In Vivo Arterial Lipoprotein Lipase Expression Augments Inflammatory Responses and Impairs Vascular Dilatation

Mayumi Takahashi, Yaeko Hiyama, Masayoshi Yokoyama, Shuiqing Yu, Yunying Hu, Kristan Melford, André Bensadoun, Ira J. Goldberg

Objective—Although epidemiologic data suggest that hypertriglyceridemia and elevated plasma levels of fatty acids are toxic to arteries, in vitro correlates have been inconsistent. To investigate whether increased endothelial cell expression of lipoprotein lipase (LpL), the primary enzyme creating free fatty acids from circulating triglycerides (TG), affects vascular function, we created transgenic mice that express human LpL (hLpL) driven by the promoter and enhancer of the Tie2 receptor.

Methods and Results—Mice expressing this transgene, denoted EC-hLpL and L for low and H for high expression, had decreased plasma TG levels compared with wild-type mice (WT): 106±31 in WT, 37±17 (line H), and 63±31 mg/dL (line L) because of a reduction in VLDL TG; plasma cholesterol and HDL levels were unaltered. Crossing a high expressing EC-hLpL transgene onto the LpL knockout background allowed for survival of the pups; TG in these mice was approximately equal to that of heterozygous LpL knockout mice. Surprisingly, under control conditions the EC-hLpL transgene did not alter arterial function or endothelial cell gene expression; however, after tumor necrosis factor (TNF)-α treatment, arterial vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and endogenous TNF-α mRNA levels were increased and arteries had impaired endothelium-dependent vasodilatation. This was associated with reduced eNOS dimers.

Conclusions—Therefore, we hypothesize that excess vascular wall LpL augments vascular dysfunction in the setting of inflammation. (Arterioscler Thromb Vasc Biol. 2008;28:455-462)

Key Words: triglyceride □ fatty acids □ lipolysis □ adhesion molecules □ endothelial cells

Epidemiologic data have implicated circulating levels of triglyceride (TG) with increased cardiac events.1 One of the mechanisms for this is thought to be via toxic effects of lipolysis products such as fatty acids (FAs) and lysolecithin on endothelial cells. Many tissue culture studies have supported this by showing increased expression of adhesion molecules by lysolecithin2 or FAs,3 plasminogen activator inhibitor (PAI)-1 by FAs,4 and reduced eNOS activity by FAs in the presence of excess glucose.5

LpL is the rate-limiting enzyme for hydrolysis of plasma TG. LpL is synthesized by myocytes and adipocytes but is thought to function primarily on the luminal surface of endothelial cells. Therefore, along the arterial wall much of the exposure of endothelial cells to FAs is modulated by LpL actions.

LpL has been suggested to have a dual effect on atherosclerosis development.6 We and others have demonstrated that LpL contributes to lipid accumulation by stimulating the cellular binding and uptake of atherogenic lipoproteins in different vascular cell types including smooth muscle cells and macrophages.7,8 Moreover, loss of macrophage LpL reduces atherosclerosis.9 LpL acts as an activator of macrophage function, inducing TNF alpha (TNF-α),10 augmenting monocyte adhesion11,12 and increasing phagocytosis in low glucose conditions.13 Although these data suggest that LpL has atherogenic and inflammatory effects, there are contradictory data. LpL is reported to play an antiinflammatory role through generation of PPAR ligands for endothelial cells14 and macrophages.15 In human endothelial cells, LpL alone attenuated the inflammatory cascade produced by TNF-α.16 Thus, the function and role of LpL in inflammatory responses are not clear, but may vary depending on the in vitro conditions studied.

To study the actions of LpL in arteries, we created mice with human LpL (hLpL) expression specifically in endothelial cells using the Tie2 promoter-enhancer17 to drive an hLpL minigene. These transgenic mice (denoted EC-hLpL) have reduced plasma levels of VLDL-TG. The transgene rescued...
LPL knockout mice from neonatal death and allowed us to assess the roles of LpL in arterial biology under control and inflammatory conditions.

