Endothelial lipase is a major determinant of HDL level

See the related Commentary beginning on page 318.

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A new member of the lipase gene family, initially termed endothelial lipase (gene nomenclature, *LIPG*; protein, EL), is expressed in a variety of different tissues, suggesting a general role in lipid metabolism. To assess the hypothesis that EL plays a physiological role in lipoprotein metabolism in vivo, we have used gene targeting of the native murine locus and transgenic introduction of the human *LIPG* locus in mice to modulate the level of EL expression. Evaluation of these alleles in a C57Bl/6 background revealed an inverse relationship between HDL cholesterol level and EL expression. Fasting plasma HDL cholesterol was increased by 57% in $LIPG^{-/-}$ mice and 25% in $LIPG^{+/-}$ mice and was decreased by 19% in *LIPG* transgenic mice as compared with syngeneic controls. Detailed analysis of lipoprotein particle composition indicated that this increase was due primarily to an increased number of HDL particles. Phospholipase assays indicated that EL is a primary contributor to phospholipase activity in mouse. These data indicate that expression levels of this novel lipase have a significant effect on lipoprotein metabolism.

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Introduction

Study of the association of serum lipid levels with atherosclerosis has led to the characterization of HDL level as an inverse correlate of the risk of coronary heart disease (1, 2). Up to 70% of the variation in HDL cholesterol levels in humans is genetically determined, but the genes contributing to this variation are incompletely defined (3, 4). It is generally postulated that variations in plasma HDL cholesterol levels are determined by both the rate at which HDL cholesterol is produced and the rate of catabolism of HDL particles (5, 6). There are several known molecules that play a role in regulating plasma HDL cholesterol levels, including hepatic lipase (HL), lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP). Intense study over the last few years has focused on the HDL particle in the context of reverse cholesterol transport and antioxidant properties, providing significant insights into the mechanism of the antiatherogenic properties of HDL (7, 8). Despite significant insights gained through these studies, there continue to be large gaps in our understanding of HDL metabolism. Further study is required to better understand how HDL particles may be manipulated with regard to both level and composition to affect the atherosclerotic disease process through therapeutic intervention.

Serum HDL cholesterol levels are regulated in part by members of the lipase enzyme family. The lipases have highly conserved structural domains, and these enzymes function to metabolize triglycerides and phospholipids. Two members of this family, HL and lipoprotein lipase (LPL), are important in the processing of lipids carried within lipoproteins and probably also in the uptake of lipoprotein particles into cells (9). Although synthesized in nonendothelial cells, these two secreted enzymes translocate to the surface of endothelial cells, where they carry out their metabolic function. Both HL and LPL have been implicated in atherosclerotic vascular disease (10–15). Recently, the lipase gene family was extended through the characterization of endothelial lipase (protein, EL; gene, *LIPG*; NCBI Locuslink 9388) (16, 17). *LIPG* has been shown to be expressed by vascular cells in vivo and to be highly regulated in vitro in endothelial cells by cytokines and biophysical forces (18). This novel lipase is also expressed by a wide range of nonendothe-

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Nonstandard abbreviations used: endothelial lipase gene (*LIPG*); endothelial lipase protein (EL); hepatic lipase (HL); lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP); phospholipid transfer protein (PLTP); lipoprotein lipase (LPL); neomycin phosphotransferase (neo); thymidine kinase (TK); bacterial artificial chromosome (BAC); fast protein liquid chromatography (FPLC); lysophosphatidyl choline (Lyso-PC); phosphatidyl ethanolamine (PE); phosphatidyl choline (PC).

lial cells in a variety of tissues, including the placenta, liver, lung, ovary, thyroid gland, and testis (16, 17). EL has been shown to have phospholipase activity and relatively little triglyceride lipase activity (16, 19). One previous study has demonstrated that high-level overexpression of EL in the liver by adenovirus-mediated gene transfer results in a significant decrease in HDL cholesterol and apoA-I, suggesting that EL could be a physiological regulator of HDL metabolism (17).

In this study, we have sought to further explore a potential role for EL in HDL cholesterol metabolism. Toward this end, we have generated murine genetic models with altered levels of EL expression. Decreased *LIPG* expression was accomplished by gene targeting and development of mice functionally lacking one or two *LIPG* alleles, and increased expression was accomplished by the development of transgenic animals carrying copies of the human *LIPG* locus. Study of lipid levels in these animals revealed a strong inverse relationship between EL expression and HDL cholesterol levels. The genetic models revealed a strong correlation between phospholipase activity and EL expression and demonstrated that, in the mouse, EL is responsible for the bulk of heparin-releasable phospholipase activity. A modest correlation was found between EL expression and triglyceride levels. These studies thus establish a physiological role for EL in lipoprotein metabolism.

Methods

Generation of EL genetic models. A mouse *LIPG* cDNA probe (*Eco*RI-*Pst*I fragment) was employed for screening a λ phage 129/SvJ genomic library (Stratagene, La Jolla, California, USA), providing two overlapping clones of 15 and 13 kb. These phage clones were restriction mapped, and the exon/intron structure was partially determined by mapping and nucleotide sequence analysis. The replacement targeting vector was constructed in the pKO Scrambler NTKV-1901 vector (Stratagene) using a 4.6-kb *Hind*III-*Xba*I fragment for the 5′ arm and a 3.6-kb *Kpn*I-*Xho*I fragment for the 3′ arm (Figure 1). The homology regions used and their organization in the vector was designed to replace exon 1 of *LIPG* with a neomycin phosphotransferase (neo) cassette. Also, a cassette for herpes simplex virus thymidine kinase (TK) was provided outside the region of homology to allow negative selection.

TL-1 129 ES cells (Brigid Hogan, Vanderbilt University, Nashville, Tennessee, USA) were grown in DMEM/10% FCS containing1000 U/ml leukemia inhibitory factor (GIBCO BRL/Invitrogen, Carlsbad, California, USA). A total of 20×10^6 cells, at passage 10, were electroporated with 50 μg of linearized targeting vector with a single pulse at 800 V and 3 μF. The next day, selection was started with 300 μg/ml G418, followed 2 days later with the addition of gancyclovir at a concentration of 2 μmol/L. After 10–14 days, double resistant clones were picked and expanded, and genomic DNA was isolated and evaluated by Southern blot with 5′ and 3′ probes outside the homology domains (Figure 1). One of the correctly targeted ES cell clones was injected into C57Bl/6 blastocysts to generate chimeric animals. A single chimeric male was bred to C57Bl/6 females (Jackson Labs, Bar Harbor, Maine, USA) to obtain heterozygous pups. Male heterozygous pups were bred with C57Bl/6 female mice eight times before homozygous animals were generated. Littermates served as controls for all lines studied. Genotyping of knockout animals was performed with DNA isolated from tail tissue, which was digested with *Eco*RI and separated on 0.65% agarose gels. After transfer to nylon membranes, blots were hybridized with [^{32}P]2′-deoxycytidine 5′-triphosphate–labeled probes synthesized using 5′(*Eco*RI-*Bam*HI) or 3′ (*Hind*III-*Bgl*II) fragments by random priming (Figure 1). Genotypes of mice were verified by detection of the fragments that differ in size between the wild-type and targeted locus.

For the generation of transgenic mice carrying the human *LIPG* locus, PCR primers based on the cDNA sequence, 5′-gtaaaatgtgagcatgagcgag-3′ and 5′-ttcttg-gcattgtagccaatg-3′, were used to screen an arrayed bacterial artificial chromosome (BAC) library (20). The BAC spans from nucleotide 47,198,300 to nucleotide 47,395,100 in the June 2002 version of the human whole-genome assembly. This matches well with the estimated size of the BAC, 196 kb. The *LIPG* gene is located 5′ to 3′ from nucleotide 47,367,696 to nucleotide 47,247,058, which indicates that this BAC contains about 27.4 kb of 5′ and 48.7 kb of 3′ flanking regions. According to the whole-genome annotation, there is no other gene except a ribosomal protein gene, *RPL17*, located in an intron of the *LIPG* gene in this BAC. BAC DNA isolated with standard alkaline lysis techniques was injected into the pronuclei of C57Bl/6 fertilized eggs. Transgenic animals (hereafter referred to as h*LIPG*Tg) were identified by Southern blot of *Bgl*II-digested tail DNA with a human *LIPG* cDNA probe.

RNA and protein expression studies. Mouse tissues were homogenized in TRIZOL (Invitrogen), and the aqueous phase was extracted after adding chloroform. After precipitating the RNA with isopropanol, the RNA pellets were dissolved in the RLT lysis buffer with β-mercaptoethanol and cleaned with RNeasy columns (Qiagen, Valencia, California, USA).

For Northern blot analysis, 20 μg of total RNA was separated on formaldehyde-denaturing 1.0% agarose gels and transferred to nylon membranes. Membranes were then hybridized to a mouse *LIPG* (1.5-kb) cDNA probe radiolabeled with [^{32}P]2′-deoxycytidine 5′-triphosphate by random priming. Blots were hybridized at 42 °C for 16–24 hours in the presence of 48% formamide and 10% dextran sulfate and then washed at high-stringency conditions at 65 °C in the presence of 0.2× SSC buffer and 0.5% SDS. Visualization was achieved by exposure to Kodak Biomax MS film (Eastman Kodak, Rochester, New York, USA).

For RNase protection assays, a *Sac*I-*Nde*I fragment of human *LIPG* was cloned into the pSP72 vector, and an

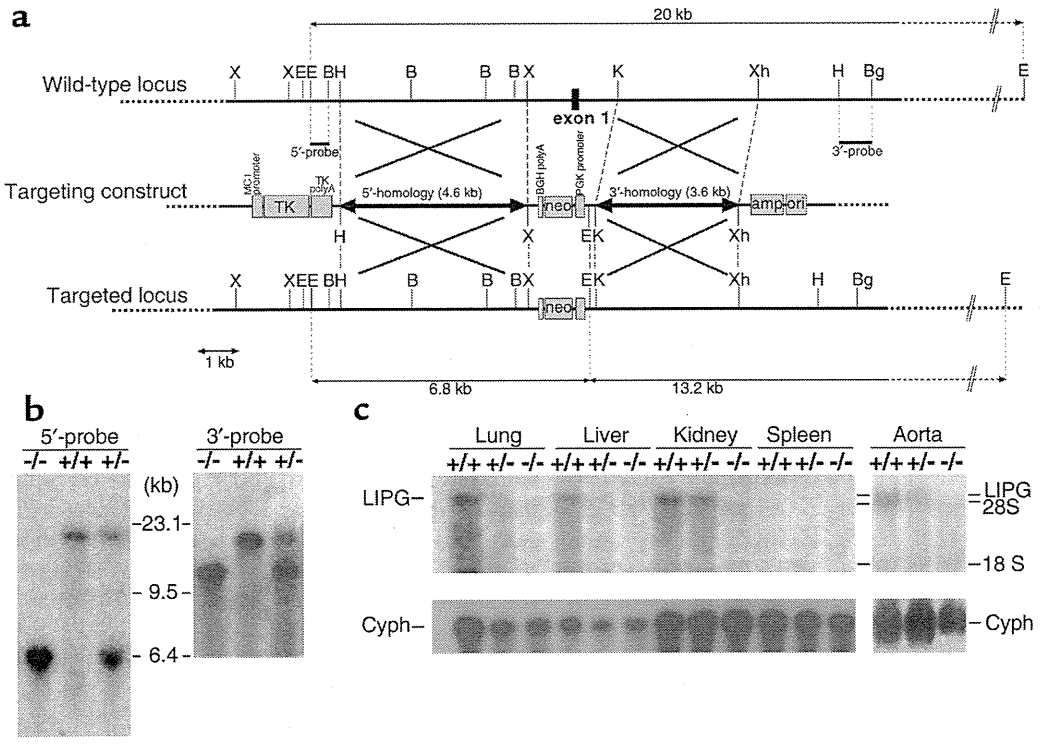

Figure 1

Targeting of the mouse endothelial lipase gene. (**a**) The wild-type locus of mouse *LIPG* (top), the targeting construct (middle), and the targeted locus (bottom). Exon 1 was replaced with the neomycin phosphotransferase gene (neo), and recombination was detected by Southern blot analysis using the probes indicated. X, *Xba*I; B, *Bam*HI; K, *Kpn*I; Bg, *Bgl*II; H, *Hind*III; E, *Eco*RI; Xh, *Xho*I. (**b**) Both probes gave a 20-kb wild-type band on Southern blotting, whereas the targeted recombinants showed a 6.8-kb band with the 5′probe and a 13.2-kb band with the 3′probe. Targeted stem cells were characterized by the presence of both DNA fragments. (**c**) Northern blot analysis showed *LIPG* expression in mouse tissues. A full-length mouse *LIPG* cDNA was used as a probe. Cyph, cyclophilin.

