Macrophage Lipoprotein Lipase Promotes Foam Cell Formation and Atherosclerosis in Low Density Lipoprotein Receptor-deficient Mice

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The role of macrophage lipoprotein lipase (LPL) expression in atherosclerotic lesion formation was examined in low density lipoprotein receptor (LDLR−/−) mice using dietary conditions designed to induce either fatty streak lesions or complex atherosclerotic lesions. First, LDLR−/− mice chimeric for macrophage LPL expression were created by transplantation of lethally irradiated female LDLR−/− mice with LPL−/− (n = 12) or LPL+/+ (n = 14) fetal liver cells as a source of hematopoietic cells. To induce fatty streak lesions, these mice were fed a Western diet for 8 weeks, resulting in severe hypercholesterolemia. There were no differences in plasma post-heparin LPL activity, serum lipid levels, or lipoprotein distribution between these two groups. The mean lesion area in the proximal aorta in LPL−/− → LDLR−/− mice was significantly reduced by 33% compared with LPL+/+ → LDLR−/− mice, and a similar reduction (38%) in lesion area was found by en face analysis of the aortae. To induce complex atherosclerotic lesions, female LDLR−/− mice were lethally irradiated, transplanted with LPL−/− (n = 14), LPL+/− (n = 13), or LPL+/+ (n = 14) fetal liver cells, and fed the Western diet for 19 weeks. Serum cholesterol and triglyceride levels did not differ between the three groups. After 19 weeks of diet, the lesions in the proximal aorta were complex with relatively few macrophages expressing LPL protein and mRNA in LPL+/+ → LDLR−/− mice. Analysis of cross-sections of the proximal aorta demonstrated no differences in the extent of lesion area between the groups, whereas en face analysis of the aortae revealed a dose-dependent effect of macrophage LPL on mean aortic lesion area in LPL−/− → LDLR−/−, LPL+/− → LDLR−/−, and LPL+/+ → LDLR−/− mice (1.8 ± 0.2%, 3.5 ± 0.5% and 5.9 ± 0.8%, respectively). Taken together, these data indicate that macrophage LPL expression in the artery wall promotes atherogenesis during foam cell lesion formation, but this impact may be limited to macrophage-rich lesions.

Lipoprotein lipase (LPL) is the rate-limiting enzyme for hydrolysis of lipoprotein triglycerides (1). The majority of LPL synthesis occurs in adipose and muscle tissues, and LPL is then transported to the luminal surface of the vascular endothelium where it is bound to heparan sulfate proteoglycans (2). LPL is also synthesized by macrophages and macrophage-derived foam cells in atherosclerotic lesions (3, 4). LPL has been proposed to play a dual role in atherogenesis. The efficient lipolysis of triglyceride-rich lipoproteins, the promotion of rapid clearance of post-prandial lipoproteins, and the generation of material for HDL formation are viewed as antiatherogenic effects of LPL (5). In contrast, local LPL activity in the artery wall has been proposed to promote atherosclerosis (6). Recently, LPL has been proposed to influence atherogenesis by mechanisms that are independent from its catalytic actions on the plasma lipoproteins. An increasing amount of evidence indicates that LPL also functions as a ligand, associating with lipoproteins and promoting their binding to the LDLR-related protein (7, 8), LDLR (9, 10), VLDL receptor (11), and extracellular proteoglycans (12, 13). LPL may promote atherogenesis by increasing the binding and retention of LDL cholesterol by proteoglycans of the subendothelial matrix (14–16).

