Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis

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Abstract Lipoprotein lipase (LPL) is a key enzyme in lipoprotein metabolism, and has been hypothesized to exert either pro- or anti-atherogenic effects, depending on its localization. Decreased plasma LPL activity is associated with the high triglyceride (TG)–low HDL phenotype that is often observed in patients with premature vascular disease. In contrast, in the vessel wall, decreased LPL may be associated with less lipoprotein retention due to many potential mechanisms and, therefore, decreased foam cell formation. To directly assess this hypothesis, we have distinguished between the effects of variations in plasma and/or vessel wall LPL on atherosclerosis susceptibility in apoE-deficient mice. Reduced LPL in both plasma and vessel wall ($LPL^{+/-}E^{-/-}$) was associated with increased TG and increased total cholesterol (TC) compared with $LPL^{+/+}E^{-/-}$ sibs. However despite their dyslipidemia, $LPL^{+/-}E^{-/-}$ mice had significantly reduced lesion areas compared to the $LPL^{+/+}E^{-/-}$ mice. Thus, decreased vessel wall LPL was associated with decreased lesion formation even in the presence of reduced plasma LPL activity. In contrast, transgenic mice with increased plasma LPL but with no increase in LPL expression in macrophages, and thus the vessel wall, had decreased TG and TC and significantly decreased lesion areas compared with $LPL^{+/+}E^{-/-}$ mice. This demonstrates that increased plasma LPL activity alone, in the absence of an increase in vessel wall LPL, is associated with reduced susceptibility to atherosclerosis. Taken together, these results provide in vivo evidence that the contribution of LPL to atherogenesis is significantly influenced by the balance between vessel wall protein (pro-atherogenic) and plasma activity (anti-atherogenic).—Clee, S. M., N. Bissada, F. Miao, L. Miao, A. D. Marais, H. E. Henderson, P. Steures, J. McManus, B. McManus, R. C. LeBoeuf, J. J. P. Kastelein, and M. R. Hayden. **Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis.** ***J. Lipid Res.*** **2000.** 41: **521–531.**

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Lipoprotein lipase (LPL) is a key enzyme in lipoprotein metabolism. Anchored to the luminal surface of the vascular endothelium, its primary role is in the hydrolysis of core triglycerides (TG) in circulating triglyceride-rich lipoproteins (TGRL), converting these into remnant particles (1). This process generates free fatty acids which may be taken up and used for energy or for storage, and also results in the generation of surface remnants which form the basis of high density lipoprotein (HDL) (1). Over 70 mutations in the LPL gene have now been described (reviewed in ref. 2), which, cumulatively, may be present at carrier frequencies approaching 20% in populations of European descent. Thus, understanding this enzyme is of key importance in furthering our understanding of atherogenesis. Various lines of evidence have suggested that LPL may confer either increased or decreased risk for atherosclerosis depending on its site of expression (reviewed in ref. 3).

Higher levels of plasma LPL activity are associated with decreased TG and increased HDL cholesterol levels in humans. Heterozygotes for LPL deficiency have increased TG and decreased HDL cholesterol levels (4–6), a profile associated with increased atherogenic risk (7–9). Increased LPL activity serves to decrease the concentration of plasma TGRL, which may themselves be atherogenic (10). Furthermore, the LPL protein may play a structural role in the bridging and uptake of lipoproteins in the liver, and has been hypothesized to aid hepatic clearance

Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; HDL, high density lipoprotein; apo, apolipoprotein; TGRL, triglyceride-rich lipoproteins; Tg, transgenic; GGE, gradient gel electrophoresis; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; ORO, oil red O.

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of remnant lipoproteins (11–14). These data suggest that increased plasma LPL activity is associated with protection against atherosclerosis.

In the vessel wall, however, increased LPL mass and/or activity may be pro-atherogenic. Macrophages are the primary source of LPL within the vessel wall (15, 16) and higher levels of macrophage LPL have been correlated with increased susceptibility to atherosclerosis in mice (17). LPL has been shown to aid uptake of lipoproteins by macrophages (18–20). Furthermore, LPL within the vessel wall increases lipoprotein retention within the subendothelial cell matrix (21–23) and in aortic segments (24, 25). Such trapped lipoproteins are more susceptible to atherogenic modification, and may be more rapidly taken up by macrophages, aiding foam cell formation (26, 27). In addition, macrophage uptake of lipoproteins in the vessel wall may be enhanced by LPL lipolytic activity via the local generation of smaller remnants that are more amenable to uptake (19, 20). Aortic LPL has been positively correlated with cholesterol uptake in the aorta of cholesterol-fed rabbits (28). Furthermore, it was shown recently that LPL may act as a monocyte adhesion protein (29, 30), and may play a role in the recruitment of monocytes into the vessel wall. These data suggest that increased LPL activity and/or mass within the vessel wall may promote atherosclerosis.

We have sought to directly address the question of the relative atherogenicity of vessel wall versus plasma LPL in vivo, using both the apoE-deficient ($E^{-/-}$) mouse model and a cholesterol-fed C57BL/6 mouse model. By comparing the atherosclerosis susceptibility of mice with decreased LPL expression in both plasma and vessel wall with mice that overexpressed LPL in tissues contributing solely to plasma LPL excluding the vessel wall, we find that raising plasma LPL without increasing vessel wall LPL confers relative protection against atherosclerosis. By contrast, any increase in vessel wall LPL is associated with increased atherogenesis, providing in vivo evidence of the differing roles for LPL in atherosclerosis susceptibility, influenced directly by its site of expression.

METHODS

Animals

Decreased LPL expression was examined in a line of mice heterozygous (+/−) for a targeted (null) LPL allele (31), and compared to their normal $LPL^{+/+}$ siblings. For the model of increased plasma LPL expression, transgenic mice (LPL^{Tg}) that overexpress human LPL in heart, skeletal muscle, adipose tissue, kidney, and stomach (32) were used. Macrophages, the primary source of vessel wall LPL, do not express the human LPL transgene. No LPL expression has been detected from peritoneal macrophages of LPL knockout mice rescued with this CMV-human LPL transgene. The mean LPL activity in media after a 24-h culture of these macrophages was 1.59 ± 0.33 in macrophages from the rescued knockouts (n = 3), compared to 8.54 ± 0.44 mU/mL in normal mice (n = 3; media alone gave a measurement of 2.4 mU/mL). Also, no human LPL has been detected in lesions of these mice by immunohistochemistry with the 5D2 monoclonal antibody (R. C. LeBoeuf, unpublished data), indicating that LPL is not expressed in the vessel wall of these mice.

In addition, the above three groups of mice were bred onto the $E^{-/-}$ mouse line obtained from the Jackson Laboratory (Bar Harbor, ME), producing mice that were deficient in apoE, and either +/− or +/+ at the mouse LPL locus, or containing the human LPL cDNA transgene (Tg, +/+ at mouse LPL locus). All mice in the study are estimated to contain a greater than 90% C57BL/6 genetic background. Our study groups comprised only female mice.

Animals were housed in microisolator cages in groups of 3–4 mice per cage, in an environmentally controlled facility, with 12-h light and dark cycles (7 am–7 pm). Animals had free access to food and water, except where indicated. All procedures were approved by the University of British Columbia Committee on Animal Care.

Diets

Mice were fed a standard mouse chow (Purina Laboratory Rodent Diet, 5001, PMI Feeds) that contained approximately 4.5% fat and 23.4% protein, with no more than 270 ppm cholesterol. At 12 weeks of age, the BL/6 mice ($E^{+/+}$) were then fed an atherogenic diet (Harlan Teklad #88051) containing approximately 15% cocoa butter, 6.2% carbohydrate, and 20.6% protein, with 1.25% cholesterol and 0.5% cholate for a period of 12 weeks.

Animal procedures

At 10 weeks of age, mice were fasted overnight (from approx. 10 pm–8 am) prior to withdrawing blood samples for lipid measurements. At 12 weeks of age the BL/6 mice were placed on the atherogenic diet for a period of 12 weeks. Blood samples were withdrawn after an overnight fast (as above) at 22 weeks of age (after 10 weeks on the diet) for lipid analysis, and mice were killed at 24 weeks of age.