*Eco*RI-*Eco*RI fragment of mouse *LIPG* was cloned into the pBluescript SK(+) vector. Both plasmids were linearized with *Xho*I. [^{32}P]UTP-labeled antisense riboprobe was synthesized with T7 RNA polymerase, and an RNase protection assay was performed as described previously (21). Protection of human *LIPG* transcripts resulted in a labeled fragment of 694 nucleotides, protection of mouse *LIPG* transcripts resulted in a labeled fragment of 610 nucleotides, and protection of β-actin transcripts resulted in a labeled fragment of 250 nucleotides.

For Western blot analysis, mouse tissues from *LIPG* transgenic mice were homogenized in lysis buffer (10 mmol/l Tris [pH 7.4], 150 mmol/l NaCl, 2 mmol/l $CaCl_2$, 1% NP-40, 1% Triton X-100, 1 mmol/l PMSF, 40 U/ml aprotinin, 15 μg/ml leupeptin). Aliquots (50 μg) of protein solution were boiled with Laemmli buffer and subjected to SDS-PAGE. The gel was transferred to polyvinylidene fluoride membrane and blocked with 5% nonfat dry milk. Anti-human EL antibody was used as the primary antibody. The primary antibody employed was an anti-human polyclonal EL antibody raised in rabbit against a synthetic peptide encoding residues 422–445 of processed EL, SWYNLWKEFRSYLSQPRNPGRELN. The membrane was incubated with anti-EL antibody (1:1000) and then anti-rabbit IgG-HRP (Amersham-Pharmacia, Piscataway, New Jersey, USA). The signal was detected by chemiluminescent reaction.

Immunohistochemistry. Wild type and h*LIPG*Tg mice were anesthetized by administration of pentobarbital and perfused with 4% paraformaldehyde/PBS at 10 mm Hg for lungs and at 130 mm Hg for other tissues. Tissues were excised and further fixed in 4% paraformaldehyde/PBS, and then paraffin-embedded sections were made by use of standard methods. The sections were incubated with 3% H_2O_2/methanol for 20 minutes to eliminate endogenous peroxidase, washed with PBS, blocked with carrier protein (DAKO LSAB kit, DAKO A/S, Glostrup, Denmark) for 1 hour at room temperature, and then incubated with primary antibody overnight at 4°C. The primary antibody was an anti-human monoclonal EL antibody raised in mouse against a synthetic peptide encoding residues 18–32 of processed EL, AGSPVPFGPEGRLE. This antibody does not cross-react with murine EL. After washing with PBS, sections were incubated with biotinylated goat anti-mouse IgG (1:300), washed with PBS, and then incubated with streptavidin–horseradish peroxidase conjugate (DAKO A/S). After color development with 0.03% diaminobenzidine, the sections were counterstained with Mayer's hematoxylin for 120 seconds. As a negative control, the primary antibody was replaced with mouse nonspecific Ig.

Analysis of plasma lipids. Following an overnight fast, 200–400 μl of blood was collected by retro-orbital bleeding into tubes containing 0.3 mg of EDTA. Plasma was collected by centrifugation at 8000 *g* for 10 minutes. Total cholesterol, HDL and LDL cholesterol, and triglycerides were measured with standard enzymatic assays by Anylitics Inc. (Gaithersburg, Maryland, USA), using the HITACHI 717 Chemical Analyzer (Rankin Biomedical Corp., Clarkston, Michigan, USA). Also, total plasma cholesterol levels were determined by the cholesterol oxidase method, and total plasma triglyceride levels were determined by the glycerin-depletion method, using commercially available kits (Wako Pure Chemicals, Osaka, Japan).

Separation of plasma lipoproteins by fast protein liquid chromatography (FPLC) was performed using two Suprose 6 columns (Amersham-Pharmacia) as described previously (22). Briefly, 100 μl of mouse plasma samples were applied to FPLC, and 37 fractions (1 ml) were collected and analyzed for total cholesterol and triglycerides. Total cholesterol and triglycerides were assayed using enzymatic kits from Sigma-Aldrich (St. Louis, Missouri, USA). Some fractions were further analyzed by SDS-PAGE followed by Coomassie brilliant blue staining of the separated proteins. For Western blotting, 100 μl of FPLC fractions containing HDL were used for 12% SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad, Hercules, California, USA). The membrane was incubated with a 1:500 dilution of mouse human apoA-I antibodies (ICN Biochemicals, Aurora, Ohio, USA) followed by incubation with secondary antibody conjugated with HRP (1:4000). The apoA-I was then visualized using an ECL technique (Amersham-Pharmacia). The numbers of animals used for FPLC analysis were as follows: 8 for *LIPG*$^{-/-}$, 7 for wild type, and 5 for h*LIPG*Tg.

For ultracentrifugation apolipoprotein analysis, plasma samples were collected by heart puncture in a syringe containing EDTA. Two separate pooled plasma samples were analyzed, with five animals included in each pooled sample. Lipoproteins were fractionated by density-gradient ultracentrifugation as described by Havel et al. (23), and the HDL fraction corresponding to d = 1.063–1.21 was used for analyses. Protein was quantitated by the Lowry biochemical method and evaluated by SDS-polyacrylamide gel electrophoresis followed by Coomassie brilliant blue staining of the gels. Cholesterol and phospholipid were measured with kits from Sigma-Aldrich and Wako Pure Chemicals, respectively, as described above. TLC was performed as follows. A total of 35 μg of the HDL fraction was extracted using a method described by Bligh and Dyer (24). The chloroform phase was dried under nitrogen, and the lipid was redissolved with 50 μl of a chloroform/methanol (1:1) solution and applied on a TLC plate. Purified lysophosphatidyl choline (Lyso-PC), phosphatidyl ethanolamine (PE), and phosphatidyl choline (PC) (Sigma-Aldrich) were used as standards. The lipids were separated using a solvent mixture of methanol, chloroform, and water (130:70:12) and visualized by staining with iodine vapor.

Biochemical determination of phospholipid concentrations in mouse plasma and HDL fractions was performed using the Phospholipid-TestWako kit (Wako Pure Chemicals). Phospholipase activity was quantitated as follows. Mouse plasma was collected into EDTA-containing tubes before and 10 minutes after intravenous injection of heparin (150 U/kg). PC emulsion was made by combining [^{14}C]dipalmitoyl PC (1 μCi) (New England Nuclear, Boston, Massachusetts, USA) and lecithin followed by evaporation under nitrogen. One milliliter of 100 mM Tris-HCl (pH 7.4) containing 1% Triton X-100, 5 mM $CaCl_2$, 200 mM NaCl, and 0.1% BSA was added and vortexed. Sixty microliters of emulsion were then added to 100 μl of plasma samples and incubated for 2 hours at 37°C. Labeled fatty acids were separated by the liquid-liquid separation as described previously (25). Briefly, the reaction was terminated by the addition of 3 ml of Dole's reagent (isopropanol/n-heptane/0.5M H_2SO_4, 78:20:2 [vol/vol]). The tubes were vortexed vigorously, and distilled water (1.6 ml) and heptane (1.8 ml) were added and vortexed. The emulsion was separated by centrifugation at 3000 *g* for 15 minutes. One and a half milliliters of the upper phase was removed to another tube containing 100 mg of silica gel. After vortexing for 20 seconds, the silica gel was precipitated by centrifugation at 100 *g* for 10 minutes. Heptane (1 ml) was removed for radioactivity counting in a scintillation counter. Four animals were included in each of the different genotype groups for this analysis.

Statistical analysis. Data are shown as means ± SEM. The t test for unequal variances or the Student's t test (two tailed) was used to determine the statistical significance of differences between the groups of animals. Differences with a P value less than 0.05 were considered significant.

Results

Production of EL genetic mouse models. Mapping and nucleotide sequence analysis revealed a 5′-most exon that contained 5′ untranslated sequence, the initiating methionine and signal sequence of the murine *LIPG* gene, corresponding to published cDNA sequence. The organization of this exon (hereafter referred to as exon 1) (Figure 1a) is similar to what is seen in the human *LIPG* locus. However, in humans there is an upstream alternative first exon that encodes EL isoforms that do not encode a signal sequence (T. Ishida et al., manuscript submitted for publication). Mapping and sequence analysis failed to reveal an orthologous region in the mouse genome, so the single identified 5′ exon 1 was targeted to be replaced with the neomycin resistance gene. Southern blot analysis was performed with both 5′ and 3′probes to verify substitution of the targeted region by homologous recombination (Figure 1b). To demonstrate that the *LIPG* gene was inactivated in *LIPG*$^{-/-}$ mice, several tissue RNAs were prepared and analyzed by Northern blotting using a full-length mouse *LIPG* cDNA probe. As shown in Figure 1c,

although wild-type mice expressed a large amount of *LIPG* mRNA in lung, kidney, aorta, liver, and heart, *LIPG*$^{-/-}$ mice were deficient in this mRNA in these organs. *LIPG*$^{-/-}$ mice were viable and fertile and did not exhibit overt defects. Various organs from *LIPG*$^{-/-}$ mice were further examined histologically, and no abnormalities were detected by light microscopy (data not shown). There was no difference in body weight between wild-type and *LIPG*$^{-/-}$ mice (data not shown).

