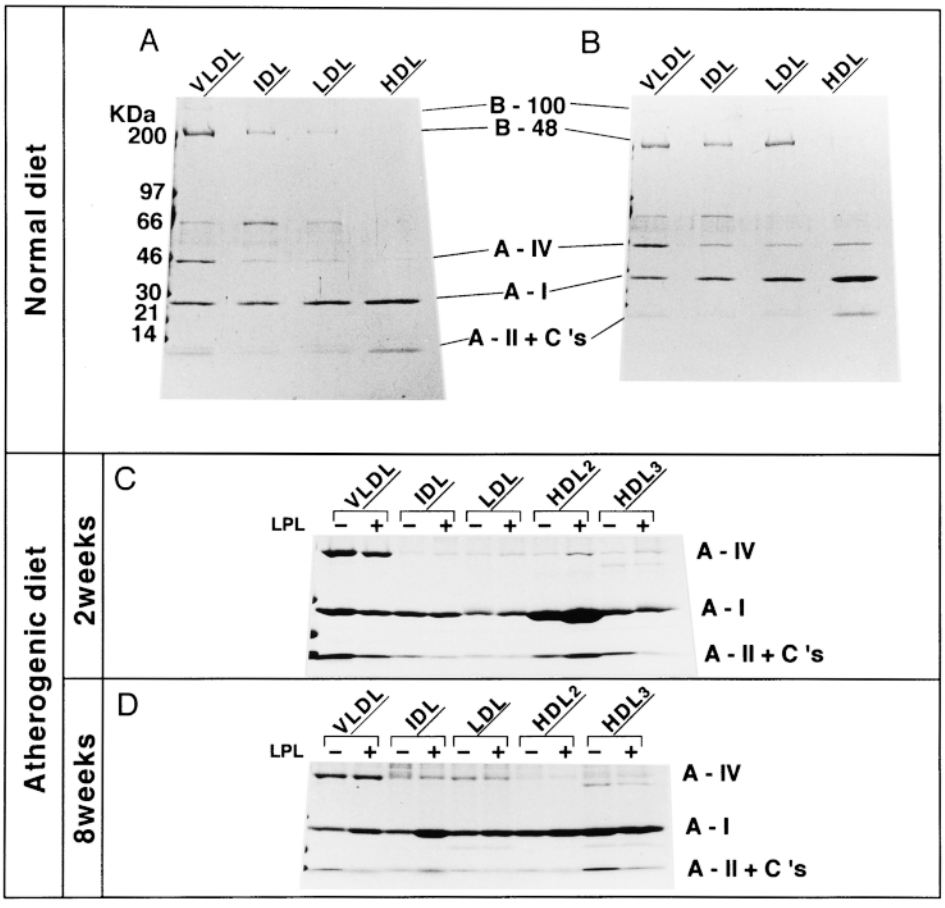

Fig. 4. Apolipoproteins in lipoprotein fractions isolated from APOEKO (A) and LPL/APOEKO (B) mice fed either a normal chow (A and B) or an atherogenic diet for 2 (C) and 8 weeks (D). The indicated lipoproteins were isolated from pooled plasma by sequential ultracentrifugation. In panels C and D, APOEKO and LPL/APOEKO are indicated by LPL (−) and LPL (+), respectively. Each fraction was dialyzed, delipidated, and subjected to SDS/5–15% polyacrylamide gel electrophoresis. Proteins were visualized by staining with Coomassie Brilliant Blue. The molecular weights of apoB-100, apoB-48, apoA-IV, apoA-I, and apoA-II+C's are indicated.

glycerides of both groups consisted of two peaks with retention times that were identical to those of the mice fed a normal chow diet (Fig. 2D). A significant reduction was observed in both peaks in LPL/APOEKO mice compared to APOEKO mice.

Agarose gel electrophoresis of the plasma showed patterns which were essentially similar to those observed in the mice fed a normal chow (Fig. 3). APOEKO mice had a broad band which migrated between β and pre-β positions (lane 4), and LPL/APOEKO mice had a sharper band with β mobility (lane 5).