After exsanguination, mice were perfused with 4% paraformaldehyde in phosphate-buffered saline for approximately 5 min at a flow rate of 3.5 mL/min. Hearts and upper aortae were then removed and fixed in the same solution prior to embedding and sectioning.

Genotyping

All genotypes were determined by PCR. The presence of the LPL transgene was determined as described previously (33). The presence of the *neo* insertion in exon 8 of the mouse *lpl* gene was assessed using multiplex PCR including 3 primers. The forward primer is located complementary to the junction of intron 7 and exon 8 (LPLK3, 5′GAAATTTTCACCCAGGCCGGAGG), while there are two reverse primers, one in the neomycin resistance insertion as described (31) (Neo, 5′TCGCCTTCTATCGCCTTCT TGAC) and one at the 3′ end of exon 8 distal to the insertion site (LPLK1, 5′CCTCTCGATGACGAAGCTGG). In the absence of the insertion, primers LPLK3 and LPLK1 amplify a band of approximately 150 bp of mouse LPL exon 8. In the presence of the *neo* insertion, the fragment (>1.5 kb) between LPLK3 and LPLK1 does not amplify under the given conditions, but LPLK3 and Neo produce a 600 bp product. A mouse heterozygous for the *neo* insertion thus will display both the 600 and 150 bp products, while a mouse wildtype at the *lpl* locus will only display the 150 bp band. The genotype at the mouse *lpl* locus can thus be unambiguously identified. PCR was carried out using 2 mm Mg^{2+}, 12 pmol LPLK1, 20 pmol LPLK3, and 24 pmol Neo for 35 cycles under the following thermocycling conditions: 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min.

A similar scheme was used to genotype the mouse *apoE* locus, with primers flanking the insertion site and one within the *neo* insertion. Genotyping at the *apoE* locus used 20 pmol of each

primer (E1: forward, exon 3, 5′GATGCCTAGCCGAGGGAG AGC, E2: reverse, intron 3, 5′GAATTGCAGAGCCTTCGAAGC, and Neo2: forward, 5′TGGCGGACCGCTATCAGGAC). Each reaction contained 2 mm Mg^{2+}, and used cycles consisting of 96°C for 1 min, 51°C for 30 sec and 72°C for 45 sec.

All PCR reactions were carried out in 50 μL volumes, in the presence of 200 μmol of each dNTP. Each reaction had an initial denaturation of 5 min at 96°C and a final extension of 5 min at 72°C.

Lipid analysis

Plasma was separated by microcentrifugation for 10 min at 4°C, aliquoted, and stored at −70°C until analysis. Triglycerides and total cholesterol were measured using commercially available kits (Boehringer Mannheim, numbers 450032 and 1142350, respectively). HDL was quantified after precipitation of apolipoprotein B-containing lipoproteins using polyethylene glycol, as described previously (33). NonHDL cholesterol was obtained by subtracting HDL cholesterol levels from total cholesterol values.

Lesion assessment

The ventricular apex of fixed hearts was transected inferior to the atria and discarded. The basal portion of the hearts was transferred to a 1:1 solution of Optimal Cutting Temperature (OCT™, Tissue Tek, 4583) media and buffered saline overnight. Hearts were then embedded with cut surface of the vericular myocardium down in OCT, flash frozen, and stored at −70°C until sectioning.

Sectioning was performed as described (34). Serial 10-μm sections were obtained working from the apical apex of the heart towards the aortic origin, beginning to mount sections from the point where all three aortic valve cusps became clearly visible. Every fourth section was placed on a slide for oil red O (ORO) staining (counterstained with hematoxylin), such that each slide had sections 40 μm apart. Sections immediately prior to those used for ORO staining were saved on a separate slide and stained with Movat's pentachrome. Atherosclerotic lesion areas were measured using the Bioview Color Image Analysis system (McDonald Research Laboratories-UBC, Infrascan Inc., 1993) and are reported as the average ORO staining area per section in the first five such sections for each mouse. Lesions in the aortic root were examined rather than those from the whole aorta to produce results which would be comparable for both model systems.

Gel filtration chromatography of plasma lipoproteins

Plasma lipoproteins were separated by fast performance liquid chromatography (FPLC) gel filtration using a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech LKB Biotechnology, Uppsala, Sweden). Plasma (100 μl) was loaded onto the column and eluted with PBS at a constant flow rate of 0.2 ml/min at 4°C. Sixty fractions, 500 μl each, were collected using a Frac 100 fraction collector (Pharmacia). Cholesterol and triglyceride concentrations were determined colorimetrically using 100 μl of each fraction (Diagnostic Chemicals Limited, Prince Edward Island, Canada and Boehringer Mannheim Corp., Indianapolis, IN, respectively) and adjusted to reflect plasma total cholesterol and triglyceride levels.

Gradient gel electrophoresis (GGE)

GGE was performed on plasma samples obtained when the mice were killed. In brief, plasma (50 μL) was pre-incubated with Sudan Black (25 μL of 1% w/v in ethylene glycol) at 4°C for 1 h. Samples were centrifuged at 10,000*g* for 20 min, an aliquot was mixed with an equal volume of saturated sucrose, and volumes equivalent to 4 μL plasma were loaded into a 2–8% gradient polyacrylamide gel. The samples were electrophoresed at 130 V for 18–24 h at 4°C. Lipoprotein species were identified and named according to their migration relative to the corresponding human species. The procedure allows the identification of the following lipoproteins: $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20), and LDL (S_f 0–12) within which size heterogeneity can be observed in humans. The retardation factors (R_f) for the above classes of lipoprotein are <0.45, 0.45–0.7, 0.7–0.85, and 0.85–1.0, respectively, when small human LDL is used as a reference. Analysis was performed by counting the number of mice of each genotype that displayed the various lipoprotein species.

Statistical analysis

Statistical analyses were performed using Systat (version 7.0, SPSS Inc.). Data are reported as mean ± standard deviation. Between group comparisons were made using Student's *t*-tests ($LPL^{+/-}$ vs. $LPL^{+/+}$ and LPL^{Tg} vs. $LPL^{+/+}$).

RESULTS

LPL-induced alterations in lipid profiles of $E^{-/-}$ mice

To examine the effects of decreased LPL on lipid profiles and atherogenesis, heterozygous LPL-deficient ($LPL^{+/-}$) mice (31) were bred with $E^{-/-}$ mice, resulting in mice lacking apoE and either $LPL^{+/-}$ or $LPL^{+/+}$ ($LPL^{+/-}E^{-/-}$ and $LPL^{+/+}E^{-/-}$, respectively). Mice heterozygous for LPL deficiency had an approximately 2-fold increase in TG (213 ± 92 vs. 118 ± 54 mg/dL, $LPL^{+/-}E^{-/-}$ vs. $LPL^{+/+}E^{-/-}$, $P < 0.001$; **Table 1**). Total cholesterol was mildly (20%) increased ($P = 0.03$, Table 1), caused by both an increase in HDL cholesterol (55 ± 41 vs. 34 ± 25 mg/dL, $P = 0.04$) and a smaller increase in nonHDL cholesterol (Table 1).

TABLE 1. Lipid levels in apoE-deficient mice by LPL genotype

	LPL +/− (n = 25)	LPL +/+ (n = 23)	LPL Tg (n = 18)	*P* Values +/+ vs. +/−	*P* Values +/+ vs. Tg
TG (mg/dL)	213 ± 92	118 ± 54	80 ± 37	<0.001	0.01
TC (mg/dL)	515 ± 155	433 ± 102	363 ± 92	0.03	0.03
HDL chol. (mg/dL)	55 ± 41	34 ± 25	10.9 ± 10.9	0.04	<0.001
Non-HDL chol. (mg/dL)	460 ± 156	399 ± 90	352 ± 87	0.10	0.10
	(n = 24)	(n = 21)	(n = 18)		
Lesion area (μm^2)	62792 ± 38391	87636 ± 40218	56877 ± 29533	0.04	0.01

Lipid levels were measured at 10 weeks of age, and lesion areas were measured at 16 weeks of age. Mice were fed a standard rodent chow diet for the duration of the study.