BAC DNA microinjection produced mice that had integrated the human transgene, as determined by Southern blot using a human *LIPG* cDNA probe (Figure 2a). Expression of h*LIPG*Tg in these animals was evaluated by RNase protection assay using radiolabeled human *LIPG* riboprobes that did not cross-react with the native mouse *LIPG*. The h*LIPG*Tg animals were found to have expression of the human *LIPG* transgene, with an RNase protection assay detecting message in brain, aorta, heart, lung, kidney, and spleen (Figure 2b). Overall, these data indicated that expression of the human BAC produced a 3.2-fold greater level of human *LIPG* mRNA than the endogenous murine gene. By Western blotting of murine tissue extracts, h*LIPG*Tg produced protein levels of human EL approximately 1.9-fold greater than endogenous murine EL (data not shown). Tissue localization of human EL was conducted by immunohistochemistry (Figure 2c). These data confirmed that human *LIPG* mRNA documented by RNase protection resulted in protein expression in aorta, kidney, lung, and spleen. Human EL was found to colocalize with endothelial cells associated with the aorta and large vessels in the kidney and lung as well as the microcirculation in lung and spleen (arrows, Figure 2c). Although *LIPG* has been shown to be expressed in the liver in humans, and in mice as shown here, there was no hepatic expression of the transgene. This result probably reflects the existence of distant hepatic regulatory elements of the *LIPG* gene that were not found on this BAC. The h*LIPG*Tg mice were viable and fertile and did not exhibit overt defects. Various organs from h*LIPG*Tg mice showed no histological abnormalities when evaluated by light microscopy (data not shown). There was no difference in body weight between wild-type and h*LIPG*Tg mice (data not shown).

Plasma lipid and lipoprotein profiles. Fasting plasma lipid levels of wild-type, *LIPG*$^{+/-}$, *LIPG*$^{-/-}$, and h*LIPG*Tg mice are listed in Table 1. In both male and female *LIPG*$^{-/-}$ mice, plasma levels of total cholesterol were significantly increased, with a 69% increase in male homozygous knockout mice as compared with wild-type littermate controls. This was primarily due to an increase in

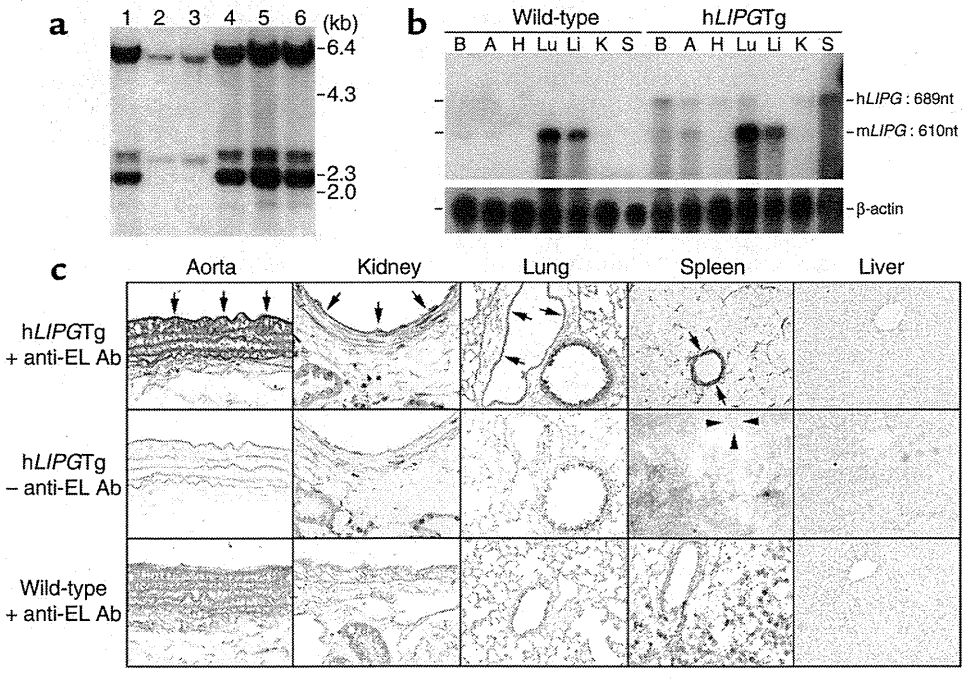

Figure 2

Generation of human endothelial lipase transgenic mice. (**a**) Genotyping of *LIPG* transgenic mice. Southern blot analysis was employed to detect the human *LIPG* transgene. Lanes 2 and 3 show wild-type mice; lanes 1, 4, and 6, heterozygous transgenic mice; and lane 5, homozygous transgenic mice. (**b**) RNase protection with h*LIPG*Tg mouse RNAs. Human *LIPG*-specific signals were detected in the brain, aorta, heart, lung, kidney, and spleen. B, brain; A, aorta; H, heart; Lu, lung; Li, liver; K, kidney; S, spleen. (**c**) Immunohistochemical evaluation of human EL expression in transgenic mice. Tissues harvested from h*LIPG*Tg mice were studied with an anti-human EL monoclonal antibody raised against a synthetic peptide. Human EL was primarily associated with the endothelium of the aorta and large vessels in the kidney, lung, and spleen as well as the microcirculation in the spleen and lung (arrows). Arrowheads in the spleen indicate the central artery, which does not stain when the primary antibody is omitted. No expression was detected in the liver. Original magnification: aorta, ×400; lung and liver, ×200; kidney and spleen, ×630.

Table 1
Lipid and lipoprotein profile in fasted EL knockout and transgenic mice on normal chow

	Chol	TG	HDL-C	LDL-C	PL
Male					
LIPG$^{-/-}$	162.0 ± 7.3	85.6 ± 6.0	119.2 ± 5.2	11.8 ± 0.6	218.4 ± 7.6
P value	<0.001	0.83	<0.001	<0.001	0.0017
n	11	11	11	6	8
LIPG$^{+/-}$	124.4 ± 5.9	97.2 ± 9.2	95.1 ± 4.1	6.6 ± 0.4	188.8 ± 6.4
P value	0.00119	0.2856	0.00265	0.665	0.0177
n	8	8	8	5	8
Wild-type	95.6 ± 4.0	83.3 ± 8.8	76.0 ± 3.4	6.2 ± 0.7	142.8 ± 7.2
n	10	10	10	6	5
h*LIPG*Tg	78.9 ± 4.0	62.1 ± 5.7	61.2 ± 3.3	4.5 ± 0.9	112.0 ± 7.5
P value	0.0095	0.0618	0.00687	0.214	0.098
n	8	8	8	5	5
Female					
LIPG$^{-/-}$	99.5 ± 5.6	86.0 ± 8.7	75.0 ± 6.1	8.3 ± 0.7	154.6 ± 11.2
P value	0.0061	0.055	0.0045	0.905	0.0921
n	11	11	11	6	5
LIPG$^{+/-}$	93.0 ± 12.8	89.4 ± 13.1	69.8 ± 9.7	6.0 ± 1.1	133.8 ± 34.2
P value	0.0467	0.0158	0.0083	0.0674	0.0058
n	9	9	9	5	5
Wild-type	72.0 ± 0.8	66.8 ± 2.7	52.8 ± 2.0	8.4 ± 0.7	102.1 ± 10.6
n	8	8	8	5	5
h*LIPG*Tg	64.7 ± 3.7	60.8 ± 6.8	45.3 ± 2.2	8.1 ± 0.7	82.1 ± 3.4
P value	0.0021	0.426	0.0224	0.798	0.033
n	11	11	10	6	5

Data are shown as means ± SEM (mg/dl). *P* values represent comparison with the wild-type group. Chol, cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PL, phospholipids.

HDL cholesterol levels, which were increased by 57% in *LIPG*$^{-/-}$ and 25% in *LIPG*$^{+/-}$ male mice, with female mice showing similar levels of increase as wild-type controls. Conversely, h*LIPG*Tg male mice showed total and HDL cholesterol levels to be decreased by 17% and 19%, respectively. It is interesting to note that LDL cholesterol levels were increased by 90% in male *LIPG*$^{-/-}$ mice as compared with wild-type mice. The significance of this finding is unclear, however, since there was no significant change in LDL cholesterol levels in any of the other genotype groups for either male or female mice.

Measurement of triglyceride levels in the genetic mouse models indicated that EL may have a modest in vivo role in triglyceride metabolism (Table 1). In female *LIPG*$^{+/-}$ animals, a significantly greater level of triglycerides was measured than in wild-type controls. Although triglyceride levels were not statistically different among the other genetic models, for female *LIPG*$^{-/-}$ animals there was an increase that approached significance, and in the male h*LIPG*Tg mice there was a decreased mean value that approached significance.

Plasma phospholipid levels tended to be higher in animals missing functional endogenous *LIPG* alleles and lower in overexpressing animals (Table 1). Both male and female *LIPG*$^{-/-}$ animals showed a significant increase in plasma phospholipid levels of over 50%, whereas *LIPG*$^{+/-}$ animals showed a significant increase in phospholipid levels of over 30%. The overexpressing h*LIPG*Tg female mice had a significant, 19% lower phospholipid level, and the male h*LIPG*Tg mice had a 21% lower level that was not significantly different from that of wild-type controls. Thus, phospholipid levels varied with respect to genotype in a pattern similar to that observed for cholesterol level.

Plasma lipoprotein profiles were determined by FPLC (Figure 3). The *LIPG*$^{-/-}$ mice had a significant 21% increase in HDL cholesterol in fractions 28 and 29. The h*LIPG*Tg mice showed a significant 78% decrease in HDL cholesterol in fraction 29 and a significant 80% decrease in fraction 30. These cholesterol levels obtained on HDL peak fractions from FPLC were different from those obtained by biochemical measurements on whole plasma (Table 1 and Figure 3). The increase in HDL cholesterol levels for the knockout mice was smaller when measured on the FPLC fractions, and the HDL cholesterol decrease observed in the transgenic mice was greater when determined on FPLC fractions.

By FPLC, the *LIPG*$^{-/-}$ mice had a minimal increase in cholesterol in LDL-containing fractions, and h*LIPG*Tg mice had a corresponding decrease, although neither of these was significant.

Apolipoprotein analysis. Lipoprotein fractions isolated by FPLC were evaluated by polyacrylamide gel electrophoresis and Western blotting, to determine if the cholesterol changes associated with altered EL expression levels reflected alterations in the apoprotein components of the lipoprotein particles. Electrophoresis on a 15% gel of the peak HDL-containing fractions revealed that there were differences in the intensity of the Coomassie-stained bands representing apoA-I in

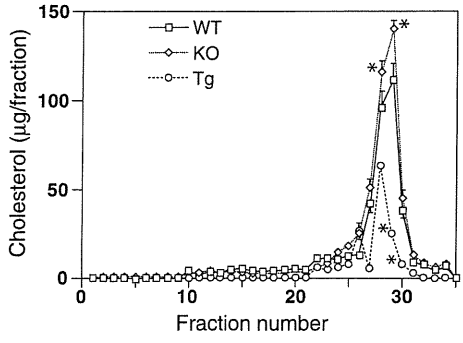

Figure 3
Cholesterol levels in lipoprotein fractions obtained by FPLC. Determination of cholesterol concentration in serum fractions obtained by FPLC revealed a correlation between *LIPG* genotype and HDL cholesterol levels. HDL cholesterol levels for *LIPG*$^{-/-}$ mice were significantly greater than for wild-type mice in fractions 28 and 29 ($P < 0.05$). HDL cholesterol levels were lower in *LIPG* transgenic animals for fractions 29 and 30 ($P < 0.05$) than in wild-type mice. KO, *LIPG*$^{-/-}$; Tg, h*LIPG*Tg. *$P < 0.05$ vs. WT.