Macrophage-derived foam cells are present in all stages of atherosclerosis and are believed to play an important role in both the initiation and progression of atherosclerotic lesions (17). In addition, lesions rich in macrophage-derived foam cells may be more prone to plaque rupture (18). A number of in vitro experiments have suggested that macrophage LPL expression may promote foam cell formation (19–21). Recently, we have reported that C57BL/6 mice reconstituted with LPL−/− macrophages develop significantly less atherosclerosis than control mice wild type for macrophage LPL expression (22). These studies demonstrated for the first time that macrophage LPL expression promotes foam cell formation and atherosclerotic lesion formation in vivo (22). However, C57BL/6 mice clearly have limitations as a model for human atherosclerosis. In response to an atherogenic diet, C57BL/6 mice develop mild hypercholesterolemia with β-VLDL accumulation and modest fatty streak lesions located exclusively in the proximal aorta (23). In contrast, LDL receptor-deficient (LDLR−/−) mice have enhanced susceptibility to diet-induced atherosclerosis (24, 25). On a high fat diet, LDLR−/− mice develop severe hypercholesterolemia with VLDL, IDL, and LDL accumulation and extensive atherosclerotic lesions throughout the aorta (24, 25). Fur-
In the current study, the contribution of macrophage LPL expression to atherosclerotic lesion formation was examined in LDLR<sup>−/−</sup> mice. LDLR<sup>−/−</sup> mice chimeric for macrophage expression of LPL were generated by transplantation of lethally irradiated LDLR<sup>−/−</sup> mice with LPL<sup>+/−</sup>, LPL<sup>+/+</sup>, and LPL<sup>++</sup> fetal liver cells (FLC) as a source of hematopoietic cells. The mice were fed the Western diet for 8 or 19 weeks to induce fatty streak lesions or complex atherosclerotic lesions. However, the impact of macrophage LPL on the extent of lesion area in the proximal aorta is lost in the setting of complex atherosclerotic lesions.

**EXPERIMENTAL PROCEDURES**

**Animal Procedures**—A colony of mice with LPL gene inactivation by homologous recombination (28) is established in our facility. Mice heterozygous for inactivation of the LPL gene were at the 7th or higher backcross into the C57BL/6 background. Recipient LDLR<sup>−/−</sup> mice were lethally irradiated (9 Gy) from a cesium gamma source, and 4 h later, 5 mice were transplanted with FLC, total RNA was extracted from bone marrow, and the mice were sacrificed and flushed with saline by injection through the left ventricle. The aorta was dissected from the proximal aorta to the iliac bifurcation, and the aortas were pinned out in an en face preparation as described previously (29). The heart with the proximal aorta was embedded in OCT and snap-frozen in liquid N2. Cryosections of 10-micron thickness were cut from the region of the proximal aorta starting from the end of the aortic sinus and for 300 μm distally, according to the method of Paigen et al. (30), adapted for computer analysis (31).

**Reverse Transcriptase PCR**—To verify genotype changes in LDLR<sup>−/−</sup> mice can be modified by varying the duration of the high fat diet, providing the opportunity to study the impact of macrophage LPL expression at different stages of atherosclerotic lesion formation. The effect of LPL-induced lipid changes on atherogenesis has been investigated previously by overexpressing human LPL in LDLR<sup>−/−</sup> mice. LDL receptor-deficient mice overexpressing human LPL were protected from hypercholesterolemia (26) and on a high fat diet, they had reduced (18-fold compared with the control) atherosclerotic lesions (27). The results are consistent with a beneficial effect of human LPL on the plasma lipoprotein profile. The goal of our current study is to examine the role of macrophage LPL in atherogenesis in LDLR<sup>−/−</sup> mice using dietary conditions designed to induce either fatty streak lesions or complex atherosclerotic lesions.

**Fetal Liver Cell Collection**—Fetal liver cells (FLC) as a source of hematopoietic cells. The recipient LDLR<sup>−/−</sup> mice were given 100 mg/liter neomycin at 55 °C, and 59 s at 72 °C. Serum Cholesterol and Triglyceride Analysis—Mice were fasted for 4 h, and blood samples were collected by retro-orbital venous puncture under metofane anesthesia. Serum was separated by centrifugation, and 1 ml phenylmethylsulfonyl fluoride was added (Sigma). The serum total cholesterol and triglycerides were determined using Sigma kit no. 352 and 339 adapted for micrometer plate assay. HDL cholesterol concentration was measured on an automated ACE analyzer using the Direct HDL Test (no. 10981) from Schiapparelli Biosystems, Inc. (Fairfield, NJ).