To examine the effects of increasing LPL in plasma, but not within the vessel wall, transgenic mice (LPL^{Tg}) containing a transgene driven by the CMV promoter (32) and overexpressing human LPL in several tissues excluding macrophages (the primary source of vessel wall LPL (15, 16)), were bred with the $E^{-/-}$ mice. The anti-atherogenic lipid profiles seen with increasing LPL were mirrored in the LPL transgenic mice. Triglycerides were further reduced versus the $LPL^{+/+}E^{-/-}$ (80 ± 37 vs. 118 ± 54 mg/dL, $P = 0.01$; Table 1), as was TC (363 ± 92 vs. 433 ± 102 mg/dL, $P = 0.03$). This was contributed to by decreased HDL cholesterol (11 ± 11 vs. 34 ± 25 mg/dL, $P < 0.001$) and nonHDL cholesterol.

LPL expression and atherosclerotic lesion formation in $E^{-/-}$ mice

Examination of atherosclerotic lesion areas revealed that $LPL^{+/-}E^{-/-}$ mice (mice with decreased LPL in plasma and the vessel wall) displayed a 30% reduction in mean ORO staining area compared with their $LPL^{+/+}E^{-/-}$ littermates (62792 ± 38391 vs. 87636 ± 40218 μm^2, $P = 0.04$), despite pro-atherogenic plasma lipid profiles (Table 1; **Fig. 1, A and B**). This suggests that the loss of LPL protein in the vessel wall was key to the finding of less atherosclerosis, and had greater effect on reducing susceptibility to atherosclerosis than the atherogenic lipid changes caused by low plasma LPL activity.

In contrast, in $LPL^{Tg}E^{-/-}$ mice overexpressing LPL only in the plasma and not in macrophages, atherosclerotic lesion areas were approximately 35% smaller (56877 ± 29533 vs. 87636 ± 40218 μm^2, $P = 0.01$, Table 1) as compared to the $LPL^{+/+}E^{-/-}$ mice (Fig. 1C). This suggests that increasing LPL expression specifically in plasma, without increasing vessel wall LPL concentrations, is associated with decreased lesion formation. Thus, increasing plasma LPL activity is associated with alterations in lipid levels, which in turn convey lowered susceptibility to atherosclerosis.

In addition to differences in mean area of ORO positivity, differences in lesion complexity were noted between the LPL genotypes (Fig. 1 D–F). In the $LPL^{+/-}E^{-/-}$ mice, lesions included numerous foam cells in clusters overlain by an elastic membrane and endothelium. Focally, there were smaller nests of smooth muscle cells. A small amount of matrix was present in certain of these lesions, and it tended to co-localize with neointimal smooth muscle cells. In the $LPL^{+/+}E^{-/-}$ mice, lesions were more prominent and had a somewhat altered character. Foam cells were more numerous and readily visible, however they constituted a lower percentage of lesion bulk, and were generally more deeply placed in the neointima, partly overlain by and in association with the matrix and smooth muscle cells. Extracellular cholesterol clefts were visible, and juxtaposed the foam cells. In addition, aortic valve cusps appeared slightly more glycosaminoglycan-rich than normal. Lesions in the $LPL^{Tg}E^{-/-}$ mice were smaller, with a less well developed matrix and a less prominent smooth muscle cell contingent. They were predominantly foam cell-rich, flat lesions. Thus, the differences noted in lesion areas were somewhat paralleled by differences in lesion complexity, with the $LPL^{+/+}E^{-/-}$ mice having the most complex lesions.

Under- and over-expression of LPL and atherosclerosis in wildtype C57BL/6 mice

In an effort to validate these findings in another mouse model, we explored the relationship between variations in LPL and atherogenesis by examining diet-induced atherosclerosis in a C57BL/6 (BL/6) mouse model with similar alterations in LPL.

The effect of LPL genotype on lipid profiles in the BL/6 model was less pronounced than in the $apoE^{-/-}$ model. Prior to the initiation of high fat/high cholesterol feeding (while mice were consuming the standard chow), $LPL^{+/-}$ mice had a trend toward increased TG as compared with $LPL^{+/+}$ mice ($P = 0.07$, **Table 2**). There were no significant differences in total cholesterol or any specific cholesterol fraction. After 10 weeks consumption of the high fat/high cholesterol diet, $LPL^{+/-}$ mice displayed over 2-fold higher TG as compared with their $LPL^{+/+}$ sibs (27 ± 20 vs. 12.5 ± 11.0 mg/dL, $P = 0.01$, Table 2). Similar to that seen in the $E^{-/-}$ mice, $LPL^{+/-}$ mice also had increased HDL cholesterol (47 ± 11 vs. 36 ± 13 mg/dL, $P = 0.02$ vs. $LPL^{+/+}$). Similar trends were seen in the mice overexpressing LPL (LPL^{Tg}), although no comparisons reached statistical significance (Table 2).

Small neointimal foam cell lesions, the equivalent of fatty streaks, were observed (**Fig. 2**) in these mice. Similar trends in lesion areas were noted as with the $E^{-/-}$ mice. Thus, mean lesion areas in the $LPL^{+/-}$ mice, with decreased vessel wall and plasma LPL, were reduced as compared with $LPL^{+/+}$ mice (2016 ± 2801 vs. 4125 ± 4122 μm^2). However, due to the large variation in lesion areas, the difference did not reach statistical significance (Table 2). Lesion areas were also decreased in the mice overexpressing human LPL in plasma but not in the vessel wall (1921 ± 2132 μm^2 vs. 4125 ± 4122 μm^2 in $LPL^{+/+}$ mice, $P = 0.06$). A few cholesterol clefts were noted in the $LPL^{+/+}$ mice. Otherwise there were no significant differences in lesion morphology between the LPL genotypes.

Lipoprotein analysis

It could be hypothesized that decreased atherosclerosis susceptibility in the mice with decreased LPL activity in the vessel wall and plasma ($LPL^{+/-}$) was due to the presence of larger, TG-rich apoB-containing lipoproteins that had a decreased ability to enter the vessel wall and/or be taken up by macrophages in these mice, and not due to decreased LPL in the vessel wall.

As an initial attempt to examine the lipoprotein distribution within the various genotypic groups, FPLC of pooled plasma was performed. As shown in **Fig.3**, $LPL^{+/-}E^{-/-}$ mice have a somewhat altered FPLC profile compared to their $LPL^{+/+}E^{-/-}$ and $LPL^{Tg}E^{-/-}$ counterparts. These mice have a tremendous increase in the amount of cholesterol-rich remnant-type particles, but not in the TG-rich, VLDL fraction. In the cholesterol-fed animals, no obvious differences in FPLC profiles were evident aside from