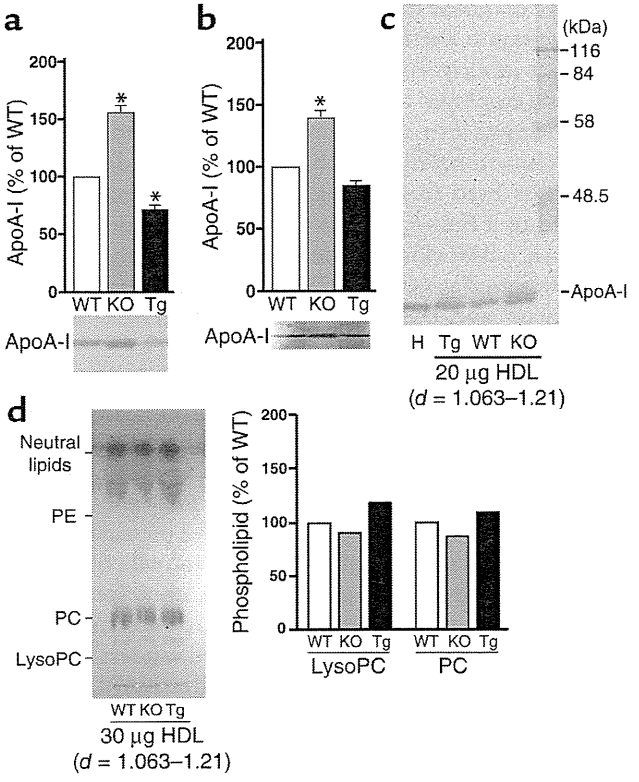

Figure 4

HDL lipoprotein particle analysis. (**a**) SDS-PAGE analysis of apoA-I in the HDL peak fractions isolated by FPLC. One hundred microliters of the HDL peak fraction from FPLC were evaluated by 15% SDS-PAGE. Gels were stained and quantitated by densitometry. (**b**) Western blot analysis of apoA-I in HDL peak fraction from FPLC. Images were quantitated by densitometry. (**c**) HDL isolated by density-gradient ultracentrifugation (d = 1.063–1.21) was evaluated by 12% SDS-PAGE, with 20 µg of protein loaded per lane. Lane 1 is human HDL standard, and lane 5 is a molecular weight marker. H, human HDL standard. (**d**) HDL isolated by density-gradient ultracentrifugation (d = 1.063–1.21) was evaluated by TLC. Equal amounts of HDL, as determined by protein quantitation, were loaded. KO, *LIPG*$^{-/-}$; Tg, h*LIPG*Tg. *P < 0.05 compared to WT.

the different genetic models (Figure 4a). Replication of this experiment and quantitation by densitometric scanning of the gels revealed a significant 52% increase in the apoA-I levels in the homozygous knockout animals and a significant 26% decrease in transgenic animals. Similar experiments were conducted with transfer of the electrophoresed protein to membranes that were probed with an antibody to apoA-I (Figure 4b). Quantitation of these results detected a significant 40% increase in apoA-I in the homozygous knockout animals and a 17% decrease in transgenic animals that was not significant. To further investigate the apolipoprotein content of the HDL fractions from FPLC, SDS-PAGE was performed on the HDL peak fraction with a 3–15% gradient gel. This gel revealed the presence of apoB in these fractions, suggesting some contamination of HDL with small LDL (data not shown). Such contamination could contribute to the observed differences in HDL cholesterol levels measured by biochemical techniques versus FPLC (Table 1 and Figure 3). Similar analysis of LDL fractions from FPLC revealed no evidence of apoA-I.

To further define the composition of HDL particles in the genetic models, ultracentrifugation fractionation of lipoprotein particles was performed. When equal amounts of protein from the HDL fraction (density, 1.063–1.21 g/ml) were analyzed by SDS-PAGE, a single band with equal density corresponding to apoA-I was obtained for the different genetic models (Figure 4c). Analysis of the same ultracentrifugation density fraction by TLC was performed to evaluate the phospholipid makeup of the HDL particles among the different mouse models (Figure 4d). When equal amounts of HDL were evaluated, the results were not statistically different among the wild-type, homozygous knockout, and transgenic lines. In particular, no accumulation of lysophosphatidyl choline was seen in the knockout or transgenic lines.

The ultracentrifugal HDL fraction was analyzed for protein, cholesterol, and phospholipid content (Table 2). These data indicated that the composition of HDL particles in the knockout and transgenic lines was similar to that of the wild-type animals.

Phospholipase activity. Phospholipase activity was compared among *LIPG*$^{-/-}$, h*LIPG*Tg, and wild-type animals before and after heparin injection (Figure 5). Before heparin injection, phospholipase activity was not statistically different among the different lines (wild-type mice, 519 ± 22 nmol FFA/ml/hr; knockout mice, 521 ± 5 nmol FFA/ml/hr; transgenic mice, 510.5 ± 20.23 nmol FFA/ml/hr). After heparin injection, there was a 32% increase in phospholipase activity in the wild-type animals. A significantly greater 45% increase in postheparin phospholipase activity was observed for transgenic animals. For *LIPG*$^{-/-}$ mice, there was a 12% postheparin increase in phospholipase activity, which was significantly less than that observed in wild-type mice. These data indicate that over 50% of heparin-releasable phospholipase activity is associated with EL.

Discussion

LIPG was originally cloned by two groups looking for differentially regulated genes in two very different in vitro systems. One group differentiated THP-1 cells and identified upregulated genes by differential dis-

Table 2

HDL lipoprotein particle composition

	Protein (%)	Cholesterol (%)	Phospholipid (%)
Wild-type	48.0	6.8	43.0
LIPG$^{-/-}$	43.5	7.1	50.0
h*LIPG*Tg	44.0	6.4	51.0

The averages of measurements for two groups of five pooled mouse plasma samples are shown. Data are expressed as mean percents by weight. Cholesterol represents the total cholesterol of the particle; free cholesterol and cholesterol ester were not measured separately.

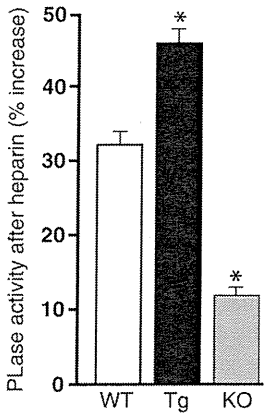

Figure 5
Phospholipase activity in genetic models. Phospholipase activity in different genetic lines was compared between pre- and postheparin injection samples. A significantly greater level of postheparin phospholipase activity was observed in the transgenic lines ($*P < 0.05$), and a significantly lower level of postheparin augmentation of phospholipase activity was found in the knockout animals ($*P < 0.05$) as compared to the wild-type controls. The preheparin levels of plasma phospholipase activity were as follows (mean ± SEM): wild-type animals, 519 ± 22.3 nmol FFA/ml/hr; knockout animals, 521 ± 5.4 nmol FFA/ml/hr; and transgenic animals, 510.5 ± 20.2 nmol FFA/ml/hr. PLase, phospholipase activity; Tg, h*LIPG*Tg; KO, *LIPG*$^{-/-}$.

play after exposure to oxidized LDL (17). Our group identified *LIPG* through a comprehensive search for genes differentially regulated in endothelial cells during angiogenesis and identified the gene as being upregulated by suppression subtraction hybridization during tube formation on Matrigel (16). The EL predicted protein sequence encoded all of the expected lipase motifs, including the catalytic triad of serine, aspartate, and histidine, as well as heparin and lipid binding regions. The lid region, the domain felt to contribute to substrate specificity, was found to be different from those of HL and LPL, suggesting a different functional profile. Indeed, EL was shown to have relatively more phospholipase activity than triglyceride lipase activity in in vitro assays. The expression pattern of EL was also distinct from the other family members, being found in thyroid, lung, placenta, kidney, liver, and testis as well as adult vasculature. EL is expressed in vivo by endothelial cells, smooth-muscle cells, and macrophages in the arterial vessel wall, and cell culture experiments have suggested that *LIPG* is highly regulated by factors such as biophysical forces and cytokines that are known to be involved in vascular disease processes (18, and our unpublished data).

The existence of EL had not been predicted by studies of lipid metabolism. Careful evaluation of the function of this new lipase is thus critically important to establish its role within the context of the known lipase family of molecules. Preliminary functional information was provided by in vivo adenoviral gene transfer experiments that indicated EL overexpression lowered HDL levels (17). Accordingly, we have established a number of mouse models on the C57Bl/6 background to investigate the actions of this new lipase family member in a rigorous fashion. Gene targeting in ES cells was employed to functionally delete the gene, and this allele was introduced into the germline and shown to be a functional null by RNA expression studies. The allele was bred from the 129/C57Bl/6 mixed background to a homogeneous C57Bl/6 background. In addition, a BAC clone carrying the entire human *LIPG* locus was used to generate C57Bl/6 mice transgenic for this locus, and these mice were characterized with respect to transgene expression.

A distinct role for EL regulation of HDL cholesterol is documented, with both knockout and transgenic mouse models showing a correlation between functional EL expression levels and HDL cholesterol. Decreased expression of native EL in mice showed a gene dosage effect, with HDL cholesterol increasing in relation to the number of null alleles. In male *LIPG*$^{-/-}$ mice, there was a 57% increase in HDL cholesterol as compared with wild-type controls. HDL cholesterol levels in transgenic animals that expressed modest levels of human EL were correspondingly decreased, suggesting that pronounced overexpression of EL is not required to alter HDL levels. Measurements on isolated lipoprotein fractions also documented the correlation between EL expression and cholesterol level in HDL particles. There were observed differences between the biochemical and the FPLC lipid analyses, with the HDL peak fractions from FPLC showing less of an increase in HDL cholesterol in knockout animals but greater decreases in HDL cholesterol in the transgenic animals. This discrepancy between the levels determined by biochemical methods, which remove apoB-containing lipoproteins to measure HDL cholesterol, and FPLC, which separates the lipoproteins by size, could be due to the presence of small LDL particles in the HDL range. Indeed, SDS-PAGE analysis of FPLC lipoprotein fractions identified apoB in the HDL fractions, indicating the presence of some LDL particles, as hypothesized.

HDL particle composition analysis on plasma fractionated by both FPLC and ultracentrifugation provided support for the hypothesis that the increase in HDL cholesterol in knockout animals was a function of an increased number of particles rather than modification of the composition of HDL particles. Quantitative SDS-PAGE and Western blotting of FPLC fractions revealed that apoA-I was the dominant protein in the HDL particles and that there was a concomitant increase in apoA-I in HDL fractions. These data also confirmed that the increased cholesterol peak on FPLC for the *LIPG* knockout animals was associated with HDL particles and that there was no shift in particle size. The increase in apoA-I in the knockout animals was confirmed by SDS-PAGE analysis of HDL density fractions from the ultracentrifugation. Corresponding data with transgenic animals revealed decreased apoA-I levels that tracked with decreased HDL cholesterol.

Quantitation of the protein, cholesterol, and phospholipid components of the density fraction corresponding to HDL revealed that the relative contribution of each of these components to the HDL particle was unchanged in the knockout and transgenic animals as compared with the controls. Chromatography of the HDL density fraction revealed that the phospholipid makeup of the HDL particles was unchanged and that there was not an enrichment with Lyso-PC. All of these data are consistent with an overall change in HDL particle number resulting from changes in EL levels in these genetic models.