Two weeks after feeding mice an atherogenic diet, apoA-I and apoA-IV were preferentially distributed in the HDL2 fraction (Fig. 4C). LPL/APOEKO mice had higher concentrations of apoA-I than APOEKO mice particularly in the HDL2 fraction. At this point, HDL2 cholesterol content was increased by 2-fold in LPL/APOEKO mice as compared to APOEKO mice (25 vs. 12 mg/dl). After 8 weeks, the preferential distribution of apoA-I in the HDL2 fraction became less prominent (Fig. 4D). There was no difference in the HDL2 cholesterol content at this point as shown in Tables 1 and 2. LPL/APOEKO mice had a higher concentration of apoA-I than APOEKO mice in VLDL and IDL, but not in HDL2. A similar increase of apoA-I in non-HDL fractions from LPL/APOEKO mice was observed in a separate experiment using different animals (data not shown).

Diet-induced atherosclerosis

Atherosclerosis was evaluated by two different methods: the en face surface lesion area of the aorta and cross-sectional analysis of aortic roots (**Fig. 5**). The en face surface lesion areas of LPL/APOEKO mice were significantly smaller than those of APOEKO mice by 30% (14.6 ± 5.1 vs. 20.6 ± 6.9%, $P < 0.05$) (Fig. 5A). Cross-sectional lesion areas of LPL/APOEKO mice were significantly smaller than those of APOEKO mice by 51% (290,000 ± 150,000 vs. 590,000 ± 160,000 μm^2, $P < 0.001$) (Fig. 5B). It is noteworthy that the two different methods showed comparable differences between the two groups.

Cholesteryl ester formation in mouse peritoneal macrophages

We isolated both VLDL and d < 1.063 g/ml lipoproteins from APOEKO and LPL/APOEKO mice, and compared their abilities to stimulate cholesteryl ester formation in peritoneal macrophages (**Fig. 6**). We used two types of mice as sources of macrophages: C57BL/6 wild-type and APOEKO mice. Both VLDL and d < 1.063 g/ml lipoproteins stimulated cholesteryl ester formation in macrophages prepared from wild-type C57BL/6 mice. The cholesteryl ester formation stimulated by the lipoproteins isolated from LPL/APOEKO mice was significantly smaller, by 30%, than that from APOEKO mice ($P < 0.05$). In contrast, neither VLDL nor d < 1.063 g/ml lipoproteins stimulated cholesteryl ester formation in macrophages prepared from APOEKO mice (<0.01 nmol/h per mg cell protein), irrespective of the lipoproteins used.

In contrast, β-VLDL obtained from LDLRKO mice fed an atherogenic diet markedly stimulated cholesteryl ester formation in wild-type macrophages (1.4 ± 0.1 nmol/h per mg cell protein).

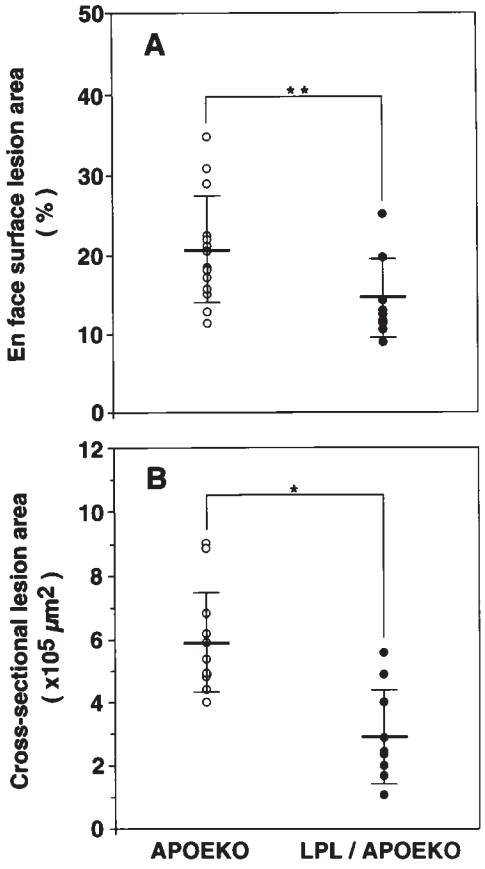

Fig. 5. En face (A) and cross-sectional (B) lesion area in the aortic sinus from APOEKO (open circle) and LPL/APOE KO mice (closed circle) fed an atherogenic diet. Male mice 8–12 weeks of age were fed an atherogenic diet for 8 weeks and the fatty streak lesion areas were determined as previously described (14, 29, 30). Mean ± SD is indicated by bold lines and error bars; * $P < 0.001$, ** $P < 0.05$.