Methods

Construction of EC-hLpL Transgene and Generation of Transgenic Mice on the Wild-Type Background

The murine Tie2 promoter and enhancer fragment (a gift from Dr Thomas N Sato, University of Texas) was used to generate transgenic mice with vascular endothelial cell specific gene expression.17 The murine Tie2 promoter and enhancer fragment (a gift from Dr Thomas N Sato, University of Texas) was used to generate transgenic mice with vascular endothelial cell specific gene expression.17

A 2.1-kbp HindIII Tie2 promoter fragment was inserted into the NotI/Smal site of pBluescript SK (+) (Stratagene) [pBl-Tie2P]. The hLpL minigene18 with muscle creatine kinase (MCK) promoter was used as template DNA and was inserted 3′ to the Tie2 promoter. The enhancer was then added distal to the LpL 3′ UTR (Figure 1A). Transfection initiation and termination sites are indicated as ATG and TGA. A NotI/NorI fragment of this construction was purified and microinjected into fertilized eggs of C57BL/6J mice. 

Breeding of Mice Expressing hLpL Only in Endothelial Cells

EC-hLpL mice were crossed with heterozygous LpL knockout mice (LpL+/−).20 Pups expressing the EC-hLpL transgene and heterozygous

for LpL deficiency (EC-hLpL/LpL−/−) were then crossed with LpL+/− mice to obtain EC-hLpL/LpL−/− mice and littermates LpL+/+ and EC-hLpL/LpL+/− mice. All procedures were in accordance with current NIH guidelines and were approved by the Columbia University Animal Care and Use Committee.

Genotyping of Transgenic Mice and Estimation of Copy Number

Genotypes were determined from tail tip DNA by polymerase chain reaction (PCR) using hLpL specific primers (sense, 5′-GAGATTT-CCTCTGTATGGGCCACC-3′; antisense, 5′-CTGCAATAGGAGACCATTTTCTCT-3′) and mouse LpL (mLpL) specific primers (sense, 5′-CTCTCTGTGCGACGTTGAGCTCCTGATACGG-3′; antisense, 5′-ACTTGGACGCGGCTGACGCGCTGAAGCA-3′, and 5′-GCGGCGCGGGGGGAACTTTCCCTGACTAGGGG-3′). To estimate the relative copy number in transgenic mouse lines, 10 ng of tail tip DNA was used for quantitative real-time PCR.

Endothelial Cell and Peritoneal Macrophage Isolations

For endothelial cell isolation, hearts were removed from anesthetized mice, rinsed with PBS, minced into 1-mm3 pieces, and incubated in a collagenase mixture (Liberase Blendzyme 4 [Roche], DME low glucose with L-glutamine [Invitrogen]) at 37°C for 30 minutes with occasional agitation. The heart digest was filtered sequentially through a sterile 100 and 35-μm nylon mesh (BD Bioscience) until it was centrifuged at 900 g for 5 minutes at 4°C, and washed twice with 0.5% BSA-PBS. The cell pellet was resuspended and incubated in 10% FBS-DMEM containing CD31-activated protein C (APC), CD45-PE, and CD44-fluorescein isothiocyanate (FITC) anti-mouse antibodies (BD Bioscience) at 4°C. After 30 minutes, the samples were centrifuged and resuspended in PBS with DAPI (Invitrogen). Live, CD31-positive, CD45/CD41 double negative cells were sorted (BD FACSAria Cell-sorting system) directly into lysis buffer (Qia-gen) for RNA isolation. Purity of the cells was confirmed by the presence of endothelial specific receptor tyrosine kinase, and absence of Cd11b, desmin, and collagen type III alpha 1.

Peritoneal macrophages were harvested 3 days after intraperitoneal injection of 40 μg concanavalin A in 0.5 mL sterile PBS. Cells were seeded in 6-well plates for RNA isolation. The isolated cells were incubated in 5 mmol/L glucose containing DMEM supplemented with 10% FBS and 20% L-cell-conditioned medium. Before isolation of RNA, the cells were washed with PBS to remove any excess medium, and cells were scraped from the plate in PBS and pelleted.