**Lipoprotein Separation**—Serum from mice was subjected to fast performance liquid chromatography analysis using a Superox 6 column from Amersham Pharmacia Biotech on a Waters high pressure liquid chromatography system, model 600 (Milford, MA). A 100-μl aliquot of serum was injected onto the column and separated with a buffer containing 0.15 mM NaCl, 0.01 mM Na2HPO4, 0.1 mM EDTA, pH 7.5, at a flow rate of 0.5 ml/min. Forty 0.5-ml fractions were collected and tubes 11–40 were analyzed for cholesterol.

**Quantitation of Atherosclerotic Lesions—**After 8 or 19 weeks on the Western diet, mice were sacrificed and flushed with saline by injection through the left ventricle. The aorta was dissected from the proximal aorta to the iliac bifurcation, and the aortas were pinned out in an en face preparation as described previously (29). The heart with the proximal aorta was embedded in OCT and snap-frozen in liquid N2. Cryosections of 10-micron thickness were cut from the region of the proximal aorta starting from the end of the aortic sinus and for 300 μm distally, according to the method of Paigen et al. (30), adapted for computer analysis (31).

**Statistical Analysis—**To examine the relationship between serum cholesterol and the extent of atherosclerosis, linear regression analysis was performed and the correlation coefficient was calculated using the SigmaStat 2.0 program (Jandel Scientific Inc.). Mean serum cholesterol levels representing an average serum cholesterol level for individual mice while on the Western diet were used for analysis of the correlation between serum cholesterol levels and the extent of atherosclerosis in the proximal aorta. The statistical significance of differences in mean aortic lesion areas between the groups were determined using the Student’s t test.

**RESULTS**

The role of macrophage LPL expression in atherosclerotic lesion formation was examined in LDLR<sup>−/−</sup> mice using dietary conditions to induce either fatty streak lesions or advanced atherosclerotic lesions. First, six-week-old female LDLR<sup>−/−</sup> mice were lethally irradiated and Gypsy transreconstituted with 5 × 10<sup>6</sup> FLC from female LDLR<sup>−/−</sup> (n = 9, experimental group) or LDLR<sup>++</sup> (n = 13, control group) embryos. Eight weeks post-transplantation, the mice were challenged with the Western type diet containing 21% fat and 0.15% cholesterol for 8 weeks. Next, 8-week-old female LDLR<sup>−/−</sup> mice were lethally irradiated and transplanted with 5 × 10<sup>6</sup> FLC from female LDLR<sup>−/−</sup> (n = 14), LDLR<sup>−/−</sup> (n = 12), or LDLR<sup>++</sup> (n = 13, control group)
donor mice, and 8 weeks post-transplantation, these mice were fed the Western diet for 19 weeks.

A change in macrophage genotype of transplanted mice was verified by reverse transcriptase PCR using total RNA extracted from bone marrow at sacrifice. LPL mRNA expression was detected in bone marrow from cells in LPL+/→ LDLR−/− but not in LPL−/→ LDLR−/− mice (Fig. 1).

Eight weeks after transplantation, the mean total serum cholesterol and triglyceride levels did not differ between the groups on a Chow diet containing 4.5% fat in either experiment (Tables I and II). When the mice were fed the Western diet, levels of serum cholesterol or triglyceride did not differ between the groups in either experiment (Tables I and II). When the mice were fed the Western diet, the mean area of cross-sections of the proximal aorta (μm² ± S.E.) in LPL−/→ LDLR−/− mice (94,026 ± 4864) was reduced by 32.6% compared with LPL+/→ LDLR−/− mice (139,569 ± 13,244; p < 0.013) (Fig. 5A). A 38% reduction in lesion area was noted by en face analysis of aortas of LPL+/→ LDLR−/− mice (0.73 ± 0.06%) compared with LPL+/→ LDLR−/− mice (1.18 ± 0.09%; p < 0.001; Fig. 5B). The ratio of macrophage area/Oil Red O staining area was 0.71–0.81. There was a significant correlation between the extent of lesion area in the proximal aorta and the aorta en face (n = 16; r = 0.65; p < 0.006; Fig. 5C).