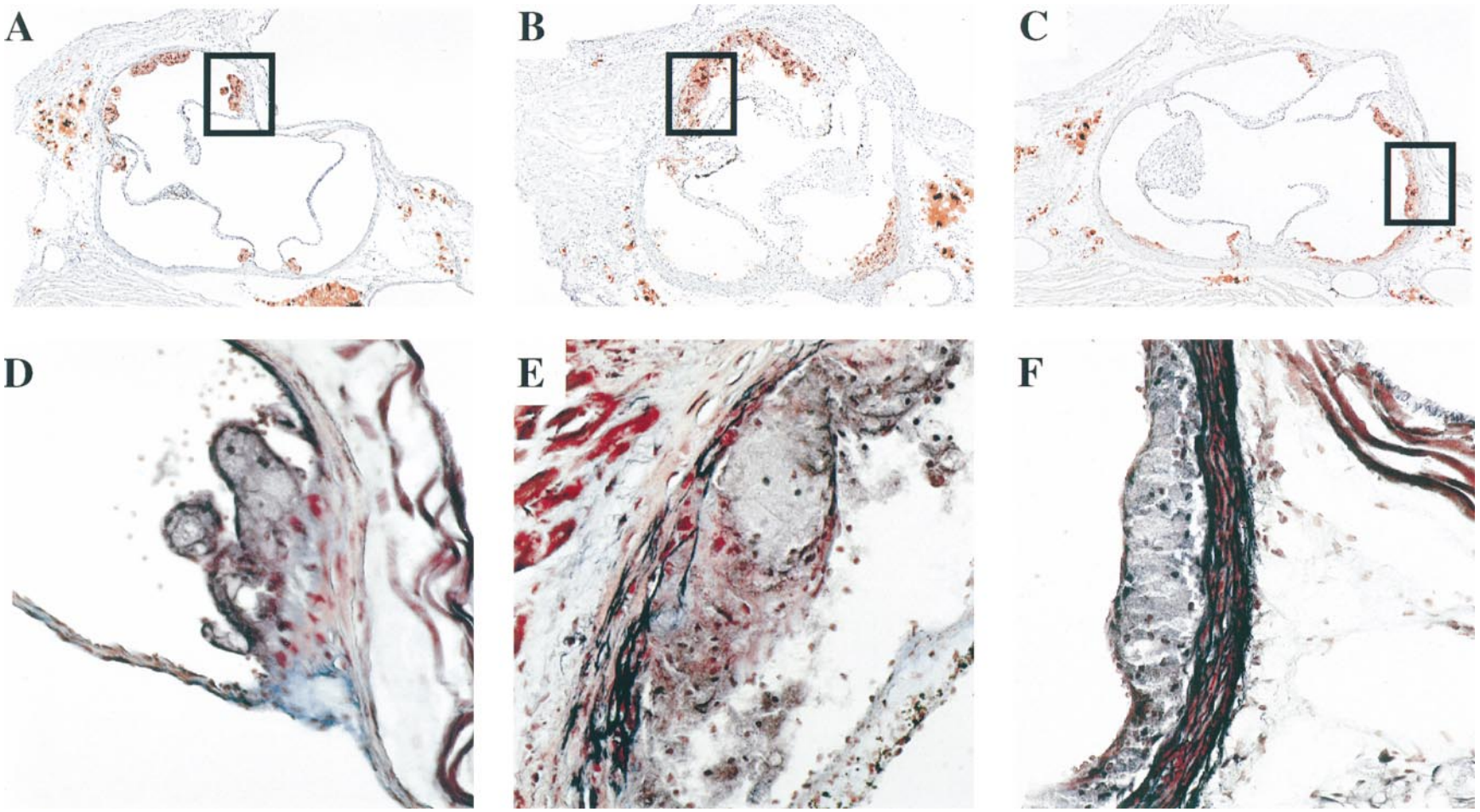

Fig. 1. Lesional constituents in apoE$^{-/-}$ mice. Panels A–C display representative aortic cross-sections. Lipid deposits are stained with oil red O, and sections were counterstained with hematoxylin, 15× magnification. Panels D–F depict magnified (50×) images from the boxed region on adjacent serial sections (from A, a commissural region, from B and C, mid-sinus regions), stained with Movat's pentachrome which stains muscle red, nuclei and elastin black, proteoglycans aqua, and collagen yellow. Lesions were predominantly foam cells (grey granular cytoplasm with small dark nuclei) with some matrix deposition, and tended to be more prominent and slightly more complex in the LPL$^{+/+}$E$^{-/-}$ mice. A., D. LPL$^{+/-}$E$^{-/-}$; B., E. LPL$^{+/+}$E$^{-/-}$; C., F. LPL^{Tg}E$^{-/-}$.

TABLE 2. Lipid levels in C57BL/6 mice, by LPL genotype

	LPL +/− (n = 22)	LPL +/+ (n = 18)	LPL Tg (n = 23)	*P* Values +/+ vs. +/−	*P* Values +/+ vs. Tg
On chow (pre-diet)					
TG (mg/dL)	100 ± 49	73 ± 46	57 ± 24	0.07	NS
TC (mg/dL)	57 ± 17	50 ± 17	49 ± 10	NS	NS
HDL chol. (mg/dL)	46 ± 13	41 ± 15	42 ± 9	NS	NS
Non-HDL chol. (mg/dL)	11 ± 6	8.7 ± 5.2	7.0 ± 3.2	NS	NS
Post atherogenic diet feeding	(n = 20)	(n = 17)	(n = 20)		
TG (mg/dL)	27 ± 20	12.5 ± 11.0	9 ± 6	0.01	NS
TC (mg/dL)	199 ± 44	186 ± 50	195 ± 45	NS	NS
HDL chol. (mg/dL)	47 ± 11	36 ± 13	31 ± 14	0.02	NS
Non-HDL chol. (mg/dL)	152 ± 43	149 ± 45	163 ± 43	NS	NS
	(n = 18)				
Lesion area (μm^2)	2016 ± 2801	4125 ± 4122	1921 ± 2132	NS	0.06

Lipid levels at 10 weeks of age, consuming a standard chow diet, and at 22 weeks of age after 10 weeks consumption of an atherogenic diet. Lesion areas were measured after 12 weeks consumption of the atherogenic diet.

changing relative amounts of the various lipoprotein species. Thus, in neither model are there more large TG-rich particles in the animals heterozygous for LPL compared to those homozygous or transgenic.

To further address whether individual mice had an altered composition or size distribution of nonHDL lipoproteins that was masked by the use of pooled plasma for the FPLC, gradient gel electrophoresis (GGE) was performed. Mouse lipoproteins were analyzed as TG-rich ($VLDL_0$, $VLDL_1$, and $VLDL_2$) and LDL-like (IDL, LDL). Compared with fasted human lipoprotein patterns, the mouse lipoproteins displayed a larger lipoprotein species (termed $VLDL_0$) and lipoproteins similar to $VLDL_1$, $VLDL_2$ and IDL. No lipoproteins similar in size to human LDL were seen. The patterns are summarized in **Table 3**. Very little difference in the distribution of lipoprotein species within each genotype is evident.

The majority of $LPL^{+/-}E^{-/-}$ and $LPL^{+/+}E^{-/-}$ mice (60–70%) typically displayed a broad band incorporating both particles similar in size to human $VLDL_1$ and $VLDL_2$. No IDL-sized species were detected in either the $LPL^{+/-}E^{-/-}$ or $LPL^{+/+}E^{-/-}$, while four $LPL^{Tg}E^{-/-}$ had lipoprotein species of a size similar to human IDL. Thus the representation of $LPL^{+/-}E^{-/-}$ and $LPL^{+/+}E^{-/-}$ mice in the various lipoprotein classes was almost identical. The $LPL^{Tg}E^{-/-}$ mice had more polarization of lipoproteins to either larger or smaller lipoproteins and less commonly displayed the broader range of both $VLDL_1$ and $VLDL_2$. While there was a trend towards larger particles, these mice also displayed an IDL-like species which could have penetrated the vessel wall more easily than the VLDL-like species. The predominant nonHDL particle in the BL/6 mice on the atherogenic diet was $VLDL_1$-like in all LPL genotypes, while IDL was found in only 10% of the $LPL^{+/-}$ mice.

In both animal models, there was very little difference in the lipoprotein species between the LPL genotypes, either comparing the lipoprotein species present within each genotype (Table 3), or comparing the genotype distribution within each lipoprotein class (data not shown). Thus, the increased TG (and TC) seen in the $LPL^{+/-}E^{-/-}$ and $LPL^{+/-}$ mice in all likelihood reflects an increased number of particles, rather than altered particle composition. This is supported by the FPLC data which depicts increased amounts of the remnant-like species, but no obvious shifts in size. The decreased susceptibility to atherosclerosis in these mice, therefore, is clearly not due to the presence of larger, less penetrant TG-rich particles in the $LPL^{+/-}$ mice.

DISCUSSION

We performed experiments allowing us to directly assess the relative atherogenicity of vessel wall and plasma LPL. In two different models of atherosclerosis, we have provided in vivo evidence for the differing influence of LPL in the development of atherosclerosis depending on its site of expression. Decreased LPL in the vessel wall due to heterozygosity for a null allele at the LPL locus was associated with decreased atherosclerotic lesion formation despite the dyslipidemia caused by low plasma LPL activity. It has been shown that atherosclerotic lesions of $LPL^{+/-}$ mice have decreased LPL mass as compared with $LPL^{+/+}$ mice (35). Furthermore, using a complementary approach, Babaev and colleagues (36) have recently shown that abolition of macrophage LPL was associated with reduced lesion formation. Mice with only partially diminished LPL activity also displayed reduced lesion formation in the proximal aorta, suggesting a dose–response relationship between macrophage LPL levels and atherosclerosis susceptibility (36). Cumulatively, these findings suggest that macrophage-derived vessel wall LPL plays a crucial proatherogenic role in determining susceptibility to atherosclerotic lesion formation (**Fig. 4**).