Quantitative Real-Time PCR Analysis

Total RNA was isolated with TRIzol reagent (Invitrogen). One microgram of total RNA was reverse transcribed by random hexamer and M-MLV reverse transcriptase (Promega). qRT-PCR was performed with the Stratagene Mx3005 using SYBR Green PCR Master Mix (Applied Biosystems). The primer sequences were as follows: hLpL (sense, 5′-GGTTGAGAGATGAGGCTGG-3′; antisense, 5′-GAATGGCGAAGCCGGGACT-3′), mLpL (sense, 5′-AAATGACTGGG-3′; antisense, 5′-CACTTTCTC-3′), and mouse LpL (mLpL) specific primers (sense, 5′-CCATCCAATCGGT-3′; antisense, 5′-CGAGATTTATCATC-3′). E-selectin (sense, 5′-TGGTTGTGGAA-3′; antisense, 5′-GGTGTTTGTGTTGGAA-3′), PAI-1 (sense, 5′-GCCAGATTTATCATC-3′; antisense, 5′-TGCGTTTTGGAGCTA-3′), TGFβ (sense, 5′-TGCGTTTTGGAGCTA-3′; antisense, 5′-GGTGTTTGTGTTGGAA-3′), ICAM-1 (sense, 5′-CCTCACTTGACAAAGATGAGC-3′), VCAM-1 (sense, 5′-CCTCACTTGACAAAGATGAGC-3′; antisense, 5′-TGGTTGTGGAA-3′; antisense, 5′-CCATCCAATCGGT-3′), and mouse LpL (mLpL) specific primers (sense, 5′-CCATCCAATCGGT-3′; antisense, 5′-GAAATGGCGAAGCCGGGACT-3′).

Figure 1. Construction and tissue expression of EC-hLpL. A, Schematic diagram for endothelial specific transgenic construct in which expression of hLpL transgene is driven by promoter and enhancer sequences from the Tie2 gene. B, Expression of EC-hLpL transgene in different tissues from EC-hLpLH (black bars, n=3) and EC-hLpLL (gray bars, n=3) were determined by quantitative real-time PCR. C, hLpL protein levels in different tissues were assessed by ELISA, WT (open bars, n=5), EC-hLpLH (black bars, n=5), and EC-hLpLL (gray bars, n=5). WAT indicates white adipose tissue. *P<0.05, **P<0.01, ***P<0.001.
LpL Mass and Activity Measurements
To obtain postheparin plasma (PHP), fasted mice were bled 5 minutes after tail vein injection of 100 U of heparin/kg body weight (Elkins-Sinn). LpL mass measurements in tissues were obtained using the homogenization buffer of Hocquette et al.21 supplemented with 20 mmol/L Hecameg; addition of Hecameg resulted in a better solubilization of tissue LpL protein. hLpL protein levels were measured by ELISA as described previously by Peterson et al.22 LpL activity was determined using a glycerol-based assay with human serum as the source of apoCII.23 The contribution of hepatic lipase activity was determined using a glycerol-based assay with human LpL. Activity, expressed as micromoles of FAs per hour per mg of PHP, was determined by comparison with a standard source of hLpL of known activity.

Lipid and Lipoprotein Determination in Plasma
Blood samples were taken by retro-orbital puncture after an overnight fast. Plasma TG and cholesterol levels were measured enzymatically using kits (Wako Chemicals). Lipoprotein separation was performed by fast performance liquid chromatography (FPLC) of 500 μL of pooled plasma on 2 serial Superose 6 columns (Pharmacia). In addition, VLDL (d <1.006 g/mL), IDL/LDL (d=1.006 to 1.063 g/mL), and HDL (d=1.063 to 1.21 g/mL) were isolated by sequential ultracentrifugation using a TLA 100 rotor (Beckman Instruments). Fractions were analyzed for TG and cholesterol.

Immunohistochemical Staining of Mouse Aortas
Five-micron paraffin sections of mouse aorta were stained with antibody. Antigen retrieval procedure was performed using Citra Solution according to the manufacturer’s protocol (BioGenex). Primary antibodies were labeled with biotinylated goat anti-rabbit secondary antibody and visualized with ABC staining system (Santa Cruz Biotechnology Inc). Endothelial staining of aorta sections was analyzed with Image-Pro Plus (Version 5.0.1) software (Media Cybernetics Inc). To compare the intensity of staining, endothelial cell areas were selected and integrated optical density was determined in sections.

VLDL Uptake Study
Human VLDL and the density >1.21 g/mL fraction of human plasma (containing cholesteryl ester transfer protein) were isolated by ultracentrifugation. VLDL and >1.21 g/mL fractions were added to dried 15 μCi glycerol [1-14C] trioleate and 150 μCi [1α,2α(n)-3H] cholesterol oleyl ether (GE Healthcare) and mixed overnight at 37°C. Labeled VLDL were then dialyzed in PBS at 4°C overnight. Mouse antibody for hLpL was used to estimate the amount of human LpL and mouse LpL. Activity, expressed as micromoles of FAs per hour per mg of PHP, was determined by comparison with a standard source of hLpL of known activity.