DISCUSSION

In the present study, we have demonstrated that overexpression of LPL protects against diet-induced atherosclerosis in the absence of apoE. We postulate that two changes in the lipoprotein profiles underlie the suppression of atherosclerosis in LPL/APOEKO mice: changes in HDL and non-HDL.

HDL-cholesterol levels were slightly elevated in LPL/APOEKO mice fed a normal chow diet (Table 1). Plasma apoA-I levels were also increased in LPL/APOEKO mice. With regard to distribution of apolipoproteins, apoA-I and apoA-IV were present in both HDL and non-HDL lipoproteins probably due to the need for VLDL surface components (34, 35). They appeared to be increased in HDL of LPL/APOEKO mice (compare Fig. 4B with Fig. 4A). Two weeks after feeding an atherogenic diet, apoA-I

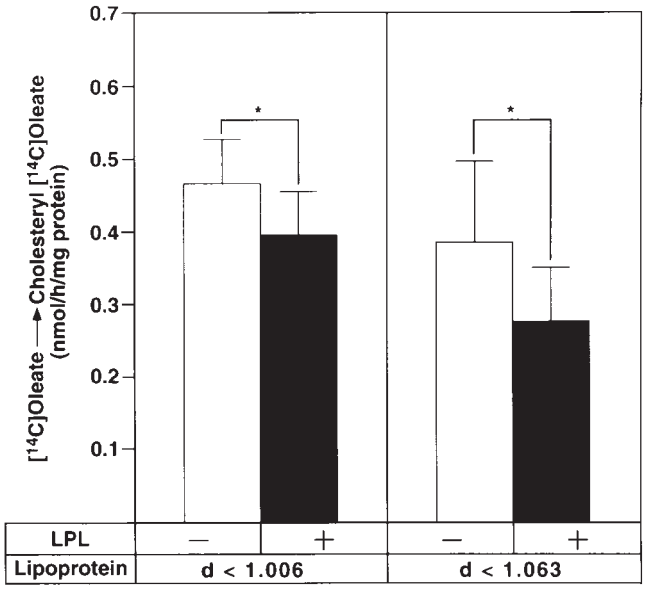

Fig. 6. Cholesteryl ester formation stimulated by either VLDL or d < 1.063 g/ml lipoproteins. Plasma was obtained from either APOEKO (open bar) or LPL/APOE KO (closed bar) mice (n = 5) which had been fed an atherogenic diet for 8 weeks. VLDL or d < 1.063 g/ml lipoproteins were isolated from pooled plasma by ultracentrifugation. Thioglycollate-elicited peritoneal macrophages were prepared from wild-type C57BL/6 mice. After treatment with LPDS for 24 h, the cells were incubated with 12.5 mg/ml of the indicated lipoproteins for 24 h, and cholesteryl ester formation was determined as described in Experimedntal Procedures. Errors bars denote SD; * $P < 0.05$ (n = 3).