After 19 weeks of the Western diet, visual inspection of the en face aorta revealed a dramatic difference in the extent of atherosclerotic lesions between the groups, with less atherosclerosis in the LPL+/→ LDLR−/− mice (Fig. 6). Surprisingly, quantitative analysis of the extent of atherosclerosis in the proximal aorta revealed no differences in the extent of lesion area between LPL+/→ LDLR−/−, LPL−/→ LDLR−/−, and LPL−/→ LDLR−/− mice with mean lesion areas (μm² ± S.E.) of 421,303 ± 25,701, 461,243 ± 47,554, and 372,897 ± 46,663, respectively (Fig. 7A). The ratio of macrophage area/Oil Red O-staining area was 0.16–0.22, indicating that the lesions were complex and contained few macrophages. In contrast, quantitative analysis of the extent of the aortic lesion area by the en face approach revealed a dose-dependent effect of LPL expression by macrophages with a stepwise decrease in the mean aortic lesion area of LPL+/→ LDLR−/−, LPL−/→ LDLR−/−, and LPL−/→ LDLR−/− mice (5.9 ± 0.8%, 3.5 ± 0.5% and 1.8 ± 0.2%, respectively; Fig. 7B). The extent of atherosclerosis is significantly reduced in LPL+/→ LDLR−/− mice and LPL−/→ LDLR−/− mice compared with LPL+/→ LDLR−/− mice by the Student’s t test (p = 0.014 and p < 0.0001, respectively). Furthermore, the extent of atherosclerosis in the LPL+/→ LDLR−/− mice is also significantly reduced compared with LPL−/→ LDLR−/− mice (p = 0.004). There was no correlation between the extent of lesion area in the proximal aorta and the aorta en face (n = 26; r = 0.14; p < 0.50; Fig. 7C). In both the short and long-term experiments, there was no correlation between the mean lesion area in the proximal aorta and serum total cholesterol levels (n = 26; r = 0.36; p = 0.11, and n = 41; r = 0.07; p = 0.69, respectively) or HDL cholesterol levels (r = 0.24; p = 0.38 and r = 0.14; p = 0.48, respectively).

**DISCUSSION**

In the present study, the role of macrophage LPL expression was examined in LDLR−/− mice transplanted with LPL−/− or LPL+/− FLC under dietary conditions designed to induce atherosclerotic lesions of varying severity, from macrophage-derived foam cells to more complex atherosclerotic lesions.
Lesion area in the LPL

Thus, macrophage LPL does not significantly influence VLDL-IDL-LDL cholesterol (Fig. 2), but levels of serum cholesterol and triglycerides. The majority of LPL is synthesized by the liver and other non-lipoprotein-associated proteins. The difference is statistically significant comparison to the control group, LPL⁻/⁻ → LDLR⁻/⁻ at that time point.

The first experiment, LDLR⁻/⁻ mice reconstituted with either LPL⁻/⁻ or LPL⁺/⁺ macrophages were fed the Western diet for 8 weeks to induce foam cell lesion formation. The mice developed severe hypercholesterolemia because of accumulations of VLDL-IDL-LDL cholesterol (Fig. 2), but levels of serum cholesterol and triglycerides did not differ between the two groups (Table I). Thus, macrophage LPL does not significantly influence plasma lipoprotein metabolism even under conditions of severe hypercholesterolemia in LDLR⁻/⁻ mice. The mean aortic lesion area in the LPL⁻/⁻ → LDLR⁻/⁻ mice was significantly reduced compared with the LPL⁺/⁺ → LDLR⁻/⁻ mice using either the en face analysis of aortic lesion area (38% reduction; Fig. 5B) or the Paigen approach to the analysis of lesions in cross-sections of the proximal aorta (33% reduction; Fig. 5A). These results extend our previous findings in C57BL/6 mice (22) by demonstrating that even in conditions of extreme hypercholesterolemia because of the absence of the LDLR, macrophage LPL expression promotes foam cell formation and atherosclerotic lesion development in vivo.