The in vivo enzymatic activity described here is in keeping with the in vitro functional data that have been obtained with recombinant EL protein. Such data have indicated that EL has minimal triglyceridase activity but functions primarily as a phospholipase (16, 17, 19). Significant increases in triglyceride levels in female knockout mice and trends toward decreased triglyceride levels in male transgenic animals suggests that EL may have some in vivo triglyceridase activity, as suggested by McCoy et al. (19). However, the changes in HDL levels noted in the genetic mouse lines was much greater. This is consistent with the observation that HDL is the lipoprotein class that is affected most by the level of EL expression. Phospholipase activity in postheparin plasma correlated with the level of EL expression. It was significantly increased in transgenic animals and decreased in knockout animals. There was a large amount of phospholipase activity contributed by circulating lipases in the mice — presumably primarily HL, which is not bound to endothelial cell surfaces in mice — and this did not change with manipulation of EL expression. However, the loss of EL in the homozygous knockout animals resulted in a significant decrease in the postheparin augmentation of phospholipase activity. These data clearly point to EL as contributing the majority of heparin-releasable phospholipase activity in mice and thus having a prominent role contributing to phospholipase activity in vivo.

Overall, findings from these experiments employing genetic models are in agreement with, and significantly extend, previous functional studies. Together, these data support the hypothesis that EL makes a physiological contribution to HDL metabolism in mice. Given the known difference in HDL lipoprotein species between mice and humans, observations made here with genetic mouse models will require confirmation in humanized mice and humans. If EL has a similar role in humans, its regulation by pathophysiological effectors will establish EL as an important independent regulator of HDL levels. At this time, the mechanism by which EL modulates HDL level is unclear. If EL hydrolyzes HDL phospholipids, it would necessitate removal of the other components of the HDL particle such as apoA-I and cholesterol by transfer to tissue or other lipoprotein classes. This should result in an increased rate of catabolism of all of the components of HDL and would be consistent with the observation that apoA-I and HDL cholesterol levels fall proportionately with HDL phospholipid.

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REVIEW

Update on the Role of Endothelial Lipase in High-Density Lipoprotein Metabolism, Reverse Cholesterol Transport, and Atherosclerosis

Tomoyuki Yasuda, MD, PhD; Tatsuro Ishida, MD, PhD; Daniel J. Rader, MD

Endothelial lipase (EL) is a phospholipase that belongs to the lipoprotein lipase (LPL) family, which includes LPL and hepatic lipase (HL). Similar to LPL and HL, EL regulates lipoprotein metabolism, mainly high-density lipoprotein (HDL) metabolism and HDL cholesterol (HDL-C) levels in humans and mice. Existing data strongly suggest that inhibition of EL in humans would be expected to increase the HDL-C level. However, it has not been definitively established whether the effect of EL activity on HDL-C levels translates into effects on reverse cholesterol transport or atherosclerosis. The available data regarding the impact of EL expression and activity on HDL metabolism, reverse cholesterol transport, and atherosclerosis are reviewed. (*Circ J* 2010; **74:** 2263–2270)

Key Words: Apolipoprotein; Atherosclerosis; High-density lipoprotein; Lipids; Lipoprotein

The benefit of low-density lipoprotein cholesterol (LDL-C) reduction therapy to avoid or decrease the likelihood of atherosclerotic disease has been widely established.[1] However, accumulating clinical evidence implies limitations of LDL reduction therapies, as there remains substantial residual risk.[2] Plasma levels of high-density lipoprotein-cholesterol (HDL-C) are a negative risk factor for cardiovascular disease (CVD).[3] The antiatherogenic function of HDL is mediated at least in part by the promotion of reverse cholesterol transport (RCT).[4] In addition, the HDL particle itself or its major associated protein, apolipoprotein A-I (apoA-I), has a variety of antioxidative, antiproliferative, and antiinflammatory activities.[5] Thus, HDL-C raising therapy would be of great interest for reducing residual risk. Current approaches to raising plasma HDL-C levels have limitations; for example, niacin modestly increases plasma HDL-C levels but there are issues with tolerability, and fibrates have relatively small effects on HDL-C in most patients.[6] Some experimental apoA-I-based infusion therapies have reportedly attenuated the progression or induced the regression of atherosclerosis.[7] A large-scale clinical trial of the cholesteryl ester transfer protein (CETP) inhibitor, torcetrapib, in combination with atorvastatin failed to reduce the incident of the major cardiovascular events, despite a marked increase in plasma HDL-C levels and a decrease in plasma LDL-C levels.[8] Although the effect of CETP inhibition will be clarified by the results of ongoing clinical studies with the next generation of CETP inhibitors, it is considered that the effectiveness of HDL-raising therapies should be evaluated by the effect on RCT or clinical outcomes.

Endothelial lipase (EL) is a phospholipase that belongs to the lipoprotein lipase (LPL) gene family. EL is a regulator of plasma HDL-C levels, and here we review recent findings regarding the role EL in RCT and atherosclerosis.

EL Belongs to the *LPL* Family

Well-characterized members of the triglyceride lipase family include LPL and hepatic lipase (HL). LPL is mainly synthesized by adipocytes, skeletal muscle cells, and cardiac muscle cells, whereas HL is synthesized by the liver. LPL and HL are well known to regulate plasma cholesterol and triglyceride (TG) levels. LPL has dominantly TG lipase activity and hydrolyzes TG-rich lipoproteins, whereas HL has both TG lipase and phosholipase activity and hydrolyzes LDL and HDL, generating small LDL and small HDL.

Mature EL is a 68-kD glycoprotein,[9] and it shares amino acid sequence similarity with lipases, having 44% amino acid identity to LPL and 41% amino acid identity to HL.[10] However, the sequence homology of the lid lesion, which determines the specificity of lipases, largely differs between EL and LPL or HL (Table). EL is a unique lipase because it is synthesized mainly by vascular endothelial cells, and to a lesser extent by macrophages and smooth muscle cells. EL has a signal sequence in the amino-terminus, and therefore is secreted from these vascular cells, after which it binds to cell surface proteoglycans where it exerts its action.

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Table. Comparison of 3 Lipases

	EL	LPL	HL
Lipase activity	PL > TG	TG >>> PL	TG > PL
Major substrate	HDL	TG-rich lipoprotein	TG-rich lipoprotein, HDL, LDL
Inflammation	↑	↓	↓
Metabolic syndrome	Mass ↑	Mass ↓	mRNA, mass ↑
Effect on in vivo RCT	KO ↔	NA	KO ↔
Effect on atherosclerosis	KO ↓ ↔	KO ↑, transgenic ↕	KO ↕
Where synthesized	Endothelial cells	Adipocytes	Hepatocytes
		Muscle cells	
	Macrophages	Macrophages	Macrophages

EL, endothelial lipase; LPL, lipoprotein lipase; HL, hepatic lipase; PL, phospholipid; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RCT, reverse cholesterol transport; KO, knockout; NA, not available.

EL and HDL Metabolism

In contrast to LPL and HL, EL is primarily an A1 phospholipase and hydrolyzes HDL-phospholipids at the sn-1 position. Strauss et al reported that EL efficiently cleaves nonesterified fatty acid (NEFA) from HDL-phospholipids, supplying NEFA for EL-expressing cells.[11] Jahangiri et al showed that the reduction in HDL particle size mediated by EL was related to the amount of phospholipid hydrolysis.[12] It has been reported that EL overexpression accelerates both renal apoA-I catabolism[13] and hepatic cholesterol uptake through scavenger receptor class B type I (SR-BI).[14] As a result, EL promotes the remodeling and elimination of HDL particles. In fact, plasma HDL-C levels are increased in *EL* knockout (–/–) mice and decreased in *EL* transgenic mice, indicating the inverse dosage effect of *EL* on plasma HDL-C levels in mice.[15] The HDL particle size is increased in $EL^{-/-}$ mice, and turnover studies demonstrated that the clearance of HDL from the plasma was delayed in these mice.[16] Similarly, studies have indicated that in humans the plasma EL mass inversely correlates with plasma HDL-C levels and positively correlates with features of atherosclerosis and metabolic syndrome.[17] These findings indicate that EL is a major determinant of plasma HDL-C levels.

EL has conserved heparin-binding properties and lipid-biding domains,[18] and therefore interacts directly with heparan sulfate proteoglycans (HSPG) and circulating lipids/lipoproteins. EL acts as bridge between lipoprotein and endothelial cells, promoting lipoprotein incorporation.[19] As is the case with LPL and HL, EL has noncatalytic bridging functions in addition to its catalytic phospholipase activity, and both of these functions are considered to participate in modulating plasma HDL-C levels in mice.[20] Adenovirus-mediated hepatic EL overexpression accelerates plasma HDL clearance through both catalytic and noncatalytic bridging functions in vivo, and both the catalytic and noncatalytic bridging functions of hepatic EL promoted HDL-C selective uptake in in-vitro studies.[21] The delayed HDL clearance in $EL^{-/-}$ mice is likely mediated by both the hydrolyzing and ligand-binding functions of EL.[22] These findings suggest that EL inhibition would increase plasma HDL-C levels by inhibiting HDL and apoA-I catabolism.

Because all of these lipases bind to the endothelium through HSPG, they are released by heparin administration and the post-heparin plasma is high in lipase activity and mass compared with preheparin plasma. Previous studies have shown that heparin treatment increases plasma EL mass 3-fold.[17] The EL mass in both the pre- and post-heparin plasma inversely correlates with the plasma HDL-C levels.

Regulation of EL Expression and Activity

It has been reported that EL expression is highly regulated by a variety of factors (**Figure 1**). For instance, tumor necrosis factor α, interleukin (IL)-1β and biomechanical forces have induced EL mRNA expression in human endothelial cells.[23] Although phospholipase- and TG-lipase activities are barely detected in unstimulated endothelial cells, cytokine treatment stimulates phospholipase- and TG-lipase activities concomitant with an increase in EL expression.[24] Moreover, lipopolysaccharide induces macrophage EL expression via activation of toll-like receptor 4.[25,26] Shimokawa et al reported that angiotensin II and hypertension induce EL expression in vascular smooth muscle cells.[27] Thus, the expression and activity of EL is induced by inflammatory stimuli in vascular cells existing in atherosclerotic lesions, whereas LPL or HL expression is reduced in response to these stimuli. Indeed, Paradis et al reported that in humans the plasma EL mass positively correlates with inflammatory makers C-reactive protein, IL-6, and the sPLA2-IIa concentration.[28] Furthermore, Badellino et al showed that experimental low-dose endotoxemia in humans resulted in a 2.5-fold increase in the EL concentration, which was associated with decreases in both total and HDL-phospholipids;[29] that study paper also demonstrated a strong correlation of EL mass with several markers of inflammation.[28] Also, statins reduce EL expression and phospholipase activity in endothelial cells and macrophages, and reduced plasma EL mass accompanies increased plasma HDL-C levels in humans.[30,31] According to those findings, upregulation of EL in inflammatory states, including atherosclerosis and metabolic syndrome,[32] may contribute to the low HDL-C levels seen in these conditions. Intervention to reduce inflammation might be expected to raise plasma HDL-C levels by decreasing EL expression.

It has been postulated that EL undergoes tissue-specific regulation in vivo. Yu et al reported that hepatic EL expression decreased in $apoE^{-/-}$ mice while aortic EL expression increased.[33] Feeding wild-type mice with a high-fat diet also decreased hepatic EL expression. A similar finding was obtained with *LDL receptor* $(LDLR)^{-/-}$ mice (Yasuda et al, unpublished data 2010). Therefore, it is considered that the cellular cholesterol content might regulate hepatic EL expression, although the precise mechanism is still unknown.