and apoA-IV were preferentially distributed in HDL2 in both LPL/APOEKO and APOEKO mice (Fig. 4C). This may result from the increased transfer of these apolipoproteins from chylomicrons whose production is stimulated upon high fat feeding. The stimulated lipolysis of chylomicrons may account for the increased amounts of cholesterol as well as apoA-I in HDL2 in LPL/APOEKO mice. After 8 weeks, their preferential distribution in HDL disappeared probably because of reaching a new steady state (Fig. 4D). In the setting of apoE deficiency, even a slight change in apoA-I levels is detectable because baseline HDL cholesterol concentrations are significantly lower than those observed in the setting of LDL receptor deficiency where a subtle change may be easily masked. It is plausible that the increases in the levels of either apoA-I, apoA-IV or both in HDL mediate the suppression of atherosclerosis in LPL/APOEKO mice. In support of this, anti-atherogenic effects of the overexpressed apoA-I has been shown in the presence (36) or absence of apoE (37, 38) and overexpression of apoA-IV has been demonstrated to protect against diet-induced atherosclerosis (39, 40). With regard to the mechanisms underlying the increase in apoA-I levels, it is reasonable to consider that LPL-mediated lipolysis of triglyceride-rich lipoproteins causes transfer of surface apolipoprotein components to nascent HDL, thereby increasing apoA-I on HDL. Indeed, there is an inverse correlation between plasma triglyceride and HDL-cholesterol levels (41), and production of HDL is partly determined by LPL activity (42). However, HDL cholesterol levels vary greatly from no change (43, 44) to increases (13) or decreases (45, 46) among the different LPL transgenic mice containing different promoters. Probably, the tissues where the LPL transgenes are expressed are important determinants of HDL cholesterol levels.

In LPL/APOEKO mice, non-HDL lipoproteins were triglyceride-poor, small, and positively charged as compared to those in APOEKO mice (Figs. 2 and 3). In order to determine whether these physical changes were responsible for the suppression of atherosclerosis in LPL/APOEKO mice, we compared the ability of non-HDL lipoproteins to elicit cholesteryl ester formation in macrophages. As expected, non-HDL lipoproteins did not elicit any detectable cholesteryl ester formation in macrophages obtained from APOEKO mice. This is reasonable because the non-HDL lipoproteins completely lacked apoE and contained only a trace of apoB-100. Both apoB-100 and apoE are ligands for the LDL receptor (47), and apoE is one of the important ligands for LRP (48). Despite the failure of atherogenic lipoproteins to stimulate cholesteryl ester accumulation in apoE-deficient macrophages, APOEKO mice develop rampant atherosclerotic lesions that are composed of cholesteryl ester-filled macrophages. These observations indicate that modification of non-HDL lipoproteins is necessary for the development of atherosclerosis in this model. Interestingly, non-HDL lipoproteins from either APOEKO or LPL/APOEKO mice induced a significant degree of cholesteryl ester formation in macrophages obtained from wild-type mice (Fig. 6). It is possible that apoE secreted by wild-type macrophages is attached to the lipoprotein surface and is recognized by the LDL receptor expressed in these cells, as proposed previously (49). Cholesteryl ester formation elicited by non-HDL obtained from LPL/APOEKO mice was significantly reduced from that of APOEKO mice by 30% (Fig. 6). We would speculate that the non-HDL of LPL/APOEKO mice have limited capacity to accommodate apoE secreted from macrophages. It is noteworthy that an overabundance of LPL did not stimulate cholesteryl ester formation, in light of the belief that LPL bridges lipoproteins and cells in vitro and thus promotes their clearance (50, 51).

Despite these alterations in the physical properties of the lipoproteins mentioned above, no difference was observed in the plasma concentrations of apoB between LPL/APOEKO and APOEKO mice. These results indicate that the excessive lipolysis did not stimulate the hepatic removal of the lipolyzed lipoproteins containing apoB-48.

As our LPL/APOEKO mice expressed the LPL transgene in the aorta, we cannot completely rule out the possibility that the LPL expressed in the vascular wall contributed to the anti-atherogenic effects of the transgene. However, there was an approximately 10-fold difference in the degree of suppression of atherosclerotic lesions when LPL was overexpressed on a background of LDLR deficiency versus apoE deficiency. Therefore, it is plausible that lipoprotein profiles play a more important role in determining athereosclerosis than local expression of LPL.

In summary, the LPL overexpression is protective against diet-induced atherosclerosis even in the setting of apoE deficiency. The reduced triglyceride content of the non-HDL, the increase in the plasma apoA-I levels, or both, may account for the anti-atherogenic effects of LPL overexpression.

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