In the second experiment, LDLR⁻/⁻ mice reconstituted with either LPL⁺/⁺, LPL⁻/⁻, or LPL⁻/⁻ macrophages were fed the Western diet for 19 weeks to induce complex atherosclerotic lesions. After 19 weeks of the Western diet, a dose-dependent reduction in atherosclerotic lesion area was seen in en face analyses of the aortae of LPL⁻/⁻ → LDLR⁻/⁻ (69%) and LPL⁺/⁺ → LDLR⁻/⁻ (41.5%) mice compared with LPL⁺/⁺ → LDLR⁻/⁻ mice. Again, these results strongly support a proatherogenic role for macrophage LPL expression in vivo. Surprisingly, the extent of the lesion area in the proximal aorta did not differ between LDLR⁻/⁻ mice reconstituted with LPL⁺/⁺, LPL⁻/⁻, or LPL⁻/⁻ macrophages fed the Western diet for 19 weeks. This may be explained by the fact that mice develop atherosclerotic lesions first in the proximal aorta and the lesions progress distally, resulting in more advanced lesions in the proximal than the distal aorta. After 19 weeks on the Western diet, the lesions were extremely complicated in the proximal aorta (Fig. 3). The fact that no correlation was found between the extent of lesion area in the proximal aorta and the en face approach in these mice after 19 weeks on the Western diet (Fig. 7C) is consistent with the proposition that atherosclerotic lesions in the proximal aorta were more advanced and thus out of step with lesions in the rest of the aorta. In support of this hypothesis, we have recently observed that, after 15 weeks on the Western diet, LPL⁺/⁺ → LDLR⁻/⁻ mice already have lesions in the proximal aorta that are complex, whereas lesions in the abdominal aorta are fatty streaks containing only macrophage-derived foam cells (data not shown). The ratio of macrophage area/Oil Red O-staining area in the proximal aorta of these mice was 4 times less compared with the ratio in mice fed the Western diet for 8 weeks. In contrast, after 8 weeks on the Western diet the lesions in the proximal aorta consisted almost entirely of macrophage-derived foam cells, and there was a significant correlation in the extent of lesion area measured by the two independent techniques (Fig. 5C). Tangirala et al. (33) have previously reported a significant correlation between the extent of lesions in the entire aorta and in the proximal aorta of LDLR⁻/⁻ mice on a high fat diet. Our results indicate that the correlation between the extent of lesion area as determined by en face analysis and the Paigen approach is lost when lesions in the proximal aorta become complicated.

LPL is the rate-limiting enzyme for the hydrolysis of lipoprotein triglycerides. The majority of LPL is synthesized by the muscle and adipose tissues, and LPL is transported to the vascular endothelium, where it hydrolyzes lipoprotein triglycerides. We have previously reported that levels of serum lipids, lipoproteins, and post-heparin plasma LPL activity did not differ between C57BL/6 mice reconstituted with LPL⁻/⁻ or LPL⁺/⁺ macrophages (22). These studies demonstrated that macrophage LPL expression does not significantly contribute

### Table I

**Total serum cholesterol and triglyceride levels in female LDLR⁻/⁻ mice transplanted with LPL⁺/⁺ or LPL⁻/⁻ fetal liver cells**

| Group of animals | Serum lipid | Baseline chow diet | 4 weeks Western diet | 8 weeks Western diet |
|-----------------|-------------|--------------------|---------------------|---------------------|
| LPL⁺/⁺ → LDLR⁻/⁻ | Cholesterol | 208 ± 6            | 700 ± 24            | 669 ± 24            |
| n = 14          | Triglycerides| 75 ± 6             | 214 ± 24            | 246 ± 23            |
| LPL⁻/⁻ → LDLR⁻/⁻ | Cholesterol | 222 ± 5            | 718 ± 41            | 689 ± 40            |
| n = 12          | Triglycerides| 65 ± 3             | 275 ± 24            | 207 ± 16            |

Values are in mg/dl (mean ± S.E.). The number of animals in each group is indicated by n. The differences were not statistically significant between the groups at all time points.