Consistent with this, we also have shown that increased LPL activity specifically in the plasma (and not in macrophages and thus the vessel wall) is protective, as might be predicted by the anti-atherogenic lipid profile changes associated with increased plasma LPL activity (Fig. 4).

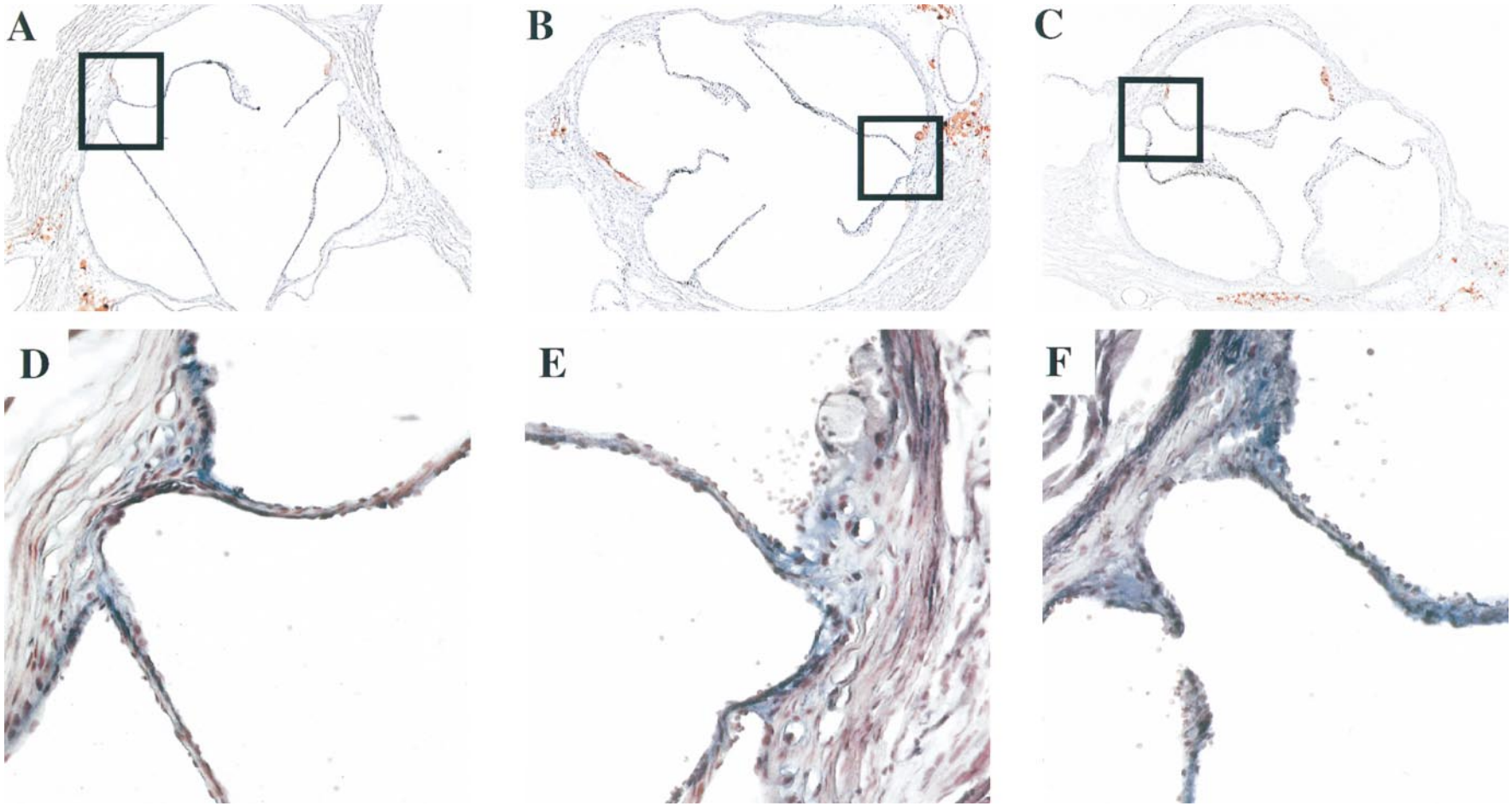

Fig. 2. Lesional constituents in BL/6 mice. Panels A–C display representative aortic cross-sections, 15× magnification. Lipid deposits are stained with oil red O, and sections were counterstained with hematoxylin. Panels D-F depict magnified (50×) images from the commissural junctions on adjacent serial sections, stained with Movat's pentachrome. Lesion areas included small collections of foam cells, especially evident in panel E. Occasional cholesterol clefts are present in the $LPL^{+/+}$ mice (panel E). As evident in panels B and E, the lesions in the $LPL^{+/+}$ mice tended to be larger than in either the $LPL^{+/-}$ or LPL^{Tg} mice. A., D. $LPL^{+/-}$; B., E. $LPL^{+/+}$; C., F. LPL^{Tg}.

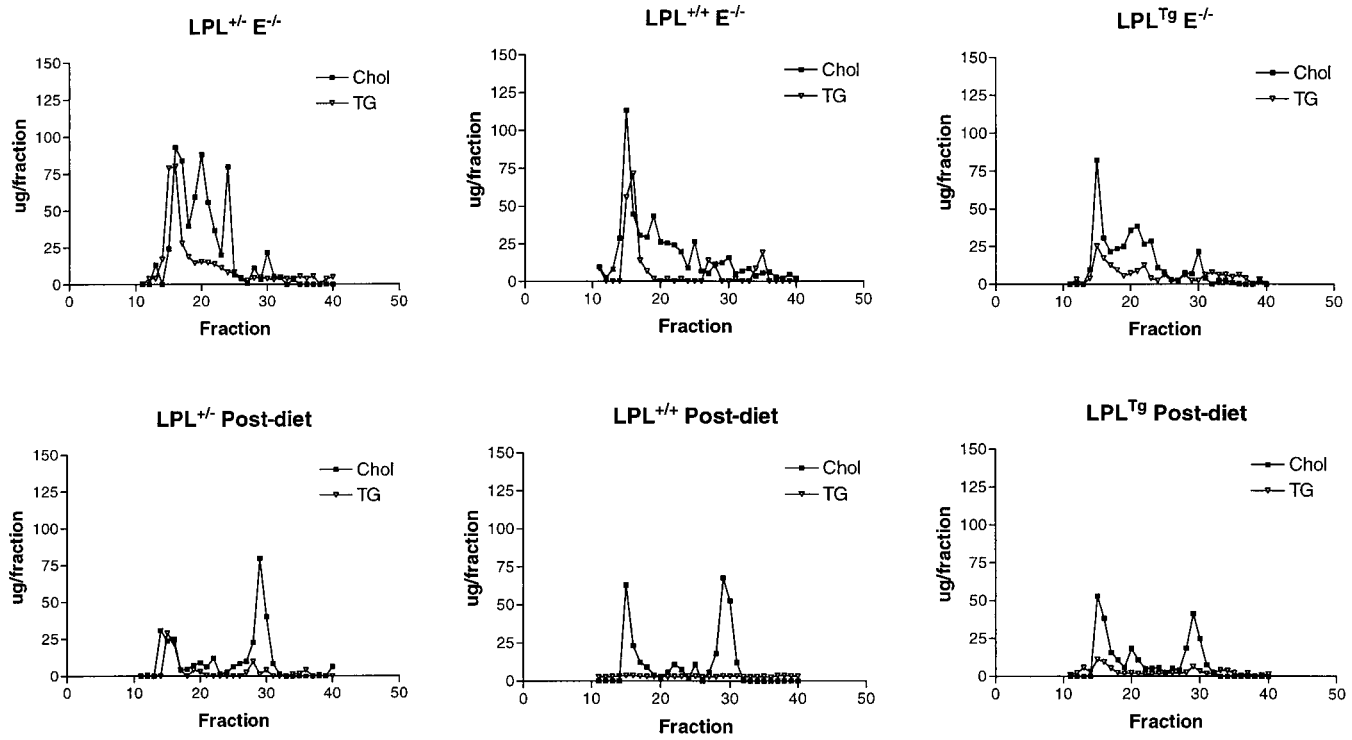

Fig. 3. FPLC profiles of pooled plasma samples from each genotype. Total cholesterol and triglycerides in each fraction are plotted for each LPL genotype within both the $E^{-/-}$ and BL/6 mice following cholesterol feeding.