EL activity is also regulated through posttranscriptional mechanisms. Griffon et al recently reported that EL forms a dimer in human plasma, and that the homo-dimer formation is essential for the maintenance of EL activity,[34] as is the case with LPL and HL. In addition, EL activity is regulated

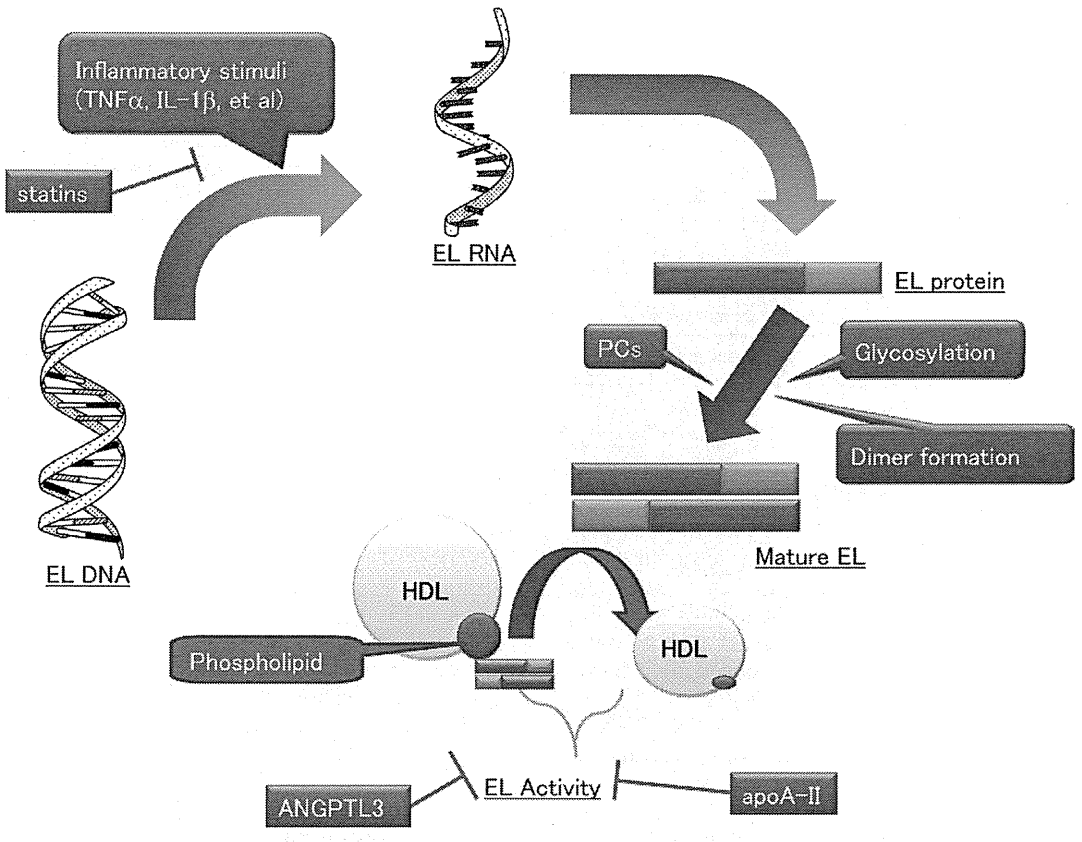

Figure 1. Endothelial lipase (EL) expression and activity are regulated by a variety of factors. Inflammatory stimuli promote EL mRNA expression. Dimer formation, glycosylation, and proprotein convertases (PCs) play a crucial role in generating mature EL. Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein A-II (apoA-II) inhibit EL lipase activity. HDL, high-density lipoprotein; IL, interleukin; TNF, tumor necrosis factor.

by proprotein convertases (PCs). PCs induce site-specific proteolysis, which plays a crucial role in regulating many fundamental biological pathways, including the sequential initiation of activation of blood coagulation factors and activation of caspases and digestive enzymes. Jin et al have shown that EL is proteolytically processed into 40-kDa and 28-kDa fragments and inactivated by PCs.[35] Overexpression of profurin, an inhibitor of PCs, results in a reduction of plasma HDL-C levels in wild-type mice, but this effect is not observed in *EL*$^{-/-}$ mice. Administration of profurin to mice inhibits the cleavage of EL and increases plasma phospholipase activity, so PC inhibitors may reduce plasma HDL-C levels by increasing EL activity. Shimamura et al reported that angiopoietin-like 3 (ANGPTL3) is known to act as an endogenous EL inhibitor.[36] *ANGPTL3* knockout mice show low plasma HDL-C levels accompanied by increased phospholipase activity, indicating that EL activity may be elevated in these mice. Furthermore, plasma ANGPTL3 levels significantly correlate with plasma HDL-C levels in humans. Inhibition of PC cleavage reduces the activation of ANGPTL3, which may attenuate the inhibitory effect of ANGPTL3 on EL activity. Thus, PCs regulate EL activity directly through cleavage of EL protein and indirectly through modulating ANGPTL3 levels in the liver. Thus, the hepatic PC–ANGPTL3–EL–HDL pathway is a novel mechanism controlling HDL metabolism and cholesterol homeostasis.[37]

McCoy et al reported that human heat-inactivated serum inhibited EL phospholipase activity,[38] indicating that some endogenous EL inhibitor might exist in human serum. Apolipoprotein A-II (ApoA-II) constitutes ~20% of the total HDL protein mass, and has been reported to inhibit several protein activities related to HDL metabolism. Broedl et al reported that EL has less activity in human apoA-I/A-II double transgenic mice compared with human apoA-I single transgenic mice.[39] These findings suggest that apoA-II may act as an inhibitor of EL activity in vivo.

EL has 5 potential N-linked glycosylation sites, and the effects of each site have been evaluated by site-directed mutagenesis of the asparagine. Miller et al reported that Asn-60 mutation markedly reduced its secretion, Asn-116 mutation increased phospholipase activity, and Asn-373 mutation reduced EL activity and its lipid-binding function.[9] Brown et al reported that the specific hydrolytic activity toward LDL by EL-N116A was significantly greater than wild-type EL in vitro.[40] In addition, adenovirus encoding wild-type EL reduced plasma HDL-C levels dominantly, whereas that encoding Asn-116 mutant EL reduced both HDL-C and LDL-C in *LDLR*$^{-/-}$ mice. In contrast, Skropeta et al reported that EL N118A, N375A, and N473A reduced phospholipase activity, and N62A increased phospholipase activity by 6-fold compared with wild-type EL.[41] There is a discrepancy between these data on the effects of glycosylation, partially because the 2 studies used different substrates, either glycerol-stabilized emulsion or reconstituted HDL. According to the data,

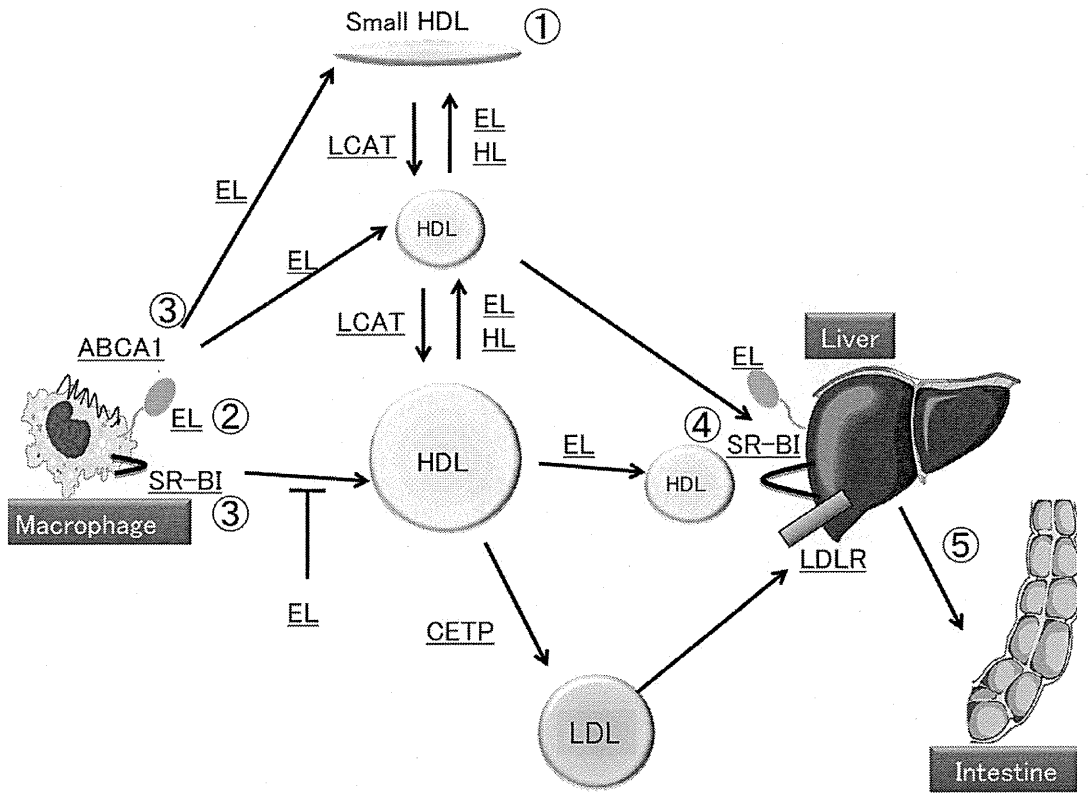

Figure 2. Effects of endothelial lipase (EL) on reverse cholesterol transport. ① EL overexpression reduces plasma high-density lipoprotein cholesterol (HDL-C) levels and generates small HDL. ② Macrophage EL promotes apolipoprotein A-I mediated cholesterol efflux. ③ EL overexpressed serum promotes ATP binding-cassette transporter 1 (ABCA1)-dependent cholesterol efflux, but reduced scavenger receptor class B type I (SR-BI)-dependent cholesterol efflux. ④ Hepatic EL promotes hepatic cholesteryl ester selective uptake through SR-BI. ⑤ Hepatic EL overexpression does not promote bile secretion. CETP, cholesteryl ester transfer protein; HL, hepatic lipase; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; LDLR, LDL receptor.

the glycosylation of the EL protein may regulate plasma HDL-C levels in vivo by affecting EL phospholipase activity.

Association of EL Single-Nucleotide Polymorphisms With HDL-C Level or CVD

Several EL gene polymorphisms have been reported to be associated with plasma HDL-C levels or cardiovascular events. The most common coding variant in *EL* is the T111I variant. Although early small reports suggested an association of the T111I variant with HDL-C levels, larger studies have definitively established a lack of association; furthermore, this variant has normal lipase activity compared with wild-type EL.[42,43] Interestingly, Shimizu et al reported that the T111I polymorphism was associated with acute myocardial infarction independently of plasma HDL-C levels.[44] Tang et al reported a similar finding in a Chinese population, which showed that the T111I variant was associated with protection from coronary artery disease (CAD).[45] The most common functional EL variant in Caucasians is the N396S variant, which has been reported to decrease EL activity in vitro and is definitively associated with elevated plasma HDL-C levels.[42] It will be important to determine whether the N396S variant is associated with CAD. Brown et al reported that a low-frequency G26S variant exists in persons of African descent and that this variant is associated with elevated plasma HDL-C levels because of reduced secretion.[46] Genome wide association studies have revealed that a common variant at the *LIPG* (EL) locus is significantly associated with plasma HDL-C levels.[47] Taking all the findings together, genetic variation in EL resulting in loss of function can elevate plasma HDL-C levels but of the relationship with CVD remains uncertain.

Does EL Inhibition Promote RCT?