### Table II

**Total serum cholesterol and triglyceride levels in female LDLR⁻/⁻ mice transplanted with LPL⁺/⁺, LPL⁻/⁻, or LPL⁻/⁻ fetal liver cells**

| Group of animals | Serum lipid | Baseline chow diet | 4 weeks Western diet | 12 weeks Western diet | 16 weeks Western diet |
|-----------------|-------------|--------------------|---------------------|----------------------|----------------------|
| LPL⁺/⁺ → LDLR⁻/⁻ | Cholesterol | 218 ± 6            | 655 ± 16            | 778 ± 24             | 849 ± 154            |
| n = 14          | Triglycerides| 90 ± 5             | 234 ± 17            | 197 ± 15             | 382 ± 38             |
| LPL⁻/⁻ → LDLR⁻/⁻ | Cholesterol | 261 ± 5            | 598 ± 17            | 841 ± 41             | 916 ± 41             |
| n = 13          | Triglycerides| 110 ± 21           | 187 ± 14            | 350 ± 51             | 382 ± 76             |
| LPL⁻/⁻ → LDLR⁻/⁻ | Cholesterol | 236 ± 6            | 634 ± 21            | 723 ± 21             | 827 ± 41             |
| n = 14          | Triglycerides| 87 ± 6             | 216 ± 15            | 270 ± 22             | 474 ± 55             |

*The difference is statistically significant comparison to the control group, LPL⁺/⁺ → LDLR⁻/⁻ at that time point.*

**Fig. 2.** Lipoprotein distribution in LDLR⁻/⁻ mice transplanted with LPL⁺/⁺ and LPL⁻/⁻ FLC after 8 weeks of the Western diet (A), and LPL⁺/⁺, LPL⁻/⁻, and LPL⁻/⁻ FLC after 19 weeks of the diet (B). Mice were fasted for 4 h. Lipoprotein distribution was determined by fast protein liquid chromatography followed by cholesterol analysis of each fraction. Data are represented as an average (n = 3) distribution of total cholesterol. Fractions 14–17 contain VLDL; fractions 18–24 are IDL/LDL; and fractions 25–28 contain HDL. Fractions 29–40 are the nonlipoprotein-associated proteins.
to the metabolism of plasma lipoproteins or the pool of LPL attached to the vascular endothelium in vivo. However, it is possible that a contribution of macrophage LPL to lipoprotein metabolism could be detected under conditions where LPL is limiting, such as in LDLR<sup>−/−</sup> mice (34). Lipolysis mediated by macrophage LPL results in the local release of free fatty acid, which could serve as potential ligands for PPARs. Recent evidence points to a role for PPARγ in foam cell formation (35, 36). Therefore, we propose that LPL might promote foam cell formation by enriching the

A number of mechanisms by which macrophage LPL may be proatherogenic have been proposed. Local hydrolysis of lipoproteins in the artery wall with release of free fatty acids may increase the local production of atherogenic remnant lipoproteins (34). Lipolysis mediated by macrophage LPL results in the local release of free fatty acid, which could serve as potential ligands for PPARs. Recent evidence points to a role for PPARγ in foam cell formation (35, 36). Therefore, we propose that LPL might promote foam cell formation by enriching the
microenvironment with free fatty acids that stimulate PPARs. Noncatalytic functions of LPL proposed to be proatherogenic include: (a) binding of both lipoproteins and cell proteoglycans, causing the retention of lipoproteins and facilitating cell lipid uptake (5, 15); (b) direct effect of LPL as a ligand for the receptors promoting cell uptake of lipoproteins (14, 16); and (c) promotion of the oxidation of LDL cholesterol (37). Mild oxidation of LDL and VLDL increases their affinity for binding to LPL, compared with native LDL and VLDL or extensively oxidized LDL (38).

FIG. 5. Atherosclerotic lesion area in the proximal aorta (A) and aorta en face preparation (B) in LDLR−/− mice transplanted with LPL+/+ and LPL−/− FLC after 8 weeks on the Western diet. The extent of atherosclerotic lesions was quantified using Oil-Red O-stained sections. Fifteen alternate 10-micron sections from the beginning of the proximal aorta were examined for each mouse, using a computer-assisted video imaging system (A). En face preparation of aortas were stained with Sudan IV and analyzed by video imaging system, and data are represented as the average mean of lesion area for each mouse and mean level for each group (B). C shows the correlation between the extent of atherosclerosis in the aortic tree and in the proximal aorta. Individual values shown are LDLR−/− mice transplanted with LPL+/+ (closed circles) and LPL−/− (open circles) FLC.

FIG. 6. Atherosclerotic lesions in aorta en face in LDLR−/− mice transplanted with LPL+/+, LPL+/−, and LPL−/− fetal liver cells. The pinned aortae were stained with Sudan IV, and the atherosclerotic lesion area was measured by image analysis.

FIG. 7. Atherosclerotic lesion area in LDLR−/− mice transplanted with LPL+/+, LPL+/−, and LPL−/− FLC after 19 weeks on the Western diet. The atherosclerotic lesions were stained and quantified as noted in Fig. 5. Data are represented as the average mean lesion area in cross-sections of the proximal aorta (A), and the percentage of lesion area in the aorta by en face analysis for each mouse and the mean for each group (B). The extent of atherosclerosis is significantly reduced in LPL+/−→LDLR−/− mice and LPL+/−→LDLR−/− mice compared with LPL+/+→LDLR−/− mice by the Student’s t test (p = 0.014 and p < 0.0001, respectively). The extent of atherosclerosis in the LPL+/−→LDLR−/− mice is also significantly reduced compared with LPL+/−→LDLR−/− mice by the Student’s t test (p = 0.004). C shows the lack of correlation between the extent of atherosclerosis in the aortic tree by en face analysis and in cross-sections of the proximal aorta. Individual values shown are LDLR−/− mice transplanted with LPL+/+ (closed circles), LPL+/− (gray circles), and LPL−/− (open circles) FLC.
If catalytically active macrophage LPL is required for the ability of LPL to promote lipid accumulation by the macrophage, the amount of LPL produced by LPL+/− macrophages might be adequate to serve this role. In contrast, a gene dosage effect might be more likely to be seen if bridging or other noncatalytic functions of LPL were the dominant mechanism.

In summary, LDLR−/− mice chimeric for macrophage LPL gene expression were created by transplanting lethally irradiated female LDLR−/− mice with fetal liver cells from day 14 LPL−/−, LPL−/+ or LPL−/+ fetuses. When challenged with an atherogenic diet for 8 or 19 weeks, LDLR−/− mice reconstituted with LPL−/− macrophages developed significantly less atherosclerosis by en face analysis of the pinned out aortae than LDLR−/− mice reconstituted with LPL−/+ or LPL−/+ macrophages in the absence of significant differences in serum lipids or lipoprotein profiles. Analysis of the extent of atherosclerosis by analysis of cross-sections of the proximal aorta revealed a similar decrease in atherosclerosis in the LDLR−/− → LDLR−/− mice after 8 weeks on the Western diet, but the difference in the proximal aorta was not present after 19 weeks on the diet when lesions were extremely complex. Therefore, we conclude that, under atherogenic conditions, macrophage LPL expression promotes foam cell formation and atherosclerosis in vivo, but the impact of macrophage LPL on the extent of atherosclerosis may be lost in complex atherosclerotic lesions.

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