These results confirm the findings of Shimada and colleagues (37) in low density lipoprotein receptor deficient mice, and very recently in apoE-deficient mice (38). We have previously shown that liver-directed LPL overexpression was associated with increased plasma catalytic activity and improved lipoprotein profiles (39). The data presented herein provide in vivo evidence that therapies designed to increase LPL activity should be targeted specifically to increasing plasma (and not vessel wall) LPL, where LPL may be of significant therapeutic potential in reducing susceptibility to atherosclerosis.

The finding of differing roles for vessel wall and plasma LPL in atherogenesis has additional clinical relevance. LPL mutations may be present at cumulative frequencies approaching 20% in Caucasian populations. Some patients have LPL mutations associated with a catalytic defect and stable LPL immunoreactive mass, for example the I194T, R243H, and G188E mutations (class II (40)), while others have mutations resulting in decreased LPL mass in addition to defective catalytic activity, such as the P207L mutation (41) and several insertions, deletions and premature truncations (class I (40)). We would thus predict that mutations such as the former may confer a significant atherosclerotic risk, as they are associated with decreased catalysis and dyslipidemia but normal vessel wall LPL mass, which may still function normally in the retention of lipoproteins. In contrast, other mutations that are associated with loss of LPL activity and less stable LPL mass, and consequently lower levels of LPL protein in the vessel wall available to retain lipoproteins, might be predicted to be less atherogenic.

Recently there have been several publications examin-

TABLE 3. Gradient gel electrophoresis characterization of nonHDL cholesterol fractions

Group	n	Percentage of mice with				Percentage of mice with	
		$VLDL_0$	$VLDL_1$	$VLDL_1$ and $_2$	$VLDL_2$	IDL	No IDL
$LPL^{+/-}$ $E^{-/-}$	24	12.5	4.2	70.8	12.5	0	100
$LPL^{+/+}$ $E^{-/-}$	21	14.3	9.5	61.9	14.3	0	100
LPL^{Tg} $E^{-/-}$	17	23.5	29.4	17.6	29.4	23.5	76.5
$LPL^{+/-}$	18	11.1	77.8	0	11.1	11.1	88.9
$LPL^{+/+}$	17	0	70.6	0	29.4	0	100
LPL^{Tg}	20	0	65.0	10.0	25.0	0	100

The TG-rich lipoproteins of all mice were classified as lipoproteins resembling $VLDL_1$, $VLDL_2$, and a larger category resembling $VLDL_0$. IDL-like lipoproteins were analyzed separately. The data for BL/6 mice are based on animals consuming an atherogenic diet for 12 weeks.

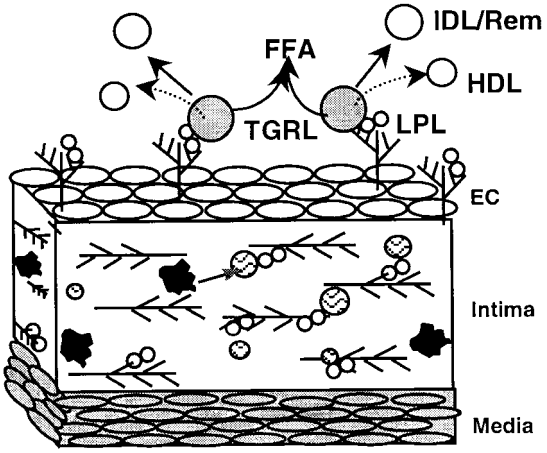

Fig. 4. Contrasting roles of plasma and vessel wall LPL in atherosclerosis. Attached to the luminal surface of the vascular endothelium, increased LPL (∞) is associated with increased hydrolysis of TGRL (○), resulting in decreased plasma TG concentrations and decreased atherosclerosis (As) susceptibility. Within the intima, however, increased LPL is associated with increased lipoprotein (⊛) retention, leading to increased macrophage (✎) lipoprotein uptake and increased foam cell formation.

ing the relationship between LPL and atherogenesis in humans (42–54). The only functional LPL variants frequent enough to allow investigators to study the relationship between LPL genetic variation and disease at the population level are three common mutations: N291S, D9N, and S447X. However, the effects of these variants on LPL catalytic function are mild (55), making it very difficult to discern differences in the absence of large sample sizes. Some studies have suggested that these variants in the LPL gene may be associated with an altered risk of developing or an increased progression or severity of atherosclerosis (42–48), while others have found no association (49–54). The data presented here provide support for a pro-atherogenic effect of the N291S and D9N variants, as these mutations retain near normal protein levels (55) but have catalytic defects associated with pro-atherogenic changes in lipids, and would thus be predicted to be associated with increased risk for atherosclerosis.

An intriguing finding in this study was that HDL cholesterol levels decreased with increasing LPL activity, particularly in the $E^{-/-}$ model. Of note, we have also shown increased HDL cholesterol levels in our heterozygous LPL-deficient feline model, another CETP-deficient system (56). Animals deficient in plasma CETP activity rely on other mechanisms of cholesterol delivery to the liver for efficient functioning of the reverse cholesterol transport pathway. Thus, selective cholesteryl ester (CE) uptake may be even more important in mice and other CETP-deficient animals. Recently, LPL has been shown to aid in the selective uptake of HDL-CE by macrophages and hepatic cells (57, 58). Thus, in the absence of CETP and apoE, which may also aid in the bridging of lipoproteins to the cell surface (59), a reduction in the amount of LPL protein may compromise the ability of HDL to deliver its CEs to the liver. This, in turn, would result in an increased plasma HDL cholesterol concentration. The lipid data presented in the $E^{-/-}$ model is thus consistent with LPL having a role in selective uptake of HDL-CE. Furthermore, this may explain why VLDL, the primary nonHDL particle in each model, was the predominant lipoprotein determining atherosclerotic susceptibility, and changes in HDL cholesterol levels did not appear to reflect changes in atherosclerosis susceptibility, as increasing HDL was not indicative of increased reverse cholesterol transport in these animals, and thus is not associated with protection against atherosclerosis.

In summary, we have demonstrated important roles for LPL in the initial stages of lesion formation, in two separate model systems. These roles in atherosclerosis are related to both the amount of vessel wall LPL protein available for functions such as trapping lipoproteins and to the level of plasma LPL activity influencing plasma lipid concentrations, as we have shown by comparing the effects of decreased plasma and vessel wall LPL with increased LPL in tissues contributing only to LPL in the plasma. Specifically, we provide in vivo evidence that increasing plasma LPL activity without altering macrophage, and hence vessel wall, LPL levels is associated with decreased lesion formation, while increased vessel wall LPL protein is pro-atherogenic. Whether these pro-atherogenic roles of vessel wall LPL are due solely to non-catalytic bridging functions or whether they are due at least in part to the localized generation of smaller particles which are more easily taken up via LPL catalytic activity cannot be determined from this study. Although these differences in lesion formation were small in relative terms, when extrapolated throughout an entire organism over a lifetime, such differences may have a significant impact on overall disease status. Our findings also provide further evidence as to the atherogenic nature of TGRL, and are consistent with a role of LPL in the selective uptake of HDL-CE. In conclusion, these findings suggest that therapies designed to increase LPL activity in the plasma, without increasing expression within the vessel wall, such as with targeted gene delivery, are likely to be of significant therapeutic potential in reducing the risk for atherosclerosis.