Endothelial Expression of Lipoprotein Lipase

Western Blot
eNOS protein expression levels were analyzed by Western blot. Fifteen micrograms of protein from each femoral artery were separated by low temperature SDS-PAGE.26 After transfer onto a polyvinylidene difluoride membrane, the membrane was blocked by 5% non-fat milk solution. Primary antibody (BD bioscience) was used at a 1:2500 dilution and the bound antibody was detected with horseradish-peroxidase-conjugated detection system (Pierce).

Liver Lipid and Histology
Anesthetized mice were perfused with 10 mL of PBS until the livers blanched. Livers were then excised, weighed, and homogenized in ice-cold PBS. Lipids were extracted with chloroform:methanol (2:1 vol/vol) and extracts were analyzed using kits (Wako). For histology, livers were frozen in OCT compound (Tissue-Tek; Sakura Finetek) and stained for lipid accumulation with Oil Red O.

Statistical Analysis
Results are given as means±SE. Statistical significance was tested using a 2-tailed Student t test. In some cases, ANOVA with a Fisher’s Protected Least Significant Difference test was used. Statistical analysis was done using the StatView version 5.0 (Abacus Concepts).

Results
Expression of the EC-hLpL Gene in Transgenic Mice
Transgenic mice were generated with an hLpL minigene driven by an endothelial specific fragment of the murine Tie2 promoter and enhancer; 2 of 4 transgenic mice born after microinjection were used to establish lines. Real-time PCR assay was used to determine the transgene copy number; the higher hLpL expressing line, denoted EC-hLpLH, had 9-fold greater copy number than the lower expressing line, denoted EC-hLpLL (supplemental Figure 1A, available online at http://atvb.ahajournals.org). EC-hLpLH transgenic mice had 928±163 ng/mL and EC-hLpLL mice 497±73 ng/mL hLpL protein in PHP.

hLpL mRNA was detected in all tissues of each transgenic mouse line. When normalized to 18S RNA, the highest expression was found in lung, white adipose tissue, and aorta (Figure 1B). In EC-hLpLH mice mRNA expression was 1.5- to 5-fold greater than EC-hLpLL mice in brain, skeletal muscle, white adipose tissue, and aorta. mLpL mRNA expression was not reduced by the transgene (supplemental Figure 1B) and several tissues—heart and lung—had modest increases in LpL activity (supplemental Table 1). hLpL protein levels were greater in lung, aorta, and skeletal muscle of line H (Figure 1C). When expressed per mg protein, aortic hLpL content exceeded that of the other tissues.

To verify the endothelial cell expression of the hLpL transgene, cardiac endothelial cells were harvested using flow cytometry. hLpL expression was present in endothelial cells derived from EC-hLpL hearts (supplemental Figure II). hLpL mRNA was not detected in peritoneal macrophages or plasma white cells.

Downloaded from http://atvb.ahajournals.org by guest on July 31, 2017
Plasma Lipid Levels in EC-hLpL Transgenic Mice

The effects of the EC-hLpL transgene on plasma lipid profiles are summarized in the Table. Relative to WT mice, TG levels decreased 65% (EC-LpLH) and 41% (EC-LpLL; 37±17 and 63±31 versus 106±31 mg/dL). Plasma cholesterol levels of transgenic mice were similar to WT mice. The FPLC elusion patterns of EC-LpL and WT plasmas are shown in Figure 2A and 2B. The 2 lines had 62% and 51% reductions in VLDL-TG. The plasma samples were also analyzed using ultracentrifugation (Figure 2C and 2D). VLDL-TG levels of EC-hLpLH and EC-hLpLL mice were decreased: WT 40.7±7.4, EC-hLpLH 17.4±2.0, and EC-hLpLL 28.2±6.3 mg/dL. HDL-cholesterol levels were unchanged by the transgene.

Hepatic Expression of EC-hLpL Transgene

Although LpL is primarily expressed in peripheral tissues, the EC-hLpL transgene was also expressed in liver, and gene expression and hLpL protein and activity in livers of the 2 lines of mice were similar (Figure 1B and 1C). This suggested that the relative expression was greater in liver than peripheral tissues of EC-hLpLL compared with EC-hLpLH; the liver to skeletal muscle hLpL mRNA ratios were 0.2 for line H and 0.8 for line L. Livers from EC-hLpLL female mice contained more TG than did WT (WT, 14.8±2.3 versus EC-hLPL, 50.5±5.5 mg/µg protein), but liver cholesterol was not altered. Livers of line EC-hLpLL female, but not male, mice were grossly steatotic and lipid deposition by Oil Red O staining was observed (supplemental Figure III).