HDL plays a central role in the process of RCT. The first step is cholesterol efflux from macrophages to HDL. A hypothesis can be made that EL inhibition would raise HDL-C levels and activate RCT in vivo. A number of studies regarding the EL effect on RCT have already been performed. Yancey et al reported that EL changes HDL composition and turns it into smaller particles.[48] In their cell culture studies, EL-rich serum decreased cholesterol efflux via SR-BI by 90% and increased efflux via ATP binding-cassette transporter 1 by 63%, probably because of increased production of pre-beta HDL in the serum. Brown et al reported that plasma and HDL from $EL^{-/-}$ mice has greater efflux capacity than those from wild-type mice.[49] Qui et al reported that macrophage EL promotes apoA-I-mediated cholesterol efflux through

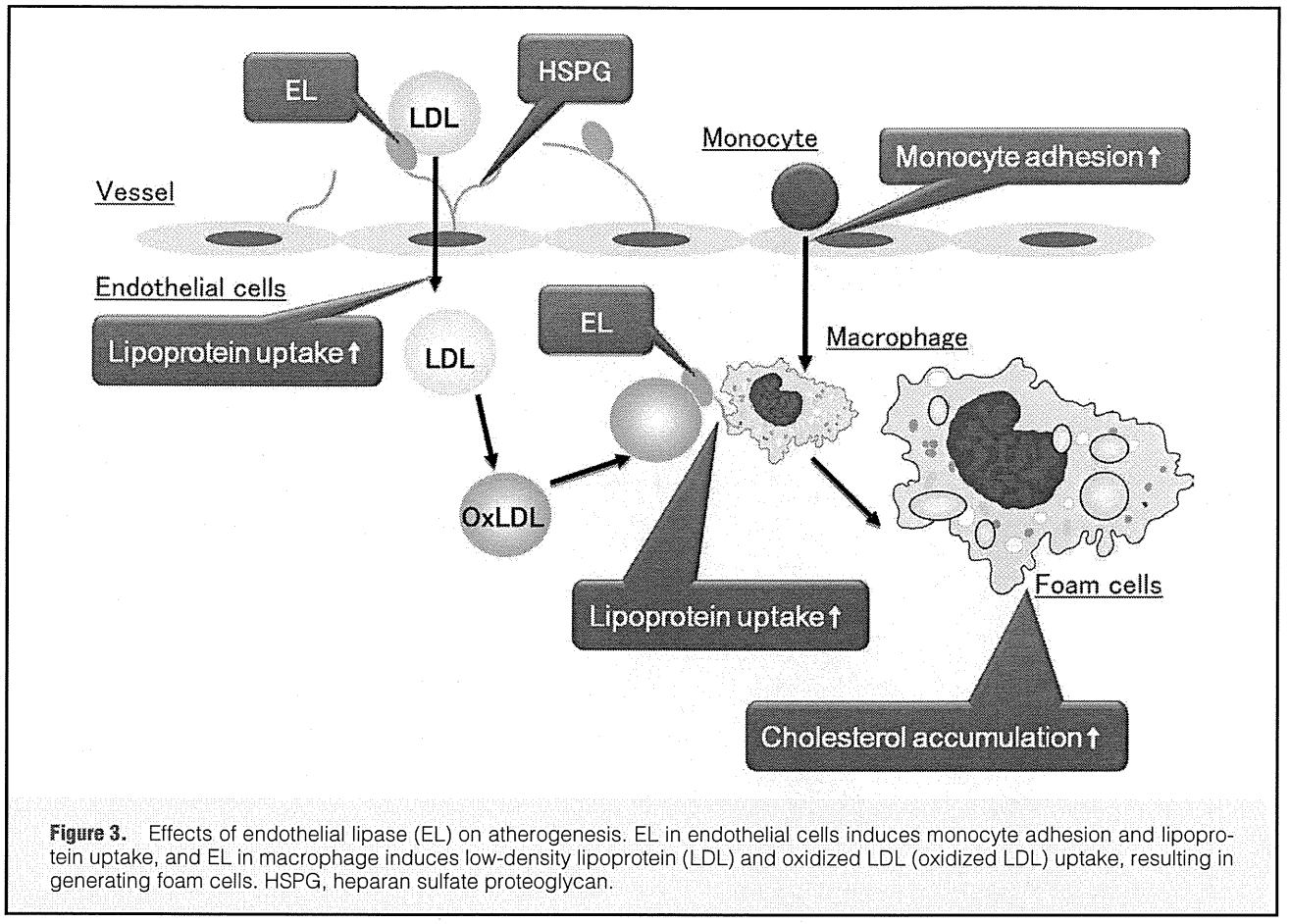

Figure 3. Effects of endothelial lipase (EL) on atherogenesis. EL in endothelial cells induces monocyte adhesion and lipoprotein uptake, and EL in macrophage induces low-density lipoprotein (LDL) and oxidized LDL (oxidized LDL) uptake, resulting in generating foam cells. HSPG, heparan sulfate proteoglycan.

catalytic- and noncatalytic-dependent mechanisms through promotion of apoA-I binding to macrophages and lysophosphatidylcholine (lyso-PC) production, which can promote apoA-I-mediated cholesterol efflux.[50] These findings indicate that EL works both ways (promoting and inhibiting) in macrophage cholesterol efflux.

The next step in RCT is hepatic cholesterol uptake. Strauss et al revealed that EL mediates the binding of HDL to the cell surface through its catalytic and noncatalytic functions in HepG2 cells, and promotes the uptake of HDL holoparticles, as well as selective uptake of its cholesterol ester (CE).[21] Additionally, Nijstad et al reported that hepatic EL overexpression promotes HDL-CE selective uptake in wild-type mice but not in *SR-BI*$^{-/-}$ mice, indicating the SR-BI-mediated selective uptake of HDL-CE requires the remodeling of large alpha-migrating HDL particles by EL.[14] These findings support the concept that EL is a key regulator of the hepatic uptake of HDL-CE.

The final step in RCT is cholesterol secretion from the liver to feces. Interestingly, EL overexpression has been reported to markedly reduce plasma HDL-C levels and increases hepatic cholesterol content without affecting biliary sterol secretion.[51] Furthermore, Brown et al have recently reported that EL- and/or HL-deficient mice exhibited the same degree of in vivo RCT as wild-type mice, despite increased plasma HDL-C levels.[49] These findings suggest that, when the complete RCT is defined as cholesterol flux from macrophages to feces, the net effect of EL on RCT remains speculative (**Figure 2**). However, if we look at the redistribution of cholesterol from the diseased vessel wall to the liver, which is a fundamental process in plaque regression, EL inhibition may increase plasma HDL-C levels without decreasing the EL ligand-binding function, which may promote cholesterol efflux from the diseased vessel wall to the liver.

Does EL Inhibition Promote or Attenuate Atherosclerosis?

The ieffect of altered expression of the TG-lipase members has been previously reported. *LPL*$^{-/-}$ mice developed spontaneous atherosclerosis,[52] whereas LPL transgenic mice had decreased atherosclerosis because of the reduction in remnant lipoprotein.[53] Moreover, LPL overexpression generates small dense LDL, resulting in an exacerbation of atherosclerosis in rabbits.[54] HL deficiency reduces atherosclerosis in *apoE*$^{-/-}$ mice,[55] but it has an antiatherogenic function in *LDLR*$^{-/-}$ mice.[56] These results indicate that the effects of the TG-lipase family on atherosclerosis have not been fully elucidated, probably because both lipases have both pro- and antiatherogenic effects on atherosclerosis depending on where is it expressed.

Although EL inactivation was previously expected to inhibit atherosclerosis by raising plasma HDL-C levels, the effect of EL inactivation on atherosclerosis seems more complex than expected. Ishida et al have previously reported that targeted inactivation of *EL* increased the plasma HDL-C level and inhibited atherosclerosis in *apoE*$^{-/-}$ mice.[57] On the other hand, Ko et al independently reported that *EL* inactivation in hyperlipidemic mice did not affect atherosclerosis even though the plasma HDL-C level was raised.[58] Because

apoE$^{-/-}$ or *LDLR*$^{-/-}$ mice are extremely high in atherogenic apoB-containing lipoproteins, it is considered to be difficult to determine the effect of relatively small changes in HDL-C levels on formation of atherosclerosis in these mice. In this context, it still remains unclear whether EL acts as a pro- or antiatherogenic molecule overall. Brown et al showed that EL and HL deficiency increases small dense LDL partly through LPL activation.[49] In addition, EL overexpression reduces plasma apoB-containing lipoprotein levels in mice,[59] while EL deficiency increases very-LDL-C (VLDL-C) levels against an apoE- or LDL-receptor deficient background.[57,58] Therefore, EL can promote the catabolism of apoB lipoproteins as well as HDL. However, considering that plasma EL mass does not correlate with plasma LDL-C or TG levels, the action of EL on apoB lipoproteins is limited in humans. Ahmed et al reported that EL activity is essential for HDL-induced adhesion molecule inhibition through PPARα activation in endothelial cells.[60] These lines of evidence imply that EL has some antiatherogenic functions.

In contrast, Kojima et al reported that monocyte adhesion was reduced on endothelial cells from *EL*$^{-/-}$ mice and increased on that from *EL* transgenic mice.[61] It is also reported that macrophages from *EL*$^{-/-}$ mice or *EL*-depleted macrophages have less capacity to take up LDL and oxidize LDL independently of its lypolytic function.[25,62] These findings suggest that EL acts as a bridge between monocytes and endothelial cells, lipoproteins and macrophages, which are the initial steps of atherosclerosis. Azumi et al reported that EL is expressed in endothelial cells, smooth muscle cells, and macrophages within human atherosclerotic lesions.[63] Hence, considering that EL is upregulated by inflammatory stimuli, EL may play a principal role in the vicious cycle of inflammation at the site of atherosclerotic lesions. EL inhibition by statins decreases the hydrolysis of phospholipids and subsequent lyso-PC productions, thus EL may increase proinflammatory lyso-PC production.[31] The data from human and mouse studies indicate that EL has some proatherogenic effects, especially locally at atherosclerotic lesions (**Figure 3**).

All in all, EL seems to have diverse effects on the formation of atherosclerosis, depending on the cell type, the degree of inflammation, species etc. The net effects of these functions may explain the inconsistent results of EL inactivation on atherosclerosis that have been reported previously. Although there are conflicting results regarding EL's pro- or antiatherogenic functions, speculation is raised that vascular EL, particularly at the site of atherosclerotic lesions, may accelerate the process of atherosclerosis in *apoE*$^{-/-}$ mice,[57] whereas elevation of the VLDL fraction affected by hepatic EL deficiency might be crucial for developing atherosclerosis in *LDLR*$^{-/-}$ mice.[64] These findings might explain the result that EL deficiency reduces atherosclerosis against the apoE knockout background, but not the LDLR knockout background.