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REFERENCES

1. Brunzell, J. D. 1995. Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Inc., New York. 1913–1932.
2. Murthy, V., P. Julien, and C. Gagné. 1996. Molecular pathobiology

of the human lipoprotein lipase gene. *Pharmacol. Ther.* **70:** 101–135.
3. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
4. Wilson, D. E., M. Emi, P-H. Iverius, A. Hata, L. L. Wu, E. Hillas, R. R. Williams, and J-M. Lalouel. 1990. Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. *J. Clin. Invest.* **86:** 735–750.
5. Bijvoet, S., S. E. Gagné, S. Moorjani, H. E. Henderson, J-C. Fruchart, J. Dallongeville, P. Alaupovic, M. Prins, J. J. P. Kastelein, and M. R. Hayden. 1996. Alterations in plasma lipoproteins and apolipoproteins before the age of 40 in heterozygotes for lipoprotein lipase deficiency. *J. Lipid Res.* **37:** 640–650.
6. Nordestgaard, B. G., S. Abildgaard, H. H. Wittrup, R. Steffensen, G. Jensen, and A. Tybjærg-Hansen. 1997. Heterozygous lipoprotein lipase deficiency. Frequency in the general population, effect on plasma lipid levels, and risk of ischemic heart disease. *Circulation.* **96:** 1737–1744.
7. Gaziano, J. M., C. H. Hennekens, C. J. O'Donnell, J. L. Breslow, and J. E. Buring. 1997. Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. *Circulation.* **96:** 2520–2525.
8. McGill, H. C., Jr., A. McMahan, G. T. Malcom, M. C. Oalmann, and J. P. Strong. 1997. Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. *Arterioscler. Thromb. Vasc. Biol.* **17:** 95–106.
9. Mack, W. J., R. M. Krauss, and H. N. Hodis. 1996. Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiographic progression. *Arterioscler. Thromb. Vasc. Biol.* **16:** 697–704.
10. Alaupovic, P., W. J. Mack, C. Knight-Gibson, and H. N. Hodis. 1997. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler. Thromb. Vasc. Biol.* **17:** 715–722.
11. Mann, W. A., N. Meyer, W. Weber, F. Rinninger, H. Greten, and U. Beisiegel. 1995. Apolipoprotein E and lipoprotein lipase coordinately enhance binding and uptake of chylomicrons by human hepatocytes. *Eur. J. Clin. Invest.* **25:** 880–882.
12. Skottova, N., R. Savonen, A. Lookene, M. Hultin, and G. Olivecrona. 1995. Lipoprotein lipase enhances removal of chylomicrons and chylomicron remnants by the perfused rat liver. *J. Lipid Res.* **36:** 1334–1344.
13. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9375.
14. Chappell, D. A., I. Inoue, G. L. Fry, M. W. Pladet, S. L. Bowen, P-H. Iverius, J-M. Lalouel, and D. K. Strickland. 1994. Cellular catabolism of normal very low density lipoproteins via the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor is induced by the C-terminal domain of lipoprotein lipase. *J. Biol. Chem.* **269:** 18001–18006.
15. Ylä-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witztum. 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* **88:** 10143–10147.
16. O'Brien, K. D., D. Gordon, S. Deeb, M. Ferguson, and A. Chait. 1992. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* **89:** 1544–1550.
17. Renier, G., E. Skamene, J. B. DeSanctis, and D. Radzioch. 1993. High macrophage lipoprotein lipase expression and secretion are associated in inbred murine strains with susceptibility to atherosclerosis. *Arterioscler. Thromb.* **13:** 190–196.
18. Rumsey, S. C., J. C. Obunike, Y. Arad, R. J. Deckelbaum, and I. J. Goldberg. 1992. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J. Clin. Invest.* **90:** 1504–1512.
19. Lindqvist, P., A-M. Ostlund-Lindqvist, J. L. Witztum, D. Steinberg, and J. A. Little. 1983. The role of lipoprotein lipase in the metabolism of triglyceride-rich lipoproteins by macrophages. *J. Biol. Chem.* **258:** 9086–9092.
20. Aviram, M., E. L. Bierman, and A. Chait. 1988. Modification of low density lipoprotein by lipoprotein lipase or hepatic lipase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **263:** 15416–15422.
21. Eisenberg, S., E. Sehayek, T. Olivecrona, and I. Vlodavsky. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.* **90:** 2013–2021.
22. Saxena, U., M. G. Klein, T. M. Vanni, and I. J. Goldberg. 1992. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. *J. Clin. Invest.* **89:** 373–380.
23. Edwards, I. J., I. J. Goldberg, J. S. Parks, H. Xu, and W. D. Wagner. 1993. Lipoprotein lipase enhances the interaction of low density lipoproteins with artery-derived extracellular matrix proteoglycans. *J. Lipid Res.* **34:** 1155–1163.
24. Rutledge, J. C., M. M. Woo, A. A. Rezai, L. K. Curtiss, and I. J. Goldberg. 1997. Lipoprotein lipase increases lipoprotein binding to the artery wall and increases endothelial layer permeability by formation of lipolysis products. *Circ. Res.* **80:** 819–828.
25. Rutledge, J. C., and I. J. Goldberg. 1994. Lipoprotein lipase (LpL) affects low density lipoprotein (LDL) flux through vascular tissue: evidence that LpL increases LDL accumulation in vascular tissue. *J. Lipid Res.* **35:** 1152–1160.
26. Tabas, I., Y. Li, R. W. Brocia, S. W. Xu, T. L. Swenson, and K. J. Williams. 1993. Lipoprotein lipase and sphingomyelinase synergistically enhance the association of atherogenic lipoproteins with smooth muscle cells and extracellular matrix. A possible mechanism for low density lipoprotein and lipoprotein(a) retention and macrophage foam cell formation. *J. Biol. Chem.* **268:** 20419–20432.
27. Hendriks, W. L., H. van der Boom, L. C. van Vark, and L. M. Havekes. 1996. Lipoprotein lipase stimulates the binding and uptake of moderately oxidized low-density lipoprotein by J774 macrophages. *Biochem. J.* **314:** 563–568.
28. Corey, J. E., and D. B. Zilversmit. 1977. Effect of cholesterol feeding on arterial lipolytic activity in the rabbit. *Atherosclerosis.* **27:** 201–212.
29. Obunike, J. C., S. Paka, S. Pillarisetti, and I. J. Goldberg. 1997. Lipoprotein lipase can function as a monocyte adhesion protein. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1414–1420.
30. Mamputu, J-C., A-C. Desfaits, and G. Renier. 1997. Lipoprotein lipase enhances human monocyte adhesion to aortic endothelial cells. *J. Lipid Res.* **38:** 1722–1729.
31. Coleman, T., R. L. Seip, J. M. Gimble, D. Lee, N. Maeda, and C. F. Semenkovich. 1995. COOH-terminal disruption of lipoprotein lipase in mice is lethal in homozygotes, but heterozygotes have elevated triglycerides and impaired enzyme activity. *J. Biol. Chem.* **270:** 12518–12525.
32. Liu, M-S., R. C. LeBoeuf, H. Henderson, L. W. Castellani, A. J. Lusis, Y. Ma, I. J. Forsythe, H. Zhang, E. Kirk, J. D. Brunzell, and M. R. Hayden. 1994. Alteration of lipid profiles in plasma of transgenic mice expressing human lipoprotein lipase. *J. Biol. Chem.* **269:** 11417–11424.
33. Clee, S. M., H. Zhang, N. Bissada, L. Miao, E. Ehrenborg, P. Benlian, G. X. Shen, A. Angel, R. C. Le Boeuf, and M. R. Hayden. 1997. Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. *J. Lipid Res.* **38:** 2079–2089.
34. Paigen, B., A. Morrow, P. A. Holmes, D. Mitchell, and R. A. Williams. 1987. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis.* **68:** 231–240.
35. Semenkovich, C. F., T. Coleman, and A. Daugherty. 1998. Effects of heterozygous lipoprotein lipase deficiency on diet-induced atherosclerosis in mice. *J. Lipid Res.* **39:** 1141–1151.
36. Babaev, V. R., S. Fazio, L. A. Gleaves, K. J. Carter, C. F. Semenkovich, and M. F. Linton. 1999. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in vivo. *J. Clin. Invest.* **103:** 1697–1705.
37. Shimada, M., S. Ishibashi, T. Inaba, H. Yagyu, K. Harada, J-I. Osuga, K. Ohashi, Y. Yazaki, and Y. Nobuhiro. 1996. Suppression of diet-induced atherosclerosis in low density lipoprotein receptor knockout mice overexpressing lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* **93:** 7242–7246.
38. Yagyu, H., S. Ishibashi, Z. Chen, J-I. Osuga, M. Okazaki, S. Perrey, T. Kitamine, M. Shimada, K. Ohashi, K. Harada, F. Shionoiri, N. Yahagi, T. Gotoda, Y. Yazaki, and N. Yamada. 1999. Overexpressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice. *J. Lipid Res.* **40:** 1677–1685.
39. Ashbourne Excoffon, K. J. D., G. Liu, L. Miao, J. E. Wilson, B. M. McManus, C. F. Semenkovich, T. Coleman, P. Benoit, N. Duverger,