Effects of EC-hLpL Expression on Survival of LpL Knockout Mice

Transgenic mouse lines were bred onto Lpl+/− and Lpl−/− backgrounds. Lpl+/− mice had mild hypertriglyceridemia, with 1.8-fold elevated TG levels compared with WT. Introduction of the EC-hLpL transgene lowered TG to levels equal to those of the WT mice. When the EC-hLpL transgenes were crossed onto the Lpl−/− background, only line H allowed for survival; line L embryos were found in expected numbers but did not survive. The TG levels in EC-LpLH/Lpl−/− mice were only mildly increased compared with that in Lpl−/− mice (Table). No significant changes were observed in cholesterol levels on the Lpl+/− and Lpl−/− background mice. The remaining experiments were all performed using the EC-hLpLH line.

VLDL Metabolism in EC-hLpL Mice

To determine how endothelial LpL altered VLDL metabolism in tissues, a kinetic study was performed comparing EC-hLpLH/Lpl+/− and Lpl−/− mice using double-labeled VLDL to assessed tissue uptake of hydrolyzable lipid (TG) and nonhydrolyzable core cholesteryl ether. In all high LpL-expressing tissues (heart, skeletal muscle, and adipose), more TG than cholesteryl ether was, as expected, assimilated. Aortic uptake was below the level of detection in this study. Although the ratio of TG/cholesteryl ether uptake was slightly increased in heart, skeletal muscle, and white adipose tissue in EC-hLpLH/Lpl+/− compared with Lpl−/− mice, these

Table. Plasma TG and Cholesterol Levels of Overnight-Fasted 12- to 13-Week-Old Male Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TG (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>106±31</td>
<td>93±9</td>
</tr>
<tr>
<td>EC-hLpLH</td>
<td>37±17**</td>
<td>114±27</td>
</tr>
<tr>
<td>EC-hLpLL</td>
<td>63±31*</td>
<td>99±28</td>
</tr>
<tr>
<td>Lpl−/−</td>
<td>187±29</td>
<td>81±3</td>
</tr>
<tr>
<td>EC-hLpLH/Lpl−/−</td>
<td>96±10**</td>
<td>85±5</td>
</tr>
<tr>
<td>EC-hLpLL/Lpl−/−</td>
<td>112±15*</td>
<td>89±14</td>
</tr>
<tr>
<td>EC-hLpLH/Lpl−/−</td>
<td>241±20</td>
<td>76±3</td>
</tr>
</tbody>
</table>

n=5–6. *P<0.05, **P<0.01.

Figure 2. Lipoprotein profiles and plasma lipoprotein level. Pooled plasmas were applied to columns as described in Methods. TG (A) and Cholesterol (B) distribution patterns of WT (open circle), EC-hLpLH (black circle), and EC-hLpLL (gray circle) mice are shown. Plasmas were also separated by ultracentrifugation. TG (C) and Cholesterol (D) levels of WT (open bars), EC-hLpLH (black bars), and EC-hLpLL (gray bars) were measured. (n=5 to 6). *P<0.05.
increases were not significant (supplemental Figure IV). Thus, endothelial cell expression of LpL and lack of parenchymal LpL expression did not lead to changes in tissue lipid uptake.

Vascular Gene Expression and Vascular Function in EC-hLPL

We next studied the role of LpL on vascular gene expression and endothelial function under control and inflammatory conditions. VCAM-1, a gene that has been reported to be regulated by LpL actions, was expressed at equal levels in WT and EC-hLpL aortas. However, after animals were treated with TNF-α, VCAM-1 gene expression was greater in aortas of EC-hLpL mice (Figure 3). E-selectin and endogenous TNF-α, but not ICAM-1 and MCP-1, mRNA levels were also increased. It has been suggested that FAs regulate PAI-1 expression and endothelial vasodilatation. Aortic PAI-1 mRNA levels were low and no differences could be detected between WT and EC-hLpL mice, even after TNF-α treatment.

Aortas from TNF-α-treated control and EC-hLpL mice were stained for VCAM-1 and E-selectin. Although after the TNF-α treatment robust staining was found in all vessels, quantification showed greater VCAM-1 staining intensity in the EC-hLpL vessels (supplemental Figure V).

Femoral arteries were obtained from control and TNF-α-treated mice; under control conditions, relaxation in response to increasing concentration of acetylcholine was unaltered. However, after TNF-α treatment, relaxation was reduced in arteries from EC-hLpL mice relative to that of the WT.
Thus, it is possible that this enzyme functions in a different manner depending on its location. Most importantly, a number of in vitro studies have suggested that high local concentrations of FA, including FAs generated by LpL hydrolysis of TG, modulate endothelial function and gene expression. By expressing LpL in endothelial cells we were able to study this directly and in vivo. Our data show the following: (1) The Tie2 promoter-enhancer can direct expression of LpL in endothelial cells. (2) Enzymatically active LpL can be produced by endothelial cells in vivo. (3) Endothelial cell LpL, without parenchymal cell LpL, is sufficient to rescue the newborn demise of LpL knockout mice. (4) In the heart and other tissues, Tie2 expression of LpL, alone, leads to normal TG-derived FA uptake. In addition, uptake of nonhydrolyzable core lipid, represented by cholesteryl ether, was not reduced. (5) Under control conditions, EC-hLpL did not alter endothelial cell gene expression; however, in the presence of TNF-α some inflammatory genes had enhanced expression. (6) Arterial dilatation in the presence of TNF-α was reduced in the EC-hLpL transgenic mice. This suggests that high local concentrations of de novo generated FAs lead to vascular dysfunction.

Using the Tie2 promoter-enhancer system, we created mice that expressed LpL in endothelial cells. By isolating endothelial cells, this gene expression was confirmed. Lungs and aortas expressed relatively high levels of hLpL mRNA. The organ expression profile of the two lines of mice differed, and this difference likely reflected the phenotypes found. Of interest, female mice from the lower expressing line developed fatty livers (supplemental Figure III). This line of mice had relatively greater hepatic than peripheral LpL expression (Figure 1C). Thus, as we had noted previously, LpL-mediated diversion of plasma lipids into the liver can lead to steatosis.

Endothelial cell expression of hLpL reduced plasma TG in both lines of transgenics compared with WT; this was the expected phenotype and proved that the EC-hLpL transgene created enzymatically active LpL. The higher expressing line

**Figure 4. Vasodilatation of femoral arteries from TNF-α-treated WT, EC-hLpL, and EC-hLpL/LpL-/- mice. Acetylcholine-induced relaxation and sodium nitroprusside-induced relaxation of phenylephrine pre-contracted femoral arteries. A and C, TNF-α-treated WT (open circle) and EC-hLpL (filled circle) mice. (n=5). *P<0.05, **P<0.01.**

**Figure 5. eNOS protein expression in femoral arteries of TNF-α-treated EC-hLpL/LpL-/- and LpL-/- mice. eNOS dimer and monomer determined by Western blot.
also rescued the knockout mice from neonatal demise, another proof that the enzyme was active in vivo. In contrast, although some pups were born and survived to 1 week, no lower expressing EC-hLpL transgenic mice survived to weaning. The reasons for the demise of the Lpl−/− mice are not obvious. However, previous studies found that neonatal Lpl−/− mice have very low levels of plasma glucose and, we assume, die from hypoglycemia. It is likely that EC-hLpLL mice did not produce sufficient FAs to prevent this.

One objective of this study and of previous experiments was to determine whether parenchymal cell- and endothelial cell-associated LpL have different roles in tissue lipid uptake. Mice with cardiomyocyte expression of anchored LpL have dilated cardiomyopathy and increased heart lipids, primarily cholesterol and cholesteryl ester but not TG.31 This suggested that LpL on parenchymal cells was more important for core lipid uptake than TG hydrolysis. However, EC-hLpL/LpL−/− mouse hearts did not have more VLDL TG-derived FA than cholesteryl ether uptake than did WT hearts (supplemental Figure IV). Therefore, endothelial expressed LpL allowed core lipid uptake attributable to either of 2 processes: (1) selective uptake of lipid,32 or (2) migration and association with underlying cardiomyocytes. Although both FAs and lysolecithin have been shown in vitro to have potentially toxic effects on endothelial cells,33,34 others have suggested that LpL-mediated lipolysis or LpL alone may be antiinflammatory. Whether these conditions mimic those that occur in vivo is unknown. We were, at first, surprised that under control conditions arterial gene expression did not differ between control and EC-hLpL mice; genes for PAI-1, VCAM-1, ICAM-1, and MCP-1 were not altered in vessels expressing excess LpL. In contrast to some in vitro studies, TNF-α-treated EC-hLpL mice had increased VCAM-1 expression in aortas (Figure 3). It may well be that the levels of baseline lipolysis or the presence of free FAs associated with albumin dampen the effects of excess lipolysis on gene expression in vivo such that FA effects are only found in the “stressed” TNFα-treated animals.

Because changes in endothelial cell exposure to FAs altered eNOS activity in vitro35 we assessed vascular reactivity in WT and EC-hLpL mice. After TNF-α treatment, isolated femoral arteries from EC-hLpL mice had reduced acetylcholine-induced vasodilatation. Vasodilatation in response to sodium nitroprusside was identical in the EC-hLpL−expressing and WT vessels. Therefore, the arteries were able to respond normally to an exogenous source of NO. EC-hLpL/LpL−/− mice also showed similar endothelial dysfunction.

Under normal conditions, NO released by eNOS causes vasodilatation.36 For eNOS to function, it must be an active dimer.37,38 In EC-hLpL/LpL−/− femoral arteries, eNOS dimer formation was reduced and this is likely to have created NO deficiency. Excess reactive oxygen species formation inhibits eNOS activity.39 We postulate that endothelial LpL increases local FA levels and augments reactive oxygen species production. FA treatment of cultured endothelial cells leads to reduced eNOS activity.1 This might mimic conditions that occur in diabetic arteries that, at least in experimental models, have impaired NO mediated endothelium-dependent vasodilatation associated with reduced eNOS dimers.40

In humans higher FA levels are positively correlated with vascular disease;43 these elevations are attributable to systemic factors such as obesity and diabetes. Although total plasma FA levels were not increased in the EC-hLpL mice, increased lipolysis along the endothelial surface especially in the aorta is likely to have occurred. There is no obvious way to prove this in vivo. Excess FAs along with reduced eNOS could be atherogenic. Studies are ongoing to assess whether the EC-hLpL transgene alters vascular disease in atherosclerosis-prone hyperlipidemic mice.

In summary, we created mice with endothelial-specific expression of LpL. Unlike myocytes and adipocytes, endothelial cells do not normally express this enzyme; however, our data show that they are capable of LpL production. If produced in sufficient amounts, endothelial cell LpL can prevent the early neonatal demise of LpL-deficient mice. Most of the many gene expression changes that one might expect with increased local lipolysis were not found in these animals. Only in the presence of an inflammatory stimulus, TNF-α, was arterial dysfunction evident. VCAM-1 expression was increased and there was impaired endothelium-dependent vasodilatation. Thus, arterial responses to FAs might only be defective in the setting of vascular activation. Such conditions occur commonly and are thought to underlie the advanced vascular disease seen with diabetes, dyslipidemia, and cigarette smoking.

Sources of Funding

These studies were supported by grants HL45095 and 73029 from NHLBI and a mentored postdoctoral research grant from the American Diabetes Association (to M.Y.).

Disclosures

None.

References


In Vivo Arterial Lipoprotein Lipase Expression Augments Inflammatory Responses and Impairs Vascular Dilatation

Mayumi Takahashi, Yaeko Hiyama, Masayoshi Yokoyama, Shuqing Yu, Yunying Hu, Kristan Melford, André Bensadoun and Ira J. Goldberg

Arterioscler Thromb Vasc Biol. 2008;28:455-462; originally published online February 7, 2008; doi: 10.1161/ATVBAHA.107.153239

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/28/3/455

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2008/02/26/ATVBAHA.107.153239.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Sup. Fig. I

A

Relative copy number

male

female

B

mLpL/18S rRNA ratio

Heart
Lung
Muscle
WAT
Aorta
Sup. Fig. II

Graph showing the hLpL/18S rRNA ratio for macrophage, white cell, and endothelial cell types.
Sup. Fig. III

A  WT  EC-hLpLL Female

B  WT  EC-hLpLL Female
Sup. Fig. iV

The graph illustrates the ratio of $^{14}$C-TG/^{3}$H-CE across different tissues: Heart, Liver, Muscle, WAT, Lung, and ID. The bars represent the mean values with error bars indicating the standard deviation. The x-axis represents different tissues, and the y-axis shows the ratio values.
Sup. Fig. V

A.

WT

EC-hLpL

B.

Integrated Optical Density

WT

EC-hLpL

*