Conclusions

EL is clearly a determinant of plasma HDL-C levels in humans, and inhibition of EL in humans would be expected to raise plasma HDL-C levels. However, the effect of EL inhibition on atherosclerotic CVD in humans is harder to predict. EL appears to have a variety of functions that may modulate atherosclerosis. Thus, the net effect of EL inhibition on atherosclerosis may be complicated and varied among the tissues or cells where EL is expressed, or by the presence of inflammation. The effect of EL inactivation has been proposed, mainly from findings obtained with genetically modified mice. However, it should be noted that the effects of *EL* deletion and pharmacological inactivation of EL, by EL inhibitors if available, are substantially different. Because *EL*$^{-/-}$ mice have undergone genetic deletion of *LIPG*, they do not express EL. In contrast, animals or humans administered with an EL inhibitor do express EL, but it is catalytically inactive. Thus, it is considered that the presence of EL after the administration of EL inhibitor, even if it is catalytically inactive, may promote the clearance of HDL from the plasma, and partly affect the plasma HDL level through its bridging function. From these findings, we speculate that vascular EL may have a relatively proatherogenic role by supplying lipids to the vessel wall or by reducing plasma HDL-C levels, whereas hepatic EL may have an antiatherogenic role by promoting hepatic cholesterol uptake. Therefore, selective inhibition of EL in blood vessels under inflammatory conditions may have beneficial effects on the prevention of atherosclerosis. It is expected that the pharmacological inactivation of EL may inactivate EL at the site of atherosclerosis to increase HDL-C, but would not interfere with its bridging function in the liver.

Finally, the cardiovascular effect of EL inhibition is hardly clarified in mice because there are so many differences in lipid metabolism between humans and mice. Therefore, to provide a useful insight as to whether EL inhibition is beneficial for atherosclerotic diseases in humans, the effects of EL inhibitors should be evaluated in animals that have similar lipid metabolism to humans, for example hamsters, rabbits or monkeys. Overall, EL remains an interesting potential target for therapeutic inhibition as a novel strategy to raise HDL-C and reduce the risk of atherosclerotic CVD.

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Special Report

Diagnosis and Management of Type I and Type V Hyperlipoproteinemia

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Both type I and type V hyperlipoproteinemia are characterized by severe hypertriglyceridemia due to an increase in chylomicrons. Type I hyperlipoproteinemia is caused by a decisive abnormality of the lipoprotein lipase (LPL)- apolipoprotein C-II system, whereas the cause of type V hyperlipoproteinemia is more complicated and more closely related to acquired environmental factors. Since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and V hyperlipoproteinemia are relatively rare, few guidelines for their diagnosis and treatment have been established; however, type I and V hyperlipoproteinemia are clinically important as underlying disorders of acute pancreatitis, and appropriate management is necessary to prevent or treat such complications. Against such a background, here we propose guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

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Key words; Chylomicronemia, Gene mutation, Hyperlipidemia, Lipase, Triglyceridemia

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Background

According to Fredrickson's classification of hyperlipoproteinemia (WHO classification), type I and V hyperlipoproteinemia (hyperlipidemia) are characterized by an increase in chylomicrons alone and an

increase in very low-density lipoprotein (VLDL) in addition to chylomicrons, respectively[1]. Type I hyperlipoproteinemia is a clinical condition showing the severest hypertriglyceridemia and is classically represented by two rare genetic disorders, i.e., familial lipoprotein lipase (LPL) deficiency (MIM 238600) and familial apolipoprotein C-II deficiency (MIM 207750)[2]. Even rarer conditions such as familial inhibitor of lipoprotein lipase (MIM 118830) and the presence of autoantibodies also cause type I hyperlipoproteinemia[3, 4]. More recently, patients with mutations in two additional genes have also been reported to manifest primary type I hyperlipoproteinemia, i.e., genes for glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) (MIM 612757) and for lipase maturation factor 1 (LMF1) (MIM 611761)[5, 6]. Since LPL is an insulin-dependent enzyme, diabetic lipemia observed in insulin-deficient conditions such as type 1 diabetes is well-known as secondary type I hyperlipoproteinemia. Therefore, type I hyperlipoproteinemia is caused by a decisive abnormality of either LPL, which is a rate-limiting enzyme involved in the hydrolysis of triglyceride (TG)-rich lipoproteins such as chylomicrons and VLDL, or apolipoprotein C-II, a cofactor necessary for the expression of LPL activity.

The cause of type V hyperlipoproteinemia is more complicated, and more miscellaneous clinical conditions are considered to belong to this category. It rarely shows familial occurrence, but its inheritance pattern is variable; therefore, type V hyperlipoproteinemia is usually considered to be triggered by acquired environmental factors in individuals with some congenital susceptibility to altered TG metabolism (genetic factors). While the involved environmental factors vary, involvement of heavy drinking, type 2 diabetes, hormonal therapy using steroids and estrogen, and drugs such as diuretics and β-blockers are frequently observed[7].

Many guidelines concerning the diagnosis and treatment of hypercholesterolemia have been formulated[8], and outstanding results of clinical intervention using lipid-lowering drugs, particularly statins, have been reported by large-scale clinical studies. On the other hand, since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and type V hyperlipoproteinemia, in particular, are relatively rare, few guidelines for their diagnosis and treatment have been established either in Japan or abroad; however, diagnostic criteria for primary hyperchylomicronemia were issued in the 1988 report by the Study Group on Primary Hyperlipidemia of the Ministry of Health and Welfare (Group leader: Seiichiro Tarui)[9]. Type I and V hyperlipoproteinemia are important as underlying disorders of acute pancreatitis, which is often lethal, and appropriate management, including restriction of fat intake, is necessary to prevent or treat such complications. Against such a background, the Study Group on Primary Hyperlipidemia of the Ministry of Health, Labour and Welfare (Group leader: Nobuhiro Yamada) proposes guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

Characteristics of Hyperchylomicronemia

The half-life of chylomicrons is about 5 minutes, and no chylomicron is observed in the plasma of normotriglyceridemic to moderately hypertriglyceridemic individuals after 12-hour fasting. Chylomicrons are considered to appear in fasting plasma in those with a serum TG level of about 1,000-2,000 mg/dl or above, and physical symptoms usually occur above this level ($\geq$ 2,000 mg/dl); therefore, there is a strict viewpoint defining hyperchylomicronemia as a serum TG level of 2,000 mg/dl or above accompanied by characteristic complaints or findings. However, caution is necessary, because there are patients showing no clinical symptom even at a serum TG level of 20,000-30,000 mg/dl, even though they are rare. From a clinical standpoint, it must be explained to the patient that there is risk of pancreatitis when the TG level is 1,000 mg/dl or higher even on casual sampling. This may also apply to neonates whose blood sampling after a long period of fasting is usually difficult. It must also be remembered in clinical laboratory testing that a marked increase in the serum TG level often affects the measurement system, causing apparently low serum amylase, hemoglobin, and electrolyte levels (e.g., sodium appears to be reduced by about 2-4 mEq/l with every 1,000 mg/dl increase in the TG). In particular, acute pancreatitis secondary to hypertriglyceridemia must not be misdiagnosed due to apparently low serum amylase.

Type I Hyperlipoproteinemia

A) Familial Lipoprotein Lipase (LPL) Deficiency

a) Concept and Definition

LPL is an enzyme that hydrolyzes TG of lipoprotein particles in blood, and its abnormal activity underlies type I hyperlipoproteinemia in many cases and type V hyperlipoproteinemia in some. Familial LPL deficiency is a rare monogenic disorder that exhibits the severest hyperchylomicronemia. It was first docu-

mented in 1932 in a boy born to a family with a history of consanguineous marriage[10], and the underlying abnormality was demonstrated to be a congenital defect of LPL activity, the rate-limiting enzyme of chylomicron hydrolysis, by Havel *et al.* in 1960[11]. Following the classification of familial hypercholesterolemia, it has been proposed to classify this disease as a class I defect causing complete loss of LPL protein, a class II defect characterized by the production of catalytically inactive protein, and a class III defect characterized by the production of inactive protein lacking affinity to heparan sulfate[12].

b) Etiology

The disease is caused by an abnormality of the human LPL gene, and the patients are homozygotes (including so-called compound heterozygotes) who have inherited LPL gene abnormalities from both parents in an autosomal recessive pattern with penetrance of 100%. The human LPL gene is located on the short arm of chromosome 8 (8p22), is about 35 kb in length, contains 10 exons, and codes for an enzyme protein consisting of 448 amino acids[13-15].

c) Clinical Symptoms

This disease is a relatively rare autosomal recessive disorder, and more than 30 families with this condition have been reported in Japan. The frequency of the occurrence of homozygous patients is estimated to be 1 in every 500,000 to 1 million people. Many patients have a family history of consanguineous marriage, and since patients exhibit chylous serum due to hyperchylomicronemia from early childhood and abdominal pain due to pancreatitis after the intake of fat, the disease is frequently diagnosed during the suckling period or early childhood. In females, the detection of hyperchylomicronemia during pregnancy may lead to the diagnosis. Attacks of abdominal pain due to acute pancreatitis following hyperchylomicronemia are often mistaken for acute abdomen, and the patient may undergo unnecessary laparotomy. While some patients acquire a dietary habit to avoid the intake of fat and suffer growth impairment, some show no marked attack of abdominal pain until adulthood, with consequent overlooking of the disease. It is the primary disease to be differentially diagnosed in a patient with persistent abdominal pain accompanied by hypertriglyceridemia[2].

Hyperchylomicronemia itself is also a major clinical finding, and the serum TG level reaches about 1,500 to even 20,000 mg/dl or more. The presence of chylomicrons can be confirmed by a simple method, i.e., the appearance of a top white cream layer in serum after standing at 4℃ for 24 hours or mild centrifugation. In typical cases, the lower layer is clear and transparent, reflecting an increase in chylomicrons alone. The possibility of LPL deficiency is high if the serum TG level is 1,500 mg/dl or higher, and the serum total cholesterol level is about 1/10 the serum TG level or lower. All other clinical findings are due to the marked increase in chylomicrons. First, eruptive xanthomas, which appear when the serum TG level increases to 2,000 mg/dl or above, are noted in about half of the patients, particularly on the extensor sides of the limbs, buttocks, and shoulders. They appear in association with changes in the serum TG level and disappear gradually over several weeks to a few months. When the serum TG level increases above 4,000 mg/dl, lipemia retinalis, in which the retinal vessels appear whitish pink due to chylous serum on funduscopy, appears, but vision is not impaired. Among other findings, hepatosplenomegaly due to the infiltration of macrophage foam cells that have phagocytozed lipids in the extravascular space, is observed, with hepatomegaly being frequent, but these changes are reversible and are rapidly improved (within 1 week) with correction of the serum lipid levels; however, the most serious complication is acute pancreatitis, and it must be managed carefully as it may be a prognostic determinant. From a clinical viewpoint, the possibility of acute pancreatitis must be explained to the patient if the TG level is 1,000 mg/dl or higher even on casual sampling. Dyspnea and neurological symptoms such as dementia, depression, and memory disorders have been reported as complications of this disorder.

As mentioned above, a major prognostic determinant of homozygous familial LPL deficiency is acute pancreatitis, which is often lethal. LPL deficiency has long been considered not to be closely related to atherosclerosis in humans, because no marked atherosclerotic lesion was noted at the autopsy of several homozygous patients with LPL deficiency who died due to acute pancreatitis. However, detailed research has reported that heterozygotes, which are considered to occur in 1 in every 500 individuals, usually show no marked abnormality in the lipid level but are likely to exhibit hypertriglyceridemia when they develop diabetes or are exposed to burdens such as severe obesity, excessive drinking, and pregnancy[16, 17]. There have also been reports of the frequent occurrence in heterozygotes of familial combined hyperlipidemia (FCHL)[12] and monogenic familial hypertriglyceridemia[16], which are common hyperlipidemia related to atherosclerosis; however, it remains controversial whether homozygotes with LPL gene abnormality are likely to develop atherosclerosis. A Canadian group