D. Branellec, P. Denefle, M. R. Hayden, and M. E. S. Lewis. 1997. Correction of hypertriglyceridemia and impaired fat tolerance in lipoprotein lipase-deficient mice by adenovirus-mediated expression of human lipoprotein lipase. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2532–2539.
40. Auwerx, J., P. Leroy, and K. Schoonjans. 1992. Lipoprotein lipase: recent contributions from molecular biology. *Crit. Rev. Clin. Lab. Sci.* **29:** 243–268.
41. Ma, Y., H. E. Henderson, M. R. V. Murthy, G. Roederer, M. V. Monsalve, L. A. Clarke, T. Normand, P. Julien, C. Gagné, M. Lambert, J. Davignon, P. J. Lupien, J. Brunzell, and M. R. Hayden. A mutation in the human lipoprotein lipase gene as the most common cause of familial chylomicronemia in French Canadians. *N. Engl. J. Med.* **324:** 1761–1766.
42. Peacock, R. E., A. Hamsten, P. Nilsson-Ehle, and S. E. Humphries. 1992. Associations between lipoprotein lipase gene polymorphisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. *Atherosclerosis.* **97:** 171–185.
43. Jemaa, R., F. Fumeron, O. Poirier, L. Lecerf, A. Evans, D. Arveiler, G. Luc, J-P. Cambou, J-M. Bard, J-C. Fruchart, M. Apfelbaum, F. Cambien, and L. Tiret. 1995. Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study. *J. Lipid Res.* **36:** 2141–2146.
44. Kastelein, J. J. P., B. E. Groenemeyer, D. M. Hallman, H. Henderson, P. W. A. Reymer, S. E. Gagné, H. Jansen, J. C. Seidell, D. Kromhout, J. W. Jukema, A. V. G. Bruschke, E. Boerwinkle, and M. R. Hayden. 1998. The Asn9 variant of lipoprotein lipase is associated with the -93G promoter mutation and an increased risk of coronary artery disease. *Clin. Genet.* **53:** 27–33.
45. Humphries, S. E., V. Nicaud, J. Margalef, L. Tiret, and P. J. Talmud. 1998. Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides. The European Atherosclerosis Research Study (EARS). *Arterioscler. Thromb. Vasc. Biol.* **18:** 526–534.
46. Jukema, J. W., A. J. van Boven, B. Groenemeijer, A. H. Zwinderman, J. H. C. Reiber, A. V. G. Bruschke, J. A. Henneman, G. P. Molhoek, T. Bruin, H. Jansen, E. Gagné, M. R. Hayden, and J. J. P. Kastelein. 1996. The Asp9Asn mutation in the lipoprotein lipase gene is associated with increased progression of coronary atherosclerosis. *Circulation*. **94:** 1913–1918.
47. Wittekoek, M. E., S. N. Pimstone, P. W. A. Reymer, L. Feuth, G-J. Botma, J. C. Defesche, M. Prins, M. R. Hayden, and J. J. P. Kastelein. 1998. A common mutation in the lipoprotein lipase gene (N291S) alters the lipoprotein phenotype and risk for cardiovascular disease in patients with familial hypercholesterolemia. *Circulation*. **97:** 729–735.
48. Wittrup, H. H., A. Tybjærg-Hansen, S. Abildgaard, R. Steffensen, P. Schnohr, and B. G. Nordestgaard. 1997. A common substitution (Asn291Ser) in lipoprotein lipase is associated with increased risk of ischemic heart disease. *J. Clin. Invest.* **99:** 1606–1613.
49. Mattu, R. K., E. W. A. Needham, R. Morgan, A. Rees, A. K. Hackshaw, J. Stocks, P. C. Elwood, and D. J. Galton. 1994. DNA variants at the LPL gene locus associated with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population. *Arterioscler. Thromb.* **14:** 1090–1097.
50. Fisher, R. M., F. Mailly, R. E. Peacock, A. Hamsten, M. Seed, J. S. Yudkin, U. Beisiegel, G. Feussner, G. Miller, S. E. Humphries, and P. J. Talmud. 1995. Interaction of the lipoprotein lipase asparagine 291→serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults. *J. Lipid Res.* **36:** 2104–2112.
51. Zhang, Q., J. Cavanna, B. R. Winkelman, B. Shine, W. Gross, W. Marz, and D. J. Galton. 1995. Common genetic variants of lipoprotein lipase that relate to lipid transport in patients with premature coronary artery disease. *Clin. Genet.* **48:** 293–298.
52. Gerdes, C., R. M. Fisher, V. Nicaud, J. Boer, S. E. Humphries, P. J. Talmud, and O. Faergeman. 1997. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations. Studies in the fasting and postprandial states: the European Atherosclerosis Research Studies. *Circulation*. **96:** 733–740.
53. Mailly, F., R. M. Fisher, V. Nicaud, L-A. Luong, A. E. Evans, P. Marques-Vidal, G. Luc, D. Arveiler, J. M. Bard, O. Poirier, P. J. Talmud, and S. E. Humphries. 1996. Association between the LPL-D9N mutation in the lipoprotein lipase gene and plasma lipid traits in myocardial infarction survivors from the ECTIM study. *Atherosclerosis.* **122:** 21–28.
54. Mailly, F., Y. Tugrul, P. W. A. Reymer, T. Bruin, M. Seed, B. E. Groenemeijer, A. Asplund-Carlson, D. Vallance, A. F. Winder, G. J. Miller, J. J. P. Kastelein, A. Hamsten, G. Olivecrona, S. E. Humphries, and P. J. Talmud. 1995. A common variant in the gene for lipoprotein lipase (Asp9→Asn). Functional implications and prevalence in normal and hypertriglyceridemic subjects. *Arterioscler. Thromb. Vasc. Biol.* **15:** 468–478.
55. Zhang, H., H. Henderson, S. E. Gagné, S. M. Clee, L. Miao, G. Liu, and M. R. Hayden. 1996. Common sequence variants of lipoprotein lipase: standardized studies of in vitro expression and catalytic function. *Biochim. Biophys. Acta.* **1302:** 159–166.
56. Ginzinger, D. G., S. M. Clee, J. Dallongeville, M. E. S. Lewis, H. E. Henderson, E. Bauje, Q. R. Rogers, D. R. Jensen, R. H. Eckel, R. Dyer, S. Innis, B. Jones, J-C. Fruchart, and M. R. Hayden. 1999. Lipid and lipoprotein analysis of cats with lipoprotein lipase deficiency. *Eur. J. Clin. Invest.* **29:** 17–26.
57. Rinninger, F., T. Kaiser, W. A. Mann, N. Meyer, H. Greten, and U. Beisiegel. 1998. Lipoprotein lipase mediates an increase in the selective uptake of high density lipoprotein-associated cholesteryl esters by hepatic cells in culture. *J. Lipid Res.* **39:** 1335–1348.
58. Panzenboeck, U., A. Wintersberger, S. Levak-Frank, R. Zimmermann, R. Zechner, G. M. Kostner, E. Malle, and W. Sattler. 1997. Implications of endogenous and exogenous lipoprotein lipase for the selective uptake of HDL_3-associated cholesteryl esters by mouse peritoneal macrophages. *J. Lipid Res.* **38:** 239–253.
59. van Barlingen, H. H. J. J., H. de Jong, D. W. Erkelens, and T. W. A. de Bruin. 1996. Lipoprotein lipase-enhanced binding of human triglyceride-rich lipoproteins to heparan sulfate: modulation by apolipoprotein E and apolipoprotein C. *J. Lipid Res.* **37:** 